Enzyme-membrane-reactors: recent trends and applications for the production of fine chemicals and pharmaceutical building-blocks

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ABSTRACT

Biocatalysis has gained attention in recent decades as a green and efficient method for producing high-value chemicals. Enzymes, notably due to their high selectivity, offer significant advantages for organic synthesis. However, industrial implementation remains limited due to challenges such as free enzyme instability, enzyme inhibition, and difficulties in catalyst recovery and reuse. The coupling of biocatalysis with membrane technology in enzyme-membrane-reactors (EMR) holds significant potential for process intensification, as it paves the way to continuous flow synthesis concatenated with product purification, and biocatalyst recovery. By allowing flow hybrid processes (i.e. simultaneous biocatalytic reactions and membrane operations in one-pot), EMR have the potential to increase reaction yields and kinetics, and reduce downstream processing requirements. This review explores recent trends and advancements in EMR for the production of pharmaceutical building blocks and fine chemicals. We examine the combination of enzymes with both polymeric and ceramic membranes, highlighting their respective benefits and limitations. We cover both EMR processes where free enzymes are used separately from membrane devices, and EMR processes employing membrane-immobilized enzymatic reactors. As enzyme immobilization in/on solid supports has emerged as an effective approach for enhancing enzyme stability and reusability, we argue that the development of such membrane-immobilized enzyme reactors is of prime importance for the pharmaceutical industry. These insights aim to provide a comprehensive overview of the role and recent applications of EMRs in advancing biocatalytic processes within the fine chemistry and pharmaceutical industry.

KEYWORDS

Biocatalysis, membranes, membrane processes, intensification, enzyme immobilization, pharmaceutical industry

1 Introduction – membranes as a practical tool to intensify biocatalytic processes

Over the past decades, biocatalysis has emerged as a promising and potentially greener approach to produce value-added molecules [1]. Owing to their unique characteristics, such as their high (enantio)selectivity and stereospecificity, non-toxicity (biodegradable), and their ability operate under mild conditions (e.g., aqueous media, low temperatures), enzymes have gained significant interest in organic synthesis and pharmaceutical industry [2]. Hence, biocatalytic processes have the potential to rapidly become a powerful synthetic tool for the industrial preparation of valuable compounds, such as pharmaceuticals and fine chemicals [3–5].

However, despite multiple benefits and advantages they offer, their implementation at the industrial level in the pharmaceutical industry is not straightforward and remains limited [6], due to several challenges. These are generally related to the fact that enzymes are often used in their "free" form, functioning as soluble homogeneous biocatalysts that are difficult to reuse, restricted to batch reactors, and which typically exhibit limited stability [7]. Additionally, enzymes tend to suffer from substrate and/or product inhibition, and – in some cases – unfavorable thermodynamic equilibria for the targeted reactions. The quality of the final product can also be compromised by free enzyme deactivation, resulting in complex purification processes [8].

Some of these challenges can be mitigated by immobilizing enzymes on solid supports [9,10], which allows for their recovery and reuse, and facilitates their implementation in continuous flow processes [11]. Moreover, immobilization often results in enhancing the enzyme stability and tolerance to organic solvents [12,13]. The transfer from batch to continuous-flow processing is of major industrial interest as it increases the productivity of (bio)catalytic transformations and the efficiency of subsequent/coupled unit operations (e.g. crystallization), thus improving the overall process's economic viability [1,14–23]. Enzymatic processes arguably pave the way for the development of intensified industrially relevant organic synthesis [24]. Enzyme immobilization can be performed in various ways, and on a wide range of functional materials such as polymeric resins, inorganic powders, biopolymers, or membranes [12,25,26]. Ready-to-use synthetic resins are the traditional ubiquitous carrier, allowing to run biocatalytic reactions in heterogeneous catalysis mode, and to easily recover and reuse the biocatalysts.

Enzyme immobilization on membranes is a particular case which deserves attention, as it additionally offers the possibility to perform biocatalytic reactions along with membrane separation, in a one-pot fashion. For example, the removal of a product can favorably shift the biocatalytic reaction equilibrium toward product formation and hence increase the reaction yield. Also, membranes can be used to introduce one of the reagents at a controlled rate, to avoid enzyme inhibition. The coupling of biocatalytic reaction with membrane operation in so-called “enzyme-membrane-reactors” (EMR) has

the potential to intensify biocatalytic processes. Typically, membrane separations require only a limited amount of energy with respect to other unit operations [27–29]. Furthermore, membrane-reactors display relatively easy reactor operation and modulation, as well as straightforward scale-up to large systems [8,30–32]. Thus, integrated hybrid processes allowing to simultaneously perform flow biocatalytic reactions and product separation (e.g. to drive the equilibrium) or controlled substrate addition, are of particular interest for the pharmaceutical industry, as they can help reducing the need for additional downstream steps typically required to obtain pure APIs [33–35].

It must be noted that the concatenation of reaction and membrane separation on catalytically active membranes is already well established, and has been reviewed extensively. For example, Zhang et al.[36] reviewed the applications of a wide range of polymeric catalytic membranes to intensify chemical processes. Also, conventional enzyme immobilization methods and strategies have already been extensively and thoroughly reviewed [8,37–41]. These accounts focus on the detailed preparation and/or functionalization of various supports – including membranes – for enzyme immobilization. Also, some excellent reviews [8,34,36,39,42–44] cover the interests of implementing EMR in organic synthesis, by gathering scholarly examples and/or providing useful insights on industrial process considerations. For example, the tutorial reviews by Sitanggang et al. [44] and Dejonghe et al. [34] summarize the interest of coupling enzymes (free or immobilized) with membrane reactors and exemplified their use in a selection of chemical processes.

In this review, we aim to discuss the recent trends (i.e. 2010 or later) of EMR processes applied specifically for the production of valuable (chiral) building blocks for the pharmaceutical industry. We cover both ceramic and polymeric membranes applications. Polymeric membranes are most commonly employed support to develop biocatalytic membrane reactors, and present an array of advantages with respect to their ceramic counterparts. For example, polymeric membranes tend to be generally cheaper and offer a wider range of manufacturing techniques have been developed to enable better control and tailoring of the final membrane properties [2,36]. Yet, their organic nature often hampers their chemical stability, which could be an issue when such membranes are put in contact with organic solvents and hence, limit their applicability in multiphasic membrane reactors [45]. On the other hand, ceramic membranes are able to overcome these drawbacks thanks to their inherent outstanding chemical and thermal stability [45,46], hence we consider that it is also important to cover their applications.

Overall, one of the key advantages of coupling biocatalysis with membrane technology is the ability to run biocatalytic reactions while simultaneously performing product/substrate separation from the enzymes, and from the reaction medium. Furthermore, it allows operating the synthesis process in continuous flow mode, which can enhance productivity and economic feasibility. Using multiple membranes in series with different molecular weight cut-offs (MWCO) can also enhance product selectivity, and the final step of membrane separation allows the concentration of the non-permeable

product. The selected membrane material must be stable under the conditions (temperature, pH, presence of organic solvent) that optimize the enzyme's catalytic activity. When being coupled with membrane reactors, enzymes can be used either separately from the membrane devices, or as membrane-immobilized enzymatic reactors. This defines the two categories of hybrid processes that are discussed in this review. The coupling of enzymes with membrane reactors is exemplified in Section 2, while the use of membrane-immobilized enzymatic reactors is reviewed in details in Section 3.

2 Main synthetic routes that can benefit from the synergistic use of enzymes and membranes

Chiral compounds are the most important building blocks in the chemical and pharmaceutical industry, as they are widely employed for the production of fine chemicals and drugs [47,48]. Moreover, it is increasingly important to synthesize enantiopure drugs for the pharmaceutical industry [49,50]. More precisely, enantiopure alcohols [51] and amines [1,3,52] are key examples of prime importance in the synthesis of pharmaceuticals, but also in the flavors and fragrances industry. Small peptides and short oligosaccharides are other categories of functional molecules that can be accessed via biocatalytic synthesis processes.

Using transaminases (Figure 1a) and alcohol dehydrogenases (Figure 1b) is a conventional biocatalytic strategy to produce chiral amines and alcohol, respectively [53–55]. Most industrially relevant transaminations suffer from unfavorable thermodynamics, and transaminases tend to be inhibited by their keto substrate (amino-acceptor) and by-products. To achieve high transamination yields while avoiding transaminase inhibition, in situ (co)-product removal and/or controlled substrate addition can be performed using membrane technologies [56–58]. One possible strategy is the use of pervaporation to remove acetone (i.e. the most common co-product of transamination reactions). Pervaporation is especially attractive for the temperature-sensitive processes because it can be operated at moderate temperatures. Dejonghe et al. [58] implemented a hydrophobic pervaporation at the outlet of a transamination reactor (employing free enzymes) in order to remove the acetone by-product from the biocatalytic system. A PolyDiMethylSiloxane (PDMS) membrane module was used as pervaporation unit. Such transamination coupling with pervaporation resulted in a 13% increase in product yield after 9 hours of reaction, compared to the standard transamination process (where no pervaporation was performed). However, it was also observed that the effect of acetone removal by pervaporation is minimal at low acetone concentrations (in the biocatalytic system). This highlights the need to work at high substrate concentrations and possibly to couple pervaporation to another product separation technique (which would allow to primary push the transamination towards high product yields). This other product separation technique should target the amine product, and may be also performed via membrane technology (e.g. via membrane-extraction). Notably, Dejonghe et al. [59] conducted the flow

asymmetric synthesis of 1-methyl-3-phenylpropylamine (MPPA) from benzyl acetone using free ATA-v2 mutant enzyme in organic solvent (n-heptane), and employed polypropylene (PP) membranes contactor for the *in situ* amine product removal. An acidic aqueous solution (pH = 3) was used to efficiently extract the amine product via membrane-assisted extraction. In this study, the use of an organic solvent as reaction medium was found beneficial in the sense that it allowed to increase the optimal keto-substrate concentration (by 2.5-fold with respect to its aqueous solubility) without triggering inhibition. This approach resulted in a significant increase in terms of final product yield (i.e. 99% yield) with respect to the standard transamination process (i.e. 69% yield when run without membrane-assisted extraction). Additionnally, by demonstrating the intensified asymmetric synthesis of the R-sitagliptin drug, Yang et al. [60] successfully expanded the applicability of such synergistic coupling between transamination and membrane-assisted product separation. In this case, transamination was conducted in aqueous medium (pH = 9) and PDMS was used as membrane contactor for the solvent extraction. In all these examples, the use of membrane operations offers avenues to intensify the synthesis process.

Other (chiral) molecules such as carboxylic acids or esters can lead to the obtention of valuable compounds, including chiral intermediates [32,61–63]. To this aim, lipases have gained much attention lately as they allow for the enantioselective hydrolysis/esterification (Figure 1c) and transesterification (Figure 1d) of poorly water-soluble compounds (i.e. in biphasic media or in organic solvents) [64,65]. Enzymatic esterification reactions are commonly conducted in non-aqueous solvents, as water accumulation in the reaction medium can promote side reactions (e.g. hydrolysis) and hamper the final esterification yields [66,67]. Additionally, for enzymatic process, it is well-known that excess water should be avoided to preserve high lipase activity [68,69]. In lipase-catalyzed esterification, for example, water by-product can be removed *in situ* from the reactor using membrane technologies. Pervaporation seems particularly suited for such purpose, since it requires significantly lower energy consumption and operating costs with respect to distillation processes [70]. Notably, the pervaporation-aided enzymatic production of monoacylglycerols from lauric and caprylic acid with glycerol, in solvent-free medium, was demonstrated by Satyawali et al. [71] in 2021. Lipases immobilized on polymeric resins in packed-bed reactor (3 kg scale) operating in recirculation mode, were coupled with two zeolite membranes (in series, 56.5 cm^2 total area) for pervaporation. Such coupled esterification-pervaporation process not only allowed to push the fatty acids conversion towards completion (> 95% after 256 hours), but also afford to increase the relative monoacylglycerols content in the final product (with respect to di- and triacylglycerols). Such study demonstrates the practical applicability of zeolite based membranes for *in situ* water removal through hydrophilic pervaporation.

Various functional oligosaccharides, such as lactulose, galacto-oligosaccharides (GOS) [72], cyclodextrins [73] or oligodextran (5-8 kDA) [74,75], are known to have potential applications in the fine chemicals and pharmaceutical industries. β-galactosidase can catalyze the direct obtention of GOS

(Figure 1e) and lactulose from lactose via transgalactosylation reactions [72,76,77]. Lactose (a disaccharide composed of glucose and galactose) is generated in high contents in the dairy by-product of many (bio)chemical processes [78], and acts as a renewable feedstock to produce such building blocks. Cyclodextrin glycosyltransferase and dextranase catalyze the conversion of starch into mixtures of (cyclo)dextrins and the hydrolysis of dextran into oligodextrans of various molecular weights, respectively. A severe limitation in transgalactosylation reactions is that the targeted products tend to spontaneously undergo undesired consecutive reactions (i.e. hydrolysis reactions to monosaccharides glucose and galactose) [79]. Besides, it has been observed that such monosaccharides formation inhibits the β-galactosidase (i.e. inhibition of transgalactosylation reaction) [34], which further highlights the need of a product separation in these processes. Finally, selecting producing oligodextran of tailored molecular weight is also a complicated task. Hence, it clearly appears that, in all these biocatalytic reactions, the coupling with membrane technology is of particular interest, since it would afford to enhance the selectivity of the process, and the purity of the targeted product (i.e. desired molecular weight) by operating adequate size-exclusions membrane operations.

Peptides are another class of target compounds exhibiting biological activities (e.g. antihypertensive, antioxidant), which find nutritional, cosmetic, pharmaceutical applications [34,80]. Peptidases catalyze their production through protein hydrolysis (Figure 1f). However, it is established that the efficiency of peptidases decreases upon accumulation of the hydrolysis products (i.e. soluble peptides and amino acids) in the medium [34,81]. Here, conducting protein hydrolysis in continuous membrane reactors (i.e. through size-exclusion membrane operations) enables the separation of low-molecular-weight peptides from protein hydrolysates, thereby overcoming the drawbacks of batch reactions, such as product inhibition, low process productivity, and excessive hydrolyses (i.e., prevention of side reactions) [34]. Additionally, the continuous feeding of substrates (e.g. water in this case) to the reactor is advantageous as it allows to enhance the reaction kinetics, and to maintain a constant volume in the reactor by compensating the permeate flux. Such beneficial substrate addition was demonstrated by Ma et al.[82], which conducted the membrane-assisted enzymatic protein hydrolysis for the tailored production of antihypertensive peptides. First, the membrane ultrafiltration unit was implemented at the outlet of the reactor tank, which allowed to recycle the free enzymes (retained in the retentate) and prevent undesired product inhibition (*via* product separation). When further coupling the enzymatic hydrolysis (performed in recirculating mode) with continuous feeding of water to the reactor, the yield and productivity were respectively enhanced by 62.7% and 22.1% when compared to the standard batch operation (i.e. run without membrane separation and substrate feeding). The continuous addition of the protein substrate (in addition to water feeding) enabled to further boost the peptides productivity.

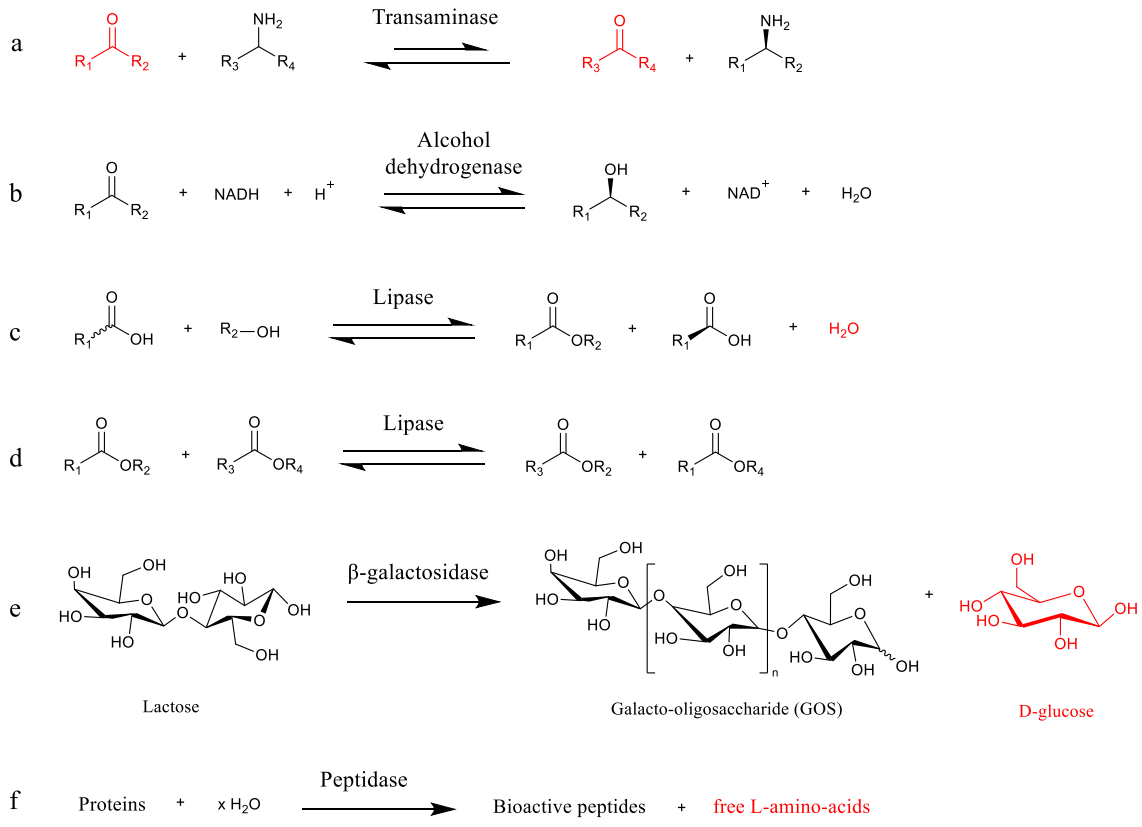

Figure 1. Main enzymatic transformations leading to pharmaceuticals and fine chemicals, and susceptible to be coupled with membrane reactors, studied in this work. Products highlighted in red are responsible for enzyme inhibition.

As mentioned above in the exposed examples, when working with free enzymes in solution, size-exclusion membrane operations are often chosen to separate the soluble biocatalyst [42] (retained in the retentate in cross-flow operations, see Figure 2) while isolating the product in the permeate [44]. In such size-exclusion operations, the membranes porosity – which defines its molecular weight cut-off (MWCO, in kDA) – has to be carefully chosen based on the molecular size of the enzyme (if present in free form), substrate(s), and product(s). Given that enzyme molecular weights typically range from 10 to 150 kDa, ultrafiltration (UF) membranes are commonly used in membrane reactors designs. The UF membrane pore size must ensure complete enzyme retention while ensuring unobstructed product transport. Nanofiltration (NF) membranes can also be employed in size-exclusion membrane operations design, especially for biocatalysts displaying small molecular weights (typically 0.2 to 10 kDA, such as for example such as for example some cysteine proteases [83])[84,85]. Additionally, NF membranes are effective in concentrating target compounds as a secondary separation step [86]. As product purification is known to be a major driver of the cost of drugs manufacturing, this is a crucial point.

Further than facilitating product separation, membrane-based operations are particularly helpful to displace the thermodynamic equilibrium and enhance synthesis yields. Table 1 gathers a list of

membrane-induced equilibrium-shifting strategies have already been applied in combination with soluble (or immobilized) enzymes, in order to intensify biocatalyzed reactions (among the selected biocatalytic transformations listed in the Figure 1). Here, soluble enzymes were typically employed separately from the membrane devices (i.e. membranes were at the boundary or at effluent side of the enzymatic reactors, acting as separation units) as represented in Figure 2, and not in a one-pot fashion. Note that other membrane-intensified transaminations employing heterogenized biocatalysts were also reported [56,57,87,88], yet these studies fall outside of the scope of such report as they involved whole-cells (instead of enzymes) as biocatalysts. This list of example shows the diversity of approaches and applications. We argue that the incorporation of enzyme into/onto membranes represents the next important step in the direction of intensification; as it is both more challenging and emerging, we discuss this in more details in the following section.

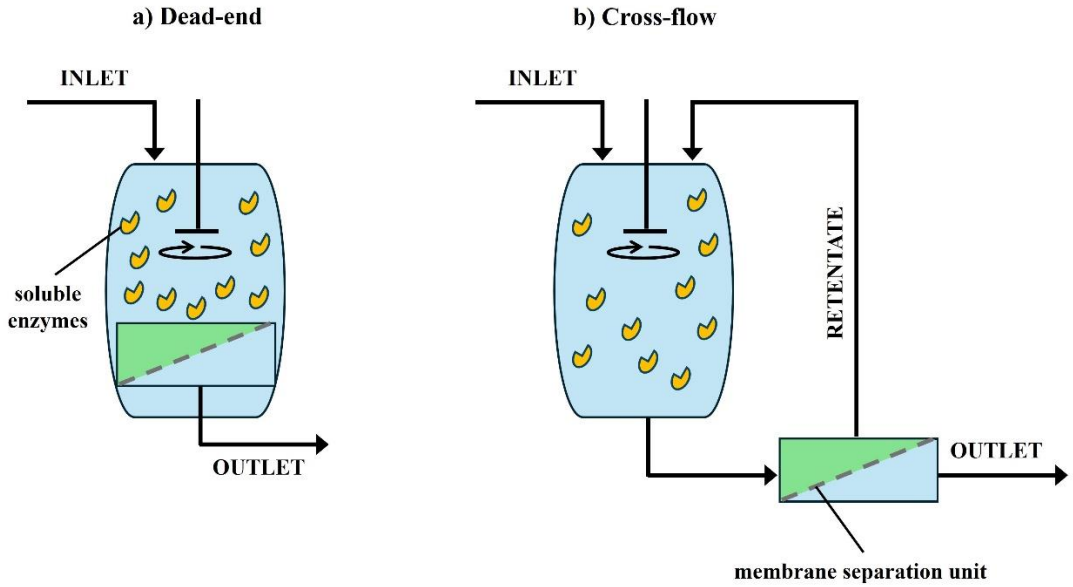

Figure 2. Schematic representation of biocatalytic processes involving free enzymes in combination with membrane separation units. In case **b)** enzymes are recycled (retained) in the retentate through membrane separation. Reproduced from Sitaggang et al (2022), with permission.

Table 1 – Exhaustive list of recent (2010-2024) examples of enzymes combination with membrane technologies for the considered biocatalytic processes intensification

Reaction(s)	Substrate	Product	Enzyme	Immob. (carrier)	Membrane	Operation	Mode	Refs
Carboligation	Benzaldehyde + acetaldehyde	(S)-2-hydroxypropiophenone	Benzoylformate decarboxylase	/ (free)	Polymeric (N.D., 10 kDA)	UF (dead-end)	Flow	[89]
Cascade (De)hydrogenation	Octanone + D-glucose	R-octanol + D-gluconolactone	Alcohol dehydrogenase + glucose dehydrogenase	/ (free)	N.D. (10kDA)	UF (dead-end)	Flow	[90]
Esterification	Fatty acids + glycerol	Monoacylglycerols	Lipase	Immob. (acrylic resin)	Ceramic (zeolites type T, N.D.)	Pervaporation	Recirculation	[71]
Esterification	Fatty acids + glycerol	Isopropyl esters	Lipase	Immob. (acrylic resin)	Ceramic (zeolites type T, N.D.) and polymeric (PVA, N.D)	Pervaporation	Recirculation	[91]
O-glycosylation	Resveratrol	Resveratrol *O*-glycosides	β-cyclodextrin–resveratrol	/ (free)	Polymeric (N.D., 0.9 or 1.4 kDA)	NF	Recirculation	[92]
Transgalactosylation	Lactose	GOS	β-galactosidase	/ (free)	Polymeric (Cellulose, 50kDA)	UF + NF	Flow	[93]
Transgalactosylation	Lactose	GOS	β-galactosidase	/ (free)	Polymeric (PES, 10 kDA)	UF	Recirculation	[94]
Transgalactosylation	Lactose	GOS	β-galactosidase	/ (free)	Ceramic (TiO_2, 20 kDA)	UF	Flow	[95]
Transgalactosylation	Lactose	GOS	β-galactosidase	/ (free)	Ceramic (50 kDA)	UF	Recirculation	[96]

Transgalactosylation	Lactose	GOS	β-galactosidase	/ (free)	Polymeric (cellulose acetate, N.D.)	UF	Recirculation	[97]
Transgalactosylation	Lactose	GOS	β-galactosidase	/ (free)	Polymeric (PES, 10 kDA)	UF	Recirculation	[98]
Transgalactosylation	Lactose + fructose	Lactulose	β-galactosidase	/ (free)	Polymeric (PES, PS, cellulose acetate, fluoro polymers 10 kDA)	UF (dead-end)	Flow	[99–101]
Hydrolysis	Phenylethanol + vinyl butyrate	Phenylethyl butyrate + acetaldehyde	Lipase (in water-oil emulsions)	/ (free)	Polymeric (PES, 10 kDA)	UF (dead-end)	Flow	[102]
Hydrolysis	Starches	Cyclodextrin	Cyclodextrin glycosyltransferase	/ (free)	Polymeric (Cellulose, 10 kDA)	UF	Flow	[103,104]
Hydrolysis	BSA	Bioactive peptides	Peptidase	/ (free)	Polymeric (PES, 3 or 10 kDA) or ceramic (ZrO_2/TiO_2, 15 kDA)	UF	Recirculation	[105]
Hydrolysis	*P. yezoensis* protein	Bioactive peptides	Peptidase	/ (free)	N.D. (3 kDA)	UF	Flow	[82]
Hydrolysis	Egg white protein	Bioactive peptides	Peptidase	/ (free)	Polymeric (PES, 10 kDA)	UF	Flow	[106]
Hydrolysis	Casein	Bioactive peptides	Peptidase	/ (free)	Polymeric (PES, 1 to 10 kDA)	UF	Recirculation	[107]
Hydrolysis	Corn gluten meal	Bioactive peptides	Peptidase	/ (free)	Polymeric (Cellulose, 1 to 5 kDA)	UF	Flow	[108]
Hydrolysis	Casein	Bioactive peptides	Peptidases	/ (free)	Polymeric (PES, 10 kDA) and ceramic (Al_2O_3, 10 kDA)	UF	Flow	[109]

Hydrolysis	Wheat gluten	Protein hydrolysate	Peptidases	/ (free)	Ceramic (hollow fibers Al_2O_3, 5 or 10 kDA)	UF	Flow	[110]
Hydrolysis	Sesame seeds	Bioactive peptides	Peptidases	/ (free)	Polymeric (PES, 5 kDA)	UF	Flow	[111]
Hydrolysis	Milk	Bioactive peptides	Peptidases	/ (free)	Polymeric (Cellulose, 5 kDA)	UF	Flow	[112]
Hydrolysis	Whey	Inhibitory peptides	Peptidase	/ (free)	Polymeric (PES, 3 kDA)	UF	Flow	[113]
Transamination	Pro-sitagliptin ketone + isopropylamine	R-Sitagliptin + acetone	Transaminase	/ (free)	Polymeric (Psf, 10 kDA) + dense PDMS	UF (for enzyme retention) + solvent extraction	Recirculation	[60]
Transamination	Benzyl acetone + isopropylamine	R-1-methyl-3-phenylpropylamine + acetone	Transaminase	/ (free)	Polymeric (PP, N.D.)	Solvent extraction	Recirculation	[59]
Transamination	Acetophenone + isopropylamine	R-MBA + acetone	Transaminase	/ (free)	Dense PDMS	Pervaporation	Batch	[58]

N.D. – non-specified ; PVDF - polyvinylidene fluoride ; PES – polyethersulfone ; Psf – polysulphone ; PS – polystyrene ; BSA – Bovine Serum Albumin ; MBA – methylbenzylamine ; PDMS – Polydimethylsiloxane ; UF – ultrafiltration ; NF – nanofiltration ; GOS – galacto-oligosaccharides

3 Membrane-immobilized enzymatic reactors

The incentives for immobilizing enzymes to intensify biocatalytic processes are evident. Enzyme immobilization is a prerequisite to envisage recovery and reuse. Immobilization usually tends to enhance enzyme stability and tolerance to organic solvents, and to allow for the use of different reactor configurations [44], which are key features industrial processes. It also paves the way to the integration of enzymes within heterogeneous catalysts, forming so-called hybrid chemo-enzymatic catalysts that are excellent candidates to run intensified cascade reactions [114–119]. A potential downside of enzyme immobilization is that enzymatic activity may – in some cases – be decreased through the immobilization process (due to active site blockage, for example). Nevertheless, free enzymes can also experience activity reduction over time due to heat and mechanical stresses during extended biocatalytic processes [120].

Immobilization on membranes is going one step further in the direction of process intensification, as it implies fixing the enzyme on a material that is itself functional in the sense that it is able to perform tailored compounds separation. As in the previous section, the membrane can be used to remove products during reaction, to inject reagents in a controlled way, etc. In some cases, the membrane itself can also be chemo-catalytically active. However, prior to enzyme immobilization on a membrane, the membrane surface often needs to be functionalized or chemically modified. Indeed, appropriate membrane materials which are directly amenable to enzyme immobilization are very rare [8]. A plethora of different surface functionalization techniques (e.g. wet chemical modifications,, plasma or UV exposure) have been reported in the literature [8,39,121,122], showing a variety of approaches, depending on the substrate chemical nature. The implementation of such surface modifications depends on the type of enzyme immobilization (e.g. covalent grafting, electrostatic-assisted adsorption, site-specific immobilization through coordination) that is envisaged, as well as the membrane operation which are targeted in the intensified biocatalytic process. Among the different existing strategies, silanization of inorganic membrane surface using (3-aminopropyl)triethoxylsilane (APTES) and polydopamine (PDA) coating deposition on polymeric membranes have been employed over the recent years [15,17,123–126] to confer amino-groups at their surfaces, serving as anchoring points for grafting. In these cases, glutaraldehyde (GA) is most conventionally used as a coupling agent between the enzyme and the functionalized membrane. Another general trend is to try and stabilize the immobilized enzymes via electrostatic interactions; polyelectrolytes such as polyethyleneimine (PEI) are often employed with this aim.

Table 2 gathers an exhaustive list of examples of membrane-immobilized enzymes exploited for the production of fine chemicals and pharmaceuticals, among the selected biocatalytic transformations listed in Figure 1. Enzymes are immobilized either in the membrane (i.e. entrapped in the membrane

pores) or onto the membrane surface. In these examples, the membrane can either be employed as a mere solid support (i.e. not exploited to perform membrane operations), or as a functional support forming an enzyme-membrane-reactor which acts as a combined reaction-separation unit (Figure 3).

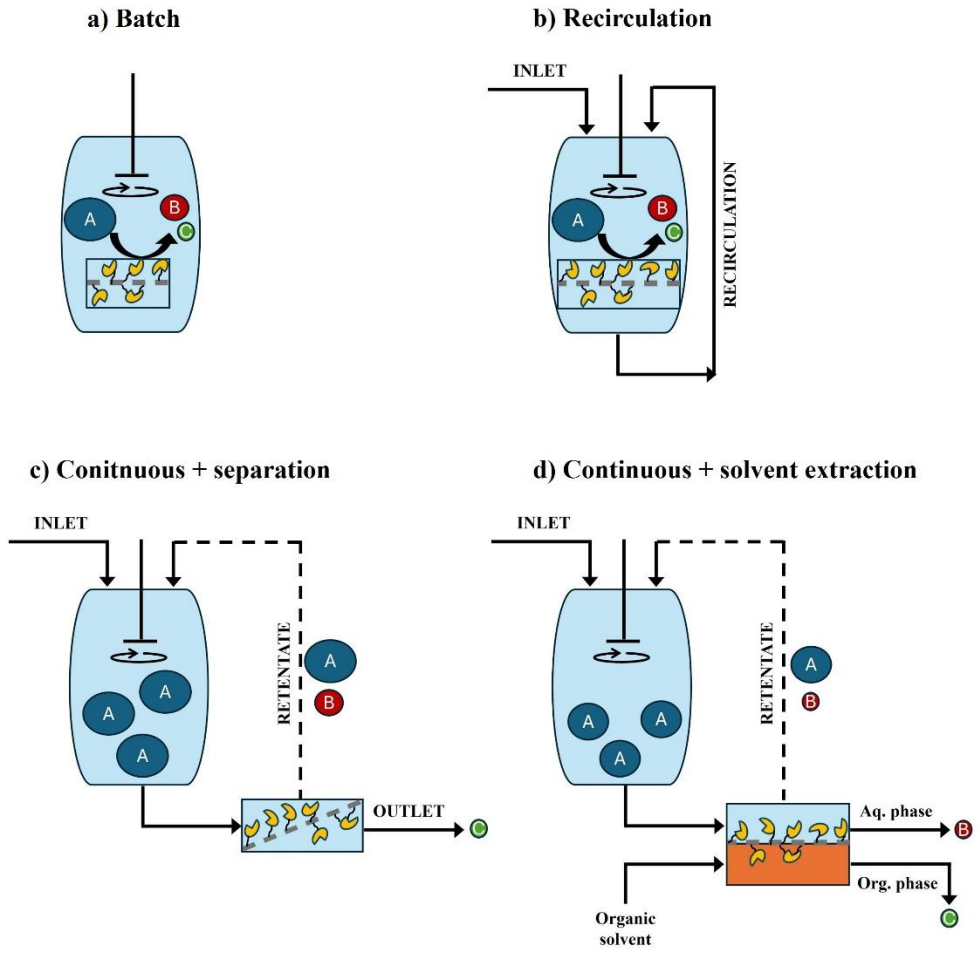

Figure 3. Schematic representation of membrane-immobilized enzymes, acting as simple heterogeneous membrane-immobilized biocatalysts (i.e. not performing membrane operations) operating in **a)** batch or **b)** in recirculating mode, or as enzyme-membrane-reactors (i.e. reaction-separation unit) in **c) compounds separation (**e.g. **via** size-exclusion, ion-exchange, etc.) or **d)** solvent extraction operations. Note that, when omitting the re-circulations, **c)** and **d)** become continuous flow operations.

Table 2 – Exhaustive list of recent (2010-2024) examples of membrane-immobilized enzymes, acting as simple heterogeneous membrane-immobilized biocatalysts (i.e. not performing membrane operations, operating in batch or in flow mode, with or without recirculation), or as enzyme-membrane-reactors (i.e. a reaction-separation unit), for the production of fine chemicals and pharmaceutical building blocks.

Reaction(s)	Substrate	Product	Enzyme(s)	Immob. (R. Sp. act)	Comments	Membrane	Separation	Mode	Refs
DKR	Ibuprofen ester	S-ibuprofen acid	Lipase	Entrapment (90%)	Retained 53% of initial sp. activity after 120 h of operation	Polymeric (PAN, 50 kDA)	Solvent extraction (interfacial; OP = isooctane)	Recirc.	[127,128]
Esterification (lipophilization)	Hesperidin + lauric acid	Hesperidin laurate	Lipase	Covalent grafting + cross-linking (c.a. 900%)	/	Polymeric (PDA + GA modified PES, 30 kDA)	/	Batch	[129]
Esterification	Stearic acid + lauryl alcohol	Lauryl stearate	Lipase	CLEAs + entrapment (142 %)	/	Polymeric (CLEAs in PVA matrix, on PVA/PES dense layer)	Pervaporation	Recirc.	[68]
Esterification	Ethanol + lactic acid	Ethyl lactate	Lipase	Entrapment (c.a. 100%)	Preserved 90% of sp. activity upon 6 cycles (i.e. 36 h of operation)	Polymeric (Sodium alginate network)	Pervaporation	Recirc.	[130]
Esterification	Valeric acid + ethanol	Ethyl valerate	Lipase	Covalent grafting (76%)	/	Polymeric (PPC, 150 kDA)	/	Batch	[131]
Transesterification	Rac-phenylethanol	S-phenylethanol + R-phenylacetate	Lipase	Covalent grafting (/)	/	Ceramic (ZrO_2, 300 kDA)	/	Recirc.	[132]

Trans-galactosylation	Lactose	GOS	β-galactosidase	Covalent grafting (c.a. 75%)	Retained 50% of its initial sp. activity after 30 days of storage	Polymeric (GA-modified PVDF)	Filtration	Recirc.	[133,134]
Trans-galactosylation	Lactose	GOS	β-galactosidase	Covalent grafting + cross-linking (/)	/	Polymeric (PES, 5-50 kDA) and NF (0.4 kDA)	UF or NF	Flow	[135,136]
Trans-galactosylation	Lactose	GOS	β-galactosidase	Covalent grafting (104 %)	Maintained 54% of its initial sp. activity after 20 cycles	Polymeric (GO + APTES modified electrospun PS nanofibers)	/	Batch	[137]
Trans-galactosylation	Lactose	GOS	β-galactosidase	Adsorption (95 %)	/	Mixed matrix (PSf + ZrO_2, 13.8 kDA)	/	Batch	[138]
Trans-galactosylation	Lactose	GOS	β-galactosidase	Covalent grafting (/)	Maintained 91% of its sp. activity after 16 h of operation	Magnetic-responsive on polymeric membrane (PES, UF 50 kDA)	UF	Flow	[139]
Trans-galactosylation	Lactose	GOS	β-galactosidase	Covalent grafting + cross-linking (140 %)	/	Polymeric (PEI + GA-modified PSf, 10 kDA)	UF	Recirc.	[140]
Hydrolysis	Oleuropein	Oleuropein aglycon	β-glucosidase	Entrapment (c.a. 100%)	Activity was unchanged after 30 h of continuous operation	Polymeric (Psf and cellulose, 30 kDA)	Solvent extraction of product (OP = Limonene)	Flow	[141–144]
Hydrolysis	Oleuropein	Oleuropein aglycon	β-glucosidase	Covalent grafting (c.a. 100%)	/	Ceramic (APTES + GA-modified Al_2O_3 capillary, 30 kDA)	Solvent extraction of product (OP = Ethyl acetate)	Flow	[45]
Hydrolysis	Dextran	Oligodextran	Dextranase	Covalent grafting	/	Polymeric (Tannic Acid +	UF	Flow	[145]

				(/)		APTES-modified PES (30 kDA) or cellulose (10 kDA))			
Hydrolysis	Dextran	Oligodextran	Dextranase	Adsorption (/)	/	Polymeric (PES, 20 or 30 kDA)	UF	Flow	[75]
Hydrolysis	Wheat gluten	Protein hydrolysate	Peptidase	Entrapment (/)	/	Polymeric (UF) (PES, 10 kDA)	Filtration (dead-end)	Flow	[146]
Hydrolysis	BSA	Bioactive peptides	Peptidase	Electrostatic interactions	/	Polymeric (PDA + PEI modified PES, 50 kDA)	UF	Flow	[147]
Hydrolysis	BSA	Bioactive peptides	Peptidase	Covalent grafting (/)	50% initial sp. activity retained after 6 cycles of 1h	Polymeric (EDC/NHS-modified PVDF, Nylon 6,6, chitosan composite)	/	Batch	[148]
Hydrolysis	Whey protein	Bioactive peptides	Peptidase	Electrostatic interactions (80 %)	/	Polymeric (PDA + PEI modified PES, 30 kDA)	/	Batch	[149]
Oligomerization	Rutin	Oligorutin	Laccase	Entrapment (/)	90% of initial sp. activity after 3 cycles of 24 h	N.D. (10 kDA)	Filtration (dead-end) of product	Batch	[150]
Oxidation	Tyrosine	L-DOPA	Tyrosinase	Entrapment (183%)	Unaltered initial sp. activity for 30 h of continuous operation	Polymeric (polyamide, 20 kDA)	UF	Flow	[151]
Oxidation	Tyrosine	L-DOPA	Tyrosinase	Covalent grafting (276%)	/	Ceramic (GA-modified NaY zeolite)	/	Recirc.	[152]
Oxidation	Tyrosine	L-DOPA	Tyrosinase	Covalent grafting (87%)	Unaltered initial sp. activity after 5 catalytic cycles (c.a. 24 h operation)	Polymeric (DAB + GA-modified PVDF)	/	Batch	[153]
Transamination (deamination)	S-MBA + pyruvate	Acetophenone + L-alanine	Transaminase	Coordination (His-tag) (23%)	Retained productivity after 5 days of continuous operation was 81 %	Polymeric blend (Cu-functionalized fibers)	/	Flow	[154]

Transamination (deamination)	S-MBA + pyruvate	Acetophenone + L-alanine	Transaminase	Covalent grafting + His-tag driving (43.6%)	/	Polymeric blend (Co-derivatized PCADE/PVDF)	/	Batch	[155]
Cascade (transamination + (de)hydrogenation)	Cinnamaldehyde	Cinnamyl amine	Transaminase + alanine dehydrogenase + formate dehydrogenase	EPC entrapment	/	N.D. (12 kDA dialysis membrane)	/	Batch	[156]
Transamination (kinetic resolution)	Rac-BMBA + pyruvate	S-BMBA + BAP + D-alanine	Transaminase	Covalent grafting (85%)	Unaltered initial sp. activity after 8 catalytic cycles (c.a. 16 h operation)	Polymeric (PDA + GDE + PEI-modified-PP)	/	Batch	[157]

R. Sp. act. – Recovered specific activity (with respect to free enzymes) ; N.D. – non-determined ; DKR – dynamic kinetic resolution ; OP – organic phase ; PAN – polyacrylonitrile ; PDA – polydopamine ; GA – glutaraldehyde ; PVA – polyvinyl alcohol ; PVDF - polyvinylidene fluoride ; CLEAs – cross-linked enzyme aggregates ; PES – polyethersulfone ; Psf – polysulphone ; PS – polystyrene ; GO – graphene oxide ; PPC – polypropylene chloride ; APTES - (3-aminopropyl)triethoxylsilane ; EDC/NHS -(1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/*N*-hydroxysuccinimide) ; BSA – Bovine Serum Albumin ; PP – polypropylene ; PEI – polyethyleneimine ; DAB - 1,4-diaminobutane ; PCADE - polycarvone acrylate di-epoxide ; Rac-MBA – Racemic methylbenzylamine ; Rac-BMBA – Racemic bromo-α-methylbenzylamine ; BAP – bromoacetophenone ; UF – ultrafiltration ; NF – nanofiltration ; GOS – galacto-oligosaccharides ; EPC – enzymes-polyelectrolytes complex

Among the (recirculating) flow operations gathered in Table 2, the dynamic kinetic resolution of ibuprofen ester is a prominent example of how the use of a lipase-membrane-reactor can intensify a chemo-enzymatic process and push such technology to the next level (Figure 4) [127,128]. In this example, a lipase was entrapped in the porosity of a polyacrylonitrile (PAN) membrane contactor. Such EMR allowed to simultaneously perform the continuous ibuprofen ester hydrolysis and the resulting S-ibuprofen product separation (i.e. membrane-assisted extraction towards an aqueous phase). The unreacted R-ibuprofen ester was then recirculated into a chemocatalytic racemization unit (i.e. Amberlist OH^- coated-resin) [127,128]. The resin is an excellent racemization catalyst due to its strong basicity and to its macro-reticular network, which provides high surface-to-volume ratio. Its large pores allow bulky molecules, such as (R)-ibuprofen ester, to diffuse effectively. This feature, combined with its strong basic properties, enables the rapid racemization of the ester through ketol-enol tautomerism mechanism [158]. The racemized ibuprofen ester substrate is then recirculated to the organic tank, and fed again to the enzyme-membrane module. Compartmentalization of the heterogeneous bio- and -chemocatalysts allows protecting the lipase from the basic catalyst and from inhibition that would otherwise happen under the effect of unreacted substrate and by-product (2-ethoxyethanol, which is absorbed by the OH^- resin).

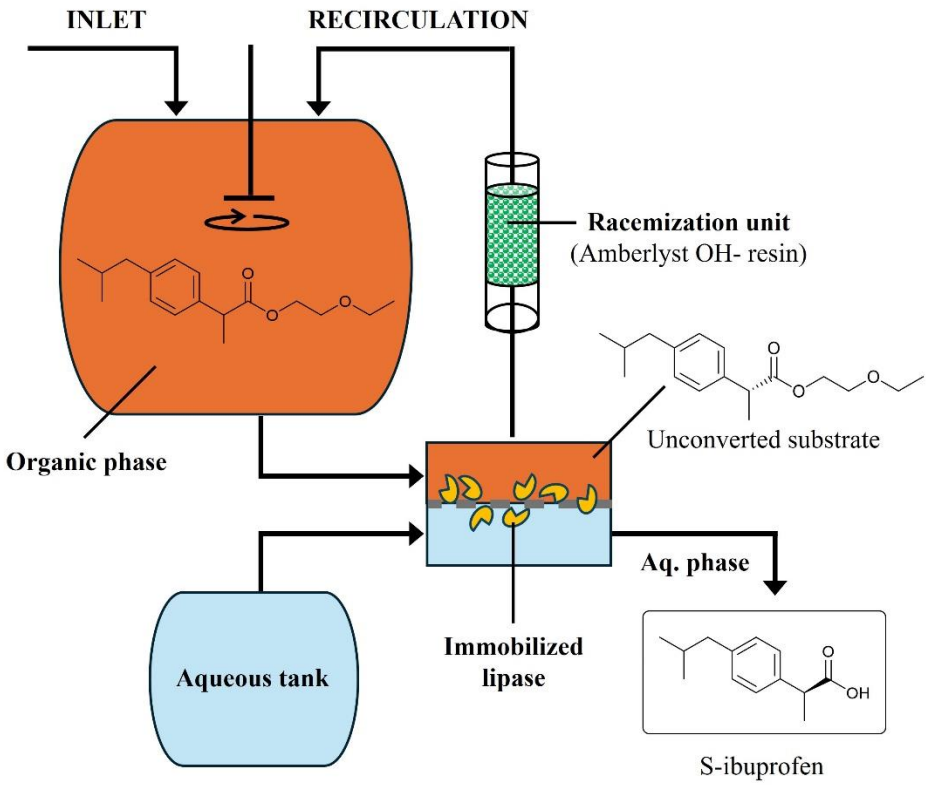

Figure 4. Schematic representation of the dynamic kinetic resolution of ibuprofen ester catalyzed by lipase-membrane-reactor (Uzir et al., 2011) [127,128].

Interestingly, the same kind of approach (membrane-assisted extraction) allowed achieving high product purity in the synthesis of oleuropein aglycone (an important antioxidant) through enzymatic

hydrolysis in an enzyme-membrane-reactor. To this aim, β-glucosidase was covalently immobilized onto ceramic membrane surfaces, and employed to hydrolyze oleuropein into oleuropein aglycone and glucose (in aqueous medium, see Figure 5) [45]. Given the differences in polarities between oleuropein aglycone and the other compounds involved in the process, a membrane-assisted solvent extraction was chosen to intensify the process. The aglycone produced in the membrane contactor was continuously extracted with an organic solvent (ethyl acetate), which allowed its separation and purification from the reaction medium. An identical strategy was implemented with polymeric (Psf) membranes containing entrapped β-glucosidase in its pores, and using limonene as organic solvent [141–143]. Yet, the use of such polymeric materials as aqueous-organic contactor for such solvent extraction processes might be less suitable than ceramic membranes, given their limited chemical stability.

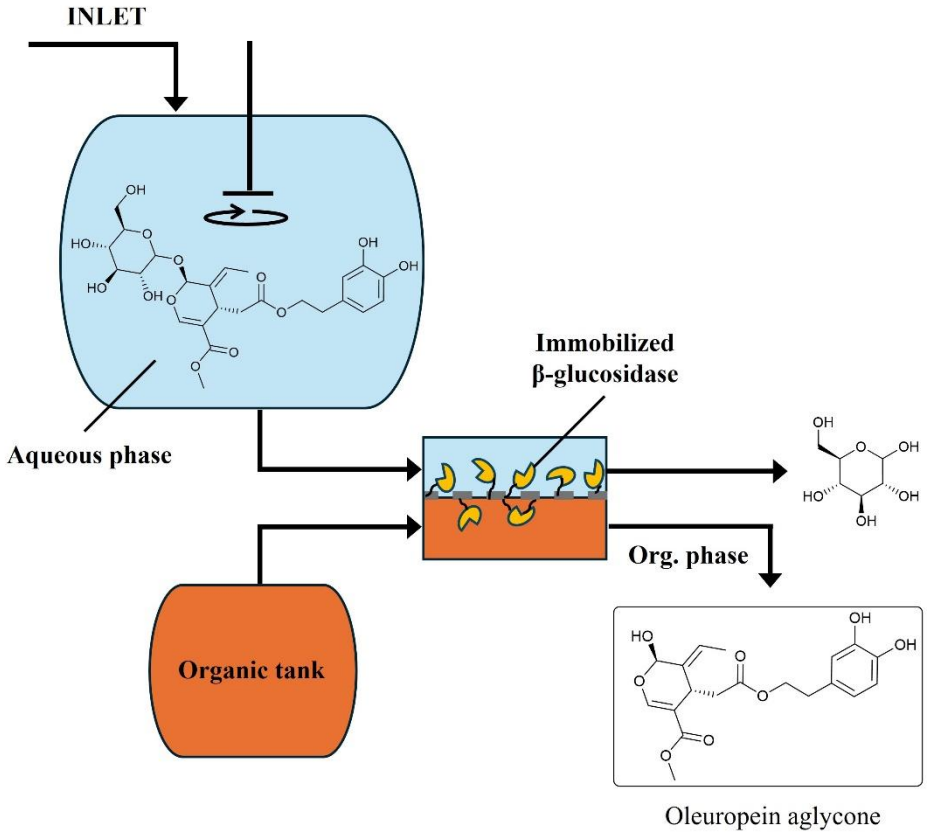

Figure 5. Schematic representation of the oleuropein aglycone production through simultaneous hydrolysis and solvent extraction using an enzyme-membrane-reactor (Giorno et al., 2018) [45].

Another elegant example of membrane-immobilized enzyme reactors applications is the *in situ* removal of water through pervaporation, as in the intensified production of lauryl stearate (which is often used as emollient and excipients in cosmetics and pharmaceuticals [159]) [68]. In this work, a "sandwich-like" membrane structure, made of a porous lipase-PVA catalytic layer coated on a polyvinyl alcohol (PVA)/polyethersulfone (PES) matrix, was employed as enzymatic-pervaporation reactor.

Interestingly, the immobilized lipase exhibited enhanced specific activity and stability compared to the soluble enzyme. This improvement was attributed to the hydrophilic microenvironment created by the hydrophilic PVA carrier, which probably absorbs the water produced during the enzymatic esterification, thereby shielding the lipase from adverse effects like enzyme inactivation. The implementation of this catalytically active membrane in a pervaporation reactor resulted in a substantial increase in conversion (i.e. from 60% to 83%) compared to the equilibrium-limited esterification process (conducted without pervaporation). A similar strategy was applied for the synthesis of ethyl lactate (a pharmaceutical-grade excipient [160]), in which a lipase entrapped in sodium alginate membrane was as enzymatic-pervaporation unit [130].

Immobilizing enzymes such as β-galactosidase, peptidase and dextranase on membranes featuring adequate MWCO, is also a practical tool to intensify the production of value-added compounds with tailored molecular weight via size-exclusion operations. For example, the production of galacto-oligosaccharides (GOS) from lactose is hindered by hydrolytic side reactions, and by enzyme inhibition caused by such monosaccharides formation and accumulation. To improve reaction yield and productivity, the continuous coupled GOS purification and monosaccharides elimination from the reaction mixture is of particular interest. To this aim, ultrafiltration membranes are employed to simultaneously host the enzymes and perform the product separation [133,134,139]. However, considering the molecular weight distribution of the carbohydrate mixture obtained after enzymatic reaction with lactose, nanofiltration also appears as an effective operation for GOS purification. Bhattacharjee et al. [135] immobilized β-galactosidase on nanofiltration (0.4 kDA) and ultrafiltration (5 to 50 kDA) membranes via covalent grafting, and compared their catalytic performance towards the continuous membrane-assisted GOS production. A higher GOS yield was obtained when using the enzyme-loaded nanofiltration membrane, which aligns with the enhanced permeation of monosaccharides and improved retention of GOS observed with the nanofiltration membrane (compared to enzyme-immobilized ultrafiltration membrane). Furthermore, the nanofiltration process resulted in substantial retention of the lactose substrate, providing extended residence time and greater interaction between the substrate and immobilized enzyme, which contributed to the increased GOS production. Similarly, Pinelo et al. [145] leveraged on the use of membrane-immobilized dextranase in order to selectively permeate the produced oligodextran featuring the desired molecular weight (5–8 kDa), and avoid over-degradation of products. In the latter study, the membrane unit consisted in a 3-layers system, composed of PS electrospun nanofibers placed between a pristine commercial membrane (PES, 30 kDA or cellulose, 10 kDA) and a macroporous support layer. To make them amenable to enzyme grafting, the PS nanofibers were functionalized with tannic acid (TA) and APTES prior enzyme immobilization. This approach allowed to significantly improve the catalytic performance (i.e. constant productivity over time and controlled saccharides molecular weight in permeate) with respect to the EMR employing free enzymes (which showed rapid deactivation over time).

The use of an enzyme-membrane-reactor in the enzymatic production of L-DOPA (which is commonly employed as a drug used for treatment of Parkinson's disease [161]) from tyrosine offers numerous advantages. By continuously supplying L-tyrosine to the biocatalytic system at a controlled rate, the EMR helps preventing enzyme inhibition and maintaining a constant substrate concentration. Additionally, it enables product separation, which is of particular interest as some by-products formed through L-DOPA spontaneous overoxidation (e.g. dopamine or dopaquinone) tend to polymerize and complicate purification. The EMR configuration facilitates the separation of these by-products via membrane filtration, ensuring continuous purification of L-DOPA on the permeate side. However, L-DOPA spontaneous oxidation remains problematic as it hampers the yields and productivity of such enzymatic process. To address this, Donato et al. [151] attempted to continuously introduce ascorbic acid [162], a reducing agent, while simultaneously removing the biocatalytically produced L-DOPA. To this aim, the authors immobilized tyrosinase on a polyamide tubular membrane sponge layer, and implemented the EMR in cross-flow configuration. It resulted that the continuous removal of L-DOPA from the reaction environment, along with the antioxidant effect of ascorbic acid, further enhanced L-DOPA productivity, reaching 1.60 $U.mg^{-1}$, i.e. higher than that of other processes reported in literature (where product separation and ascorbic acid addition were not applied).

4 Conclusion

Among the existing methods capable of intensifying biocatalytic processes in an efficient way, the coupling of enzymes with membrane technology in enzyme-membrane-reactors (EMR) is emerging as highly potent. Such integrated systems, simultaneously combining biocatalytic reactions and membrane operations, allow for more productive flow processes displaying enhanced product yield (by increasing the conversion and/or the selectivity of the process, via e.g. membrane-assisted product separation) and boosted enzyme kinetics (by preventing enzyme inhibition via controlled reagent introduction). Through this account, we aim at providing the recent trends and examples of the use of EMR for the intensified production of high-value chemicals, and in particular active pharmaceutical ingredients. Among the reviewed EMR configurations, we notably turn our attention towards membrane-immobilized enzymatic reactors. We argue that the implementation of such novel hybrid reactors which simultaneously host the immobilized enzymes and perform in-situ product separation, will catalyze further advances in the field of greener fine chemicals and pharmaceuticals manufacturing. Arguably, the coupling between the fields of biocatalysis and organic synthesis on the one hand, and process engineering on the other hand, resulting in enzyme-membrane-reactors (EMR), is crucial for both academic and industrial developments.

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