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Progress of enzyme immobilization and its potential application

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ABSTRACT

This paper briefly introduces the enzyme obvious advantages compared to the conventional chemical catalysts, and focuses on the benefits of enzyme immobilized in/onto supports, particularly, membranes, various methods of enzymes immobilized on membranes, and industrial applications. Some important research trends related to membrane bioreactors with enzyme immobilization are also listed, in which the study of enzyme stability, product separation, integrated system and waste aqueous treatment, etc., are believed to be the important areas in the future to introduce this promising technology for applied in industries.

Keywords: Enzyme; Enzyme immobilization; Membrane; Bioreactor; Integration; Separation

1. Introduction

Although the conventional methodologies of chemical processes have been successfully applied in enormous sophisticated products of synthesis, separation/purification, and analysis, with the increasing lack of sources and environmental concerns, researchers have been studying alternative methodologies which are not only efficient and safe but also environmentally benign and save resources and energy. One of the most promising strategies to achieve challengeable goals is the utilization of enzymes [1–4]. Enzymes mainly belong to proteins and peptides, which play an important role on numerous transformation reactions with high regioselectivity and stereospecificity, however, at mild conditions of temperature, pressure and pH with reaction rates of the order of those achieved by chemical catalysts at more extreme conditions. Those peculiar characteristics of enzymes provide the impetus for scientists to apply them in modern chemistry and organic synthesis processes, particularly in the development of biotechnology. Compared to conventional chemical (inorganic or organic) catalysts, enzymes exhibit high catalytic efficiency and specificity, which can make them discriminate not only between reactions and substrates but also similar parts of molecules (regiospecificity) and optical isomers (stereospecificity). Then, it will greatly reduce the possibility of a side-reaction occurrence and thus eliminate the undesirable by-products from reaction systems, which not only saves the resource but also reduces the cost of downstream separation/purification. In addition, as enzymes do not produce disposal problems as they are biodegradable and easily removed from contacting streams, they have originally attracted much interest to be used in foodstuffs, pharmaceuticals, and agrochemicals, and afterwards in organic chemical synthesises. Table 1 summarizes the most important various enzymes and their potential applications [5].

Recently, enzymes have also found potential applications in biosensors that require rapid and selective signal generation [6,7]. However, it has been noticed that there are a number of practical problems in the use of enzymes, such as higher cost of isolation and purification of, instability of their structures once separated from their microenvironment, sensitivity to reaction conditions,

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Table 1
Summary of typical enzymes and their applications in industries

Enzyme	EC number	Source	Intra/ extracellular	Scale of production, ton.y^{-1}	Industrial use
Animal enzymes					
Catalase	1.11.1.6	Liver	Intra	1	Food
Chymotrypsin	3.4.21.1	Pancreas	Extra	1	Leather
Triacylglycerol lipase	3.1.1.3	Pancreas	Extra	1	Food
Chymosin	3.4.23.4	Abomasum	Extra	1	Cheese
Trypsin	3.4.21.4	Pancreas	Extra	1	Leather
Plant enzymes					
Actinidin	3.4.22.14	Kiwi fruit	Extra	1	Food
α-Amylase	3.2.1.1	Malted barley	Extra	100	Brewing
β-Amylase	3.2.1.2	Malted barley	Extra	100	Brewing
Bromelain	3.4.22.4	Pineapple latex	Extra	1	Brewing
Endo-1,3 (4)-β-glucanase	3.2.1.6	Malted barley	Extra	10	Brewing
Ficin	3.4.22.3	Fig latex	Extra	1	Food
Lipoxygenase	1.13.11.12	Soybeans	Intra	1	Food
Papain	3.4.22.2	Pawpaw latex	Extra	10	Meat
Bacterial enzymes					
α-Amylase	3.2.1.1	*Bacillus*	Extra	100	Starch
β-Amylase	3.2.1.2	*Bacillus*	Extra	1	Starch
Asparaginase	3.5.1.1	*Escherichia coli*	Intra	1	Health
Xylose isomerase	5.3.1.5	*Bacillus*	Intra	10	Fructose syrup
Penicillin amidase	3.5.1.11	*Bacillus*	Intra	1	Pharmaceutical
Protease	3.4.21.14	*Bacillus*	Extra	100	Detergent
Pullulanase	3.2.1.41	*Klebsiella*	Extra	1	Starch
Fungal enzymes					
α-Amylase	3.2.1.1	*Aspergillus*	Extra	10	Baking
Aminoacylase	3.5.1.14	*Aspergillus*	Intra	1	Pharmaceutical
glucan 1,4-α-glucosidase	3.2.1.3	*Aspergillus*	Extra	100	Starch
Catalase	1.11.1.6	*Aspergillus*	Intra	1	Food
Cellulase	3.2.1.4	*Trichoderma*	Extra	1	Waste
Dextranase	3.2.1.11	*Penicillium*	Extra	1	Food
Glucose oxidase	1.1.3.4	*Aspergillus*	Intra	1	Food
β-galactosidase	3.2.1.23	*Aspergillus*	Extra	1	Dairy
Triacylglycerol lipase	3.1.1.3	*Rhizopus*	Extra	1	Food
Rennet	3.4.23.6	*Mucor miehei*	Extra	10	Cheese
Polygalacturonase	3.2.1.15	*Aspergillus*	Extra	10	Drinks
Pectin lyase	4.2.2.10	*Aspergillus*	Extra	1	Drinks
Protease	3.4.23.6	*Aspergillus*	Extra	1	Baking
α-galactosidase	3.2.1.22	*Mortierella*	Intra	1	Food
Yeast enzymes					
β-fructofuranosidase	3.2.1.26	*Saccharomyces*	IntraI/Extra	1	Confectionery
β-galactosidase	3.2.1.23	*Kluyveromyces*	IntraI/Extra	1	Dairy
Triacylglycerol lipase	3.1.1.3	*Candida*	Extra	1	Food
α-galactosidase	3.2.1.22	*Saccharomyces*	Intra	1	Food

inhibition by trace inhibitors, etc. The first inevitably increases the process cost and the others shorten their operational lifetime. In addition, as enzymes prefer to operate dissolved in an aqueous medium, it not only makes the operation difficult but also contaminates products and is impossibe to use for recycling. A promising method to overcome these defects is that enzymes might be immobilized in/onto supports [8]. Immobilization basically might be hinted by enzymes natural existing status in which they are usually attached on living cellular membranes where they perform according to each special case. Theoretically, if enzymes are successfully attached to solid supports with suitable methods, the immobilized enzymes should be more resistant to environmental

changes and also the reaction systems allow recycling use of enzymes and avoiding products contaminated by enzymes. Compared to other enzyme supports, it seems that the membrane would be the promising support. Recently, immobilized enzyme membrane bioreactors (E-MBR) are finding applications for many important enzymatic reactions. In E-MBR, enzymes are often immobilized onto the membrane surface or in its porous structure, in which the membrane may simply act as a barrier to retain the enzyme, while allowing the removal of products and/or the addition of reactants. A key advantage of immobilization is that it permits easier enzyme recovery. Furthermore, it increases its thermal stability and its resistance towards organic solvents.

2. Enzyme immobilization

2.1. Methods of immobilization

Enzymes may be immobilized on carriers by a variety of methods, which can be broadly classified as physical, where weak interactions between support and enzyme exist, and chemical, wherein covalent bonds are formed with the enzyme [7,8]. The physical methods include containment of an enzyme within a membrane reactor; adsorption (physical, ionic) on a water-insoluble matrix; inclusion (or gel entrapment); microencapsulation with a solid membrane; micro-encapsulation with a liquid membrane; and formation of enzymatic Langmuir–Blodgett films. The chemical immobilization methods generally contain covalent attachment to a water-insoluble matrix; cross-linking with use of a multifunctional, low molecular weight reagent; and co-cross-linking with other neutral substances, e.g. proteins. Numerous other methods which are combinations of the ones stated or original and specific of a given support or enzyme have been devised. Immobilization can also be obtained by gelification. When an enzyme solution is flushed through a membrane, e.g., an ultrafiltration membrane that rejects the enzyme molecules, the enzyme will accumulate on the membrane surface and deposit as a thin gel layer characterized by enzymatic catalytic activity. The actual gelation of enzyme protein and their dynamic immobilization on the membrane surface occurs when the protein concentration at the membrane–liquid interface reaches the gel-concentration value. When the biocatalyst is immobilized on the surface, flushing the substrate solution along the enzymatic gel also causes the conversion of substrate into product in the retentate stream. If the enzyme is inhibited by the product, thus, the reactor performance as steady state is decreased. Giorno et al. [9,10] indicated that biocatalyst, enzyme, could be flushed along a membrane module, segregated within a membrane module, or immobilized in or on the membrane by entrapment, gelification, physical adsorption, ionic binding, covalent binding or cross-linking.

During the immobilization process, various carriers including organic and inorganic, natural and synthetic, are of interest in the required characteristics [11]. Compared to other carriers, membranes are preferred to be used. However, many factors, e.g., membrane structure, immobilization methods, enzymes, etc., will affect their performance. Ulbricht et al. [12] analyzed this through immobilization enzymes using different methods on membranes in which three methods, e.g., diffusion, ultrafiltration, and cross-linking with glutaraldehyde, were used to immobilize enzymes, amyloglucosidase (AG) and invertase (INV), on membranes [polyacrylonitrile (PAN) and carboxyl-modified polyacrylonitrile (PAN-AA)]. From the first activity measurement after immobilization and rinsing with the "standard" substrate, starch, it seems that there is not much difference among the three methods. However, the membrane prepared by adsorption was not stable. In contrast, both AG cross-linking inside PAN membranes and covalent AG binding onto PAN-AA surfaces (for moderate initial loadings) produced enzyme membranes with sufficient long-term stability under storage and test conditions. Consequently, as a rule, the optimal immobilization conditions for a chosen enzyme and its application are found empirically by a process of trial and error in a way to ensure the highest possible retention of activity of the enzyme, its operational stability and durability.

2.2. Performance of immobilization enzymes

During the enzyme immobilization, in order to make the enzyme with high activity and good stability, it should be understood that there is no single method or carrier that is best for all enzymes and their applications. This is because of the widely different chemical characteristics and composition of enzymes, the different properties of substrates and products, and the different uses to which the product can be applied. Generally speaking, many factors can affect the performance of the immobilized enzymes. The most important factors that might strongly affect the immobilized enzyme performance are shown in Fig. 1.

The immobilization involves a number of factors worsening the performance of enzymes [13–15]. Compared with the free enzyme, the immobilized enzyme has its activity lowered and the Michaelis constant increased. These alterations might result from structural changes introduced to the enzyme by the applied immobilization procedure and from the creation of a microenvironment in which the enzyme works, different from the bulk solution. The latter is strongly dependent on the reaction taking place, the nature of the support and on the design of the reactor. Furthermore, being two-phase systems, the immobilized enzyme systems suffer from inevitable mass

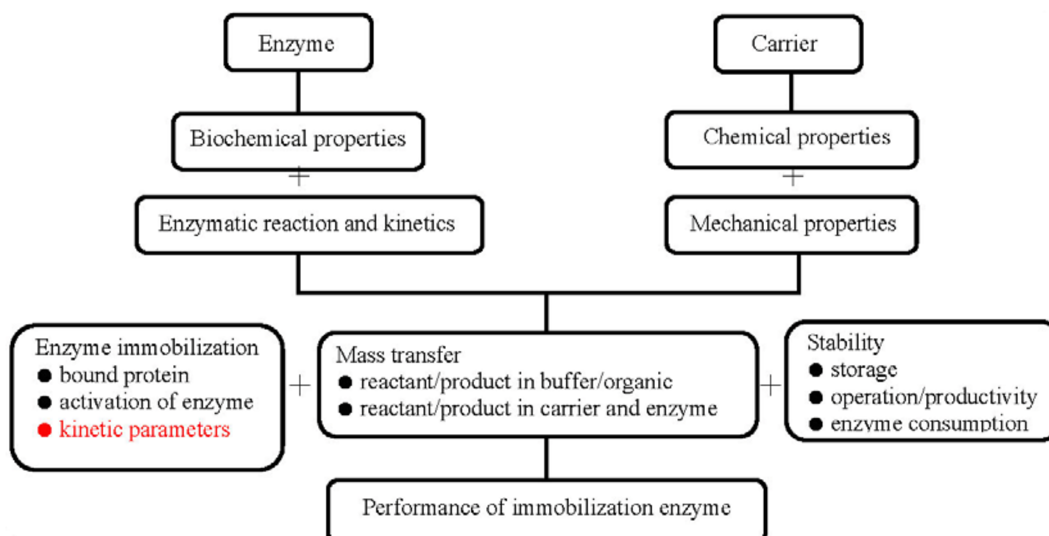

Fig. 1. Diagram showing effects of various important parameters on the performance of immobilization enzymes.

transfer limitations, producing unfavorable effects on their overall catalytic performances. Commonly, the immobilization methods must be selected to fully use the advantages of the carrier structure, particular the membrane, including its separation properties and achieve maximum and stable enzyme activity. Nevertheless, the properties of immobilized enzymes are governed by the properties of both the enzyme and the support material, as shown in Fig. 1. The interaction between the two makes an immobilized enzyme specific physicochemical and kinetic properties that may be decisive for its application, and thus, a judiciously chosen support can significantly enhance the operational performance of the immobilized system.

Although it is recognized that there is no universal support for all enzymes and their applications, a number of desirable characteristics should be common to any material considered for immobilizing enzymes. These include high affinity to proteins, availability of reactive functional groups for direct reactions with enzymes and for chemical modifications, mechanical stability and rigidity, regenerability, and ease of preparation in different geometrical configurations that provide the system with permeability and surface area suitable for a chosen biotransformation. Understandably, for food, pharmaceutical, medical and agricultural applications, nontoxicity and biocompatibility of the materials are also required. Furthermore, to respond to growing public health and environmental awareness, the materials should be biodegradable and economical.

Practically, for a selected enzyme and carrier, particularly membranes, most researchers prefer to use an adsorption method to immobilize enzymes on membranes. The ease of immobilization, absence of expensive and toxic chemicals, ability to retain the specific activity and selectivity of the lipase virtually unchanged with respect to its soluble form, and feasibility of regeneration (based on the partial reversibility of the immobilization technique) may partially account for this.

3. Enzymatic catalytic reaction systems

3.1. Operational modes

According to diversities of reactants and products in the practical application, enzymatic catalytic systems can be divided into quite different operation modes in order to fully use the enzymes, easily operation and economic view of the whole process, etc. The enzymatic catalytic reaction systems could be generally divided into the following modes, as shown in Fig. 2. A stirred tank batch reactor (STR) is where the enzyme and substrates are contained in the same container and the operation is stopped until the conversion is complete. A batch membrane reactor (MR) is where the enzyme is held within membrane tubes, which allow the substrate to diffuse in and the product to diffuse out. This reactor may often be used in a semi-continuous manner wherein the same enzyme solution is used for several batches. A packed bed reactor (PBR) or plug-flow reactor (PFR) contains the particles with enzyme immobilization in/on them. A continuous flow stirred tank reactor (CSTR) is where the enzyme and substrates are under the same conditions as the STR, except the operation is a continuous mode. A continuous flow membrane reactor (CMR) is a continuously operated version of MR. A fluidized bed reactor (FBR) is where the flow of substrate stream keeps the immobilized enzyme particles in a fluidized state.

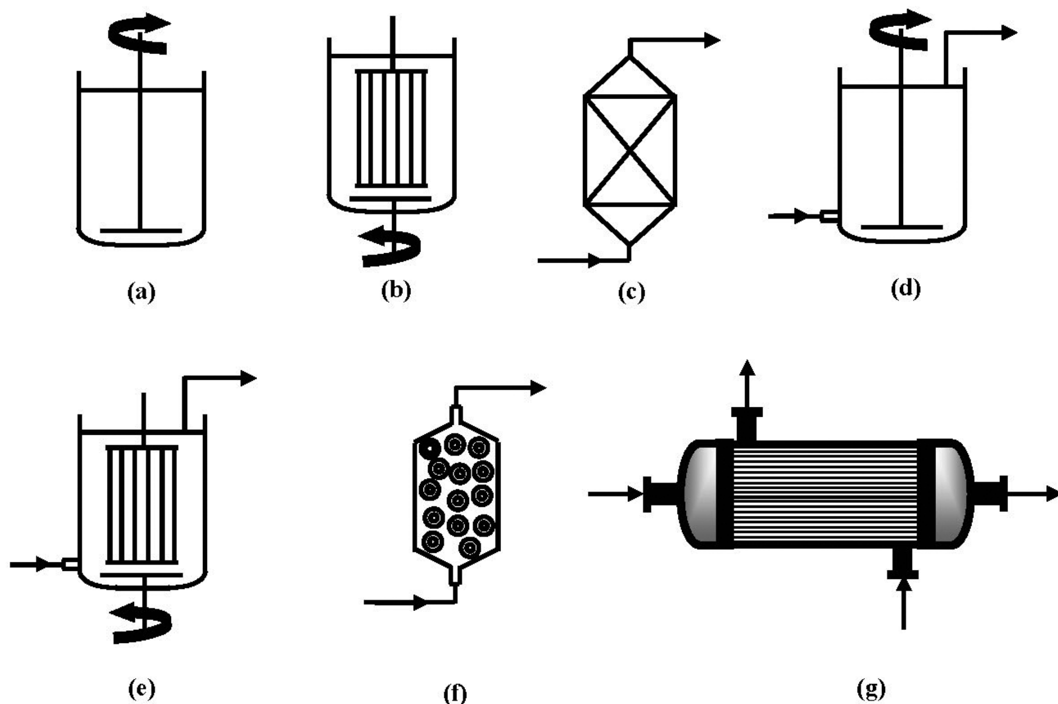

Fig. 2. Schematics of typical reactor types catalyzed by enzymes. a, stirred tank reactor; b, batch membrane reactor; c, packed-bed/plug-flow reactor; d, continuous flow stirred tank reactor; e, continuous flow membrane reactor; f, fluidized bed reactor; g, biphasic membrane reactor.

The last—and also a very important one—is the biphasic membrane reactor (BMR), in which the separated two phases, aqueous and solvent, are contacted in the pores of membranes where the enzymes are loaded. The system is by virtue of the hydrophilicity of the membrane and the slight organic-to-aqueous pressure difference imposed across the membrane, the aqueous/organic interface is fixed at that side of the membrane that is in contact with the organic process stream. In operation, the reactant is partitioned into the membrane and is converted to a water-soluble product, which subsequently diffuses out into the aqueous process stream, which simultaneously combines the reaction with separation because of the different solubility of product and sub-strate in the two phases. The hollow fiber membrane thus serves to "immobilize" enzymes while placing the enzyme-containing phase in direct contact with the substrate-containing (organic) phase, thereby avoiding the intervention of the bulk aqueous phase as occurs in dispersed-phase systems.

3.2. Advantages of biphasic membrane reactors

In the BMR, the membrane may simply act as a barrier to retain the enzyme, while allowing the removal of products and/or the addition of reactants. The potential advantages of BMR technology over conventional approaches, e.g., STR and CSTR, etc., include its higher efficiency and reduced costs owing to the integration of bioconversion and product purification, thus reducing equipment costs and the number of processing steps. For enzymatic conversions, in particular, hollow-fiber BMR find common use, with enzymes typically immobilized in the porous structure of the hollow fibers. One advantage of BMR over the conventional bioreactors is the longer contact times of reactants with the enzymes due to their high *s/v* ratios. Low residence times are a problem with conventional enzymatic bioreactors, and good efficiency is obtained only with rapid reactions. Additionally, in BMR, the two separate phases not only provides the bioreaction occurring on the membrane surface but also achieve liquid/liquid mass transfer without dispersion of one phase within another. By careful control of the pressure difference between the fluids, one of the fluids is immobilized in the pores of the membrane so that the fluid/fluid interface is located at the mouth of each pore. This approach offers a number of important advantages over conventional dispersed phase contactors, including absence of emulsions, no flooding at high flow rates, no unloading at low flow rates, no density difference between fluids required, and high interfacial area. It will effectively reduce the mass transfer effect on the reaction systems [9,16–22], leading to a remarkably low height of transfer unit (HTU) values [10,23].

4. Applications of enzyme immobilization on membranes

4.1. Application in hydrolysis and synthesis

Recently, enzyme immobilization systems, in particular BMR, have found potential applications in the enzymatic hydrolysis of macromolecules [15,16,24,25]. Protein hydrolysis is the largest area of BMR application. One of the first and more important applications is whey protein hydrolysis by pancreatine. Casein hydrolysis by alcalase in BMR has been studied by Mannheim et al. [26]. The membrane allowed them to independently control the enzyme and substrate concentrations, residence time and permeate flow; this, in turn, resulted in BMR productivity which was twelve times than that of a batch reactor [27]. Pouliot et al. [28] studied casein hydrolysis using trypsin or chymotrypsin in a hollow fiber BMR, which recently was also detailed reported by Curcio et al. [29] for the synthesis of para-k-casein and glycol-macro-peptides by chymosin. Enzyme transformation of hemoglobin, an important animal slaughterhouse by-product, has been studied in a BMR by Cempel et al. [30] and Belhocine et al. [16] where the papain enzyme was used. Some other industrial applications in related areas are listed in Table 2.

Recent studies have also reported the application of BMR in the hydrolysis of proteins derived from vegetables [17,31,32], like soy or alfalfa, e.g., a pilot-scale for alfalfa protein hydrolysis using endopeptidase delvolase. Giorno et al. [33,34] compared the efficiency of lipase to hydrolyse vegetable oil triglycerides into fatty acids and glycerol using different reactor configurations including a traditional emulsion stirred tank reactor (STR); an emulsified organic-aqueous enzyme membrane reactor (E-BMR, where the reaction occurred in emulsion and the aqueous phase was ultrafiltered through a membrane, thus separating the product); and a biphasic organic–aqueous enzyme membrane reactor (E-BMR, where the two phases were separated by the membrane that also contained the immobilized enzyme). The results showed that the apparent volumetric reaction rate of the free enzyme was higher compared with the immobilized enzyme, but the catalytic activity of the immobilized enzyme was considerably more stable. Fruit juice clarification by pectinases and cellulases is another interesting application. In the conventional process after the enzymatic reaction the pulp treatment step takes place, filtration over diatomaceous earth follows. This filtration-type process produces a lot of solid waste, and results in costly enzyme loss. E-BMR are appropriate for such application either for enzyme recovery and recycle or in the form of a more compact type system, with the biocatalyst immobilized on the membrane itself. Balcão et al. [13] recently outlined most of main features of reactors employing immobilized lipases. The other new typical systems using immobilization enzymes are listed in Table 3, which indicates that in most cases the enzyme showed good activity, particularly with high stability by using the cross-linking methods to immobilize the enzymes on the membranes.

Table 2
Typical industrial applications of enzyme immobilization systems

Enzyme (EC number)	Substrate	Product
Glucose isomerase (5.3.1.5)	Glucose	Fructose (high-fructose corn syrup)
β-Galactosidase (3.2.1.23)	Lactose	Glucose and galactose (lactose-free milk and whey)
Lipase (3.1.1.3)	Triglycerides	Cocoa butter substitutes
Nitrile hydratase (4.2.1.84)	Acrylonitrile	Acrylamide
	3-Cyanopyridine	Nicotinamide
	Adiponitrile	5-Cyanovaleramide
Aminoacylase (3.5.1.14)	D, L-Aminoacids	L-Amino acids (methionine, alanine, phenylalanine, tryptophan, valine)
Raffinase (3.2.1.22)	Raffinose	Galactose and sucrose (raffinose-free solutions)
Invertase (3.2.1.26)	Sucrose	Glucose/fructose mixture (invert sugar)
Aspartate ammonialyase (4.3.1.1)	Ammonia + fumaric acid	L-Aspartic acid (used for production of synthetic sweetener aspartame)
Thermolysin (3.4.24.27)	Peptides	Aspartame
Glucoamylase (3.2.1.3)	Starch	D-Glucose
Papain (3.4.22.2)	Proteins	Removal of “chill haze” in beers
Hydantoinase (3.5.2.2)	D, L-Amino acid hydantoins	D, L-Amino acids
Penicillin amidase (3.5.1.11)	Penicillins G and V	6-Aminopenicillanic acid (precursor of semi-synthetic penicillins, e.g. ampicillin)
β-Tyrosinase (4.1.99.2)	Pyrocatechol	L-DOPA

Table 3
Information of new typical developed enzyme immobilization reaction systems

Enzymes	Supports	Immobilization methods	Substrates	Reactor configurations	Performance		Ref.
					Activity	Stability	
Papain	Polyethersulfone	Covalent	Benzoyl arginine *p*-nitroanilide hydrochloride	STR	13.2±1.42 (μmol.g^{-1}.min^{-1})	—	[35]
		Non-covalent through avidin-biotin complex			32.3±0.54 (μmol.g^{-1}.min^{-1})	Stable	
Lipase from *Rhizopus javanicus*	Polypropylene	Cross-linking	Triglycerides and peroxidation of fatty acids	Biphasic enzyme membrane reactor	20 (μmol.m^{-2}.s^{-1})	—	[19]
Lipase from *C. rugosa*	Nylon 6	Adsorption	Diol lactone+(S)-(+)-2-methylbutyric acid	Laboratory rotator	28×10-5 (mol.h^{-1}.g^{-1})	—	[36]
Lipase from *C. rugosa*	Nylon 6 pellet support	Adsorption	Diol lactone	STR	0.024±0.006 (initial rate) (mol h^{-1}g-^{1})	—	
Amylo-glucosidase	Polyacrylinitrile (PAN) membrane	Adsorption	Starch	Ultrafiltration	490±75 (mU cm^{-2})	Unstable	[12]
		Cross-linking			480±40 (mU cm^{-2})	Stable	
	Carboxyl-modified polyacrylinitrile (PAN-AA) membrane	Covalent	Starch		980 (mU cm^{-2})	Stable	
			Maltose		300 (mU cm^{-2})		
Lipase from *C. rugosa*	Capillary polyamide membrane	Cross-linking	Vegetable oil triglycerides	Biphasic enzyme membrane reactor	4.5 (pH=5) 0.17 (pH=8) (mmol l^{-1} h^{-1})	Stable	[34]
					4.5 (50 kDa) 3.0 (10 kDa) (mmol l^{-1} h^{-1})	Stable	
Lipase from *Pseudomonas cepacea*	Zirconia ultrafiltration membrane		5,7-diacetoxyflavone	Monophasic enzyme membrane reactor	8.0 (μmol h^{-1}g^{-1})	Stable	
Lipase from *C. rugosa*	Capillary polyamide membrane	Cross-linking	(R, S)-naproxen methyl ester	Biphasic enzyme membrane reactor	8.4 (R-naproxen ester) 146(S-naproxen ester) (mmol h^{-1}g^{-1})	Stable	[18]
			Triolein		1.7±0.2 (hollow) (μmol g^{-1}s^{-1})		
			Peroxidation caprylic acid		2.4±0.4 (hollow) 22±7 (flat) (μmol g^{-1}s^{-1})		
			Epoxidation oleic acid		14±3 (flat) (μmol g^{-1}s^{-1})		
Fumarase from porcine heart	Polysulfone capillary membrane	Cross-linking	Fumaric acid	Biphasic enzyme membrane reactor	1.42 (mmol.h^{-1}.g^{-1})	Stable	[37]
Lipase from *Pseudomonas cepacia*	PAN-Daicen	Cross-linking	Racemic 2-hydroxy octanoic acid methyl ester	Biphasic enzyme membrane reactor	90 (mmol.h^{-1}.m^{-2})	—	[20]
	PES-Daicen				168 (mmol.h^{-1}.m^{-2})		
	PAN-Asahi				75 (mmol.h^{-1}.m^{-2})		
Lipase from *C. rugosa*	Polyamide capillary membrane	Cross-linking	Racemic naproxen methyl ester	Biphasic enzyme membrane reactor	14.5 (μmol.h^{-1}.g^{-1})	Stable	[21]
Pig liver esterase	UF hollow fibres	Cross-linking	*meso*-diester cis-cyclohex-4-ene-1,2-dicarboxylate	Biphasic enzyme membrane reactor	3.0×10^{-6} (M.s^{-1})	Stable	[38]

4.2. Application in the production of chiral compounds

BMRs used for the production of optically pure enantiomers have been recently described [9,36,39]. A prime driving force is the possible therapeutic potential of such compounds [40]. In this regard, the enantiomeric purity of any new enzyme inhibitor has become a very critical issue. With increased regulatory attention being given to stereoisomeric drugs, chiral synthesis and chiral resolution, techniques are increasingly in demands to obtain enantiomerically-pure drug candidates. In general, there are two approaches for obtaining enantiomerically-pure substances, that is asymmetric synthesis of the desired isomer; and resolution of the racemic mixture into individual enantiomers. It indicated that enzyme immobilized systems would have potential application in this field [19,41–44], in which the enantioselectivity might depend on the substrate concentration, amount of enzyme loaded in the membrane and immobilization in situ [18]. In fact, these parameters affect the organic/aqueous interface that plays an important role in the enhancement of enantioselectivity.

Usually, BMR operation would be superior to emulsion systems, particularly in controlling the enzyme with stable activity, easily reuse enzyme and recovery of product from reaction stream. Giorno et al. [9] also indicated that the integration of BMR with other membrane processes, such as microfiltration, ultrafiltration, reverse osmosis, membrane extraction, etc., is particularly important for products obtained by fermentation processes such as organic acids and antibiotics, and in the processing of food and beverages. Table 4 gives examples of enzyme catalysts for producing chiral compounds. The increasing demand for enantiomerically pure drugs and fine chemicals, together with the need for environmentally more benign chemistry, will lead to a rapid expansion of biocatalysis used in chiral/racemic compound productions.

4.3. Application in biosensors

Immobilized enzymes have been recently used in biosensors by integrating with transducers [46–48]. The signal produced by the interaction of the biological system with an analyte will be transduced into a measurable response [49,50]. Enzymes, which are mostly immobilized either directly on the tip of transducer or in/on a polymer membrane [51,52], are essential elements. This ingenious device could theoretically tailor for nearly any target analyte, which can be either enzyme substrates or inhibitors, due to enzymes specificity and sensitivity. In addition, as they are cost-effective and portable analytical devices, biosensors based on immobilized enzymes will be the promising tools in medicine, environmental engineering and real time monitoring, bioprocess and food control, and in biomedical and pharmaceutical analysis [53–55].

5. Further investigation on immobilization enzymes

5.1. Large-scale application

Immobilization enzyme membrane reactors, particularly BMRs, are useful for certain applications, specifically in terms of energy consumption, safety, pollution prevention and the high quality of products produced. However, the use of BMRs on an industrial scale is not yet fully established. Only a few examples have been described, such as the production of L-aspartic acid with *Escherichia coli* cells entrapped in polyacrylamides [56] and lactase (β-galactosidase) entrapped in polyacrylamides [57]. The major technological difficulties using BMRs on an industrial level are mainly because the availability of pure enzyme at an acceptable cost (often the commercial enzymes are mixtures of several proteins); difficulties in immobilizing enzymes that often need expensive cofactors; the necessity for enzyme to operate at low substrate concentrations; and the possibility of leakage of enzyme which causes microbial contaminations to products. In this case we can safely conclude that there are no general strategies for the design and preparation of such "integrated" enzyme membrane bioreactors, and their possibilities and eventual limitations cannot be fully evaluated, wherein the large-scale applications of BMRs mainly depend on the laboratory-scale studies [58,59].

In order to fully establish the use of biocatalytic membrane reactors, further studies on the design of bioprocesses, particularly for large-scale production, the control of the reaction and kinetic mechanisms, and immobilization procedures, need to be continued. The importance of controlling the quantity and the activity of the enzyme attached to the membrane was recently discussed by Ganapathi-Desai et al. [60,61] for the amidase activity of papain. In most cases, when the activity decreases with enzyme loading increases. This might be explained by the crowding of the enzyme on the surface, in which resulted in the blocking of the active site/protein denaturation. Another explanation could be that it is due to multipoint attachment of the enzyme, which would result in a decrease of the conformational flexibility at the active site, thereby, inhibiting the ability of the enzyme to bind to the substrate. It is necessary that many factors must be taken into account in order to optimize BMR performance, including enzyme and substrate concentrations, residence time, and hydraulic dynamics of lumen and shell sides. Productivity and long-term enzyme activity depend on these parameters.

5.2. Activity and stability

Technicians are well aware of enzyme versatility and possible applications in industries; however, the use of enzymes is still not significant compared to traditional

Table 4
Examples for enzyme to be used in the production of chiral compounds

Enzyme	Substrate	Product	Application
Nitrile hydratase	3-Cyano-pyridine	Nicotinamide	Pharmaceutical intermediate
Nitrile hydratase	Acrylonitrile	Acrylamide	Intermediate for water-soluble polymers
D-amino acid oxidase and glutaric acid acylase	Cephalosporin C salt	7-Amino- cephalosporanic acid	Intermediate for semisynthetic antibiotics
Penicillin acylase	7-Amino-deacetoxy-cephalosporanic acid	Cephalexin	Antibiotics
Penicillin G acylase	Penicillin G	6-Amino-penicillani acid	Intermediate for semisynthetic antibiotics
Ammonia lyase	Fumaric acid + ammonia	L-Aspartic acid	Intermediate for aspartame
Thermolysine	L-Aspartic acid + D,L-phenylalanine	Aspartame	Artificial sweetener
Dehalogenase	(R,S)-2-Chloropropionic acid	(S)-2-Chloropropionic acid	Intermediate for herbicides
Lipase	(R,S)-Glycidyl-butyrate	(S)-Glycidyl-butyrate	Chemical intermediate
Lipase	Isosorbide diacetate	Isosorbide 2-acetate	Pharmaceutical intermediate
Lipase	(R,S)-Naproxen ethyl ester	(S)-Naproxen	Drug
Lipase	Racemic 4-methoxy-phenylmethyl glycidate	(2R,3S)- 4-methoxy-phenylmethyl glycidate	Pharmaceutical intermediate
Acylase	D,L-Valine + acetic acid	L-Valine	Pharmaceutical intermediate
Acylase	Acetyl-D,L-methionine	L-Methionine	Pharmaceutical intermediate

chemical catalytic reaction systems. This is mainly because of, firstly, most detailed studies related to enzymes are recent and the usual time tag prior to full commercial exploitation has not yet elapsed; secondly, most research is conducted on a small laboratory scale, which cannot provide enough information for commercial applications; thirdly, the cost of enzyme extraction or purification is still quite high, which might be overcome via production by genetic engineering. Finally, researchers have noticed that one major disadvantage of immobilizing enzymes on polymeric microfiltration membranes is that the activity of the immobilized enzyme is often significantly decreased because the active site may be blocked from substrate accessibility, multipoint-binding may occur, or the enzyme may be denatured [62–68]. Moreover, multipoint attachment could occur, and with random immobilization high enzyme loading is not possible [69]. Additionally, most enzymes are immobilized using the adsorption method; although this method has many advantages, as it is a reverse process, it could produce the leakage of enzymes from the membrane, thus causing a series of problems, particularly, causing enzyme activity decay during the reaction process [12,70]. Recent studies showed that site-specific immobilization using the power of molecular biology can overcome those difficulties [67–73]. There are still many factors that need to be clarified on how to improve enzyme activity and stability when it is immobilized on membranes.

5.3. Recovery of product from streams

Most of earlier enzyme catalytic reaction systems were carried out in emulsion systems, which were proved they not only caused serious deactivity of enzymes and difficult economic operation but also existed in difficulties of re-breaking emulsion systems for treating waste solutions (oil–water or water–oil emulsion), and particularly recovering of products from down streams. Bioreactors containing immobilized enzymes might have various configurations. Most of them would fall within one of the following cases, e.g., enzymes in solid forms precipitated within organic liquid phases; enzyme in soluble forms in aqueous phases and restrained by solid supports (usually micro- or ultrafiltration membranes); enzymes contained in aqueous phases and restrained by surfactant liquid membranes (called dynamic membranes) within organic liquid phases; enzymes entrapped within three-dimensional polymeric matrixes dispersed within organic phases or within aqueous phases; finally, enzymes attached to solid supports and dispersed with organic liquid phases or within aqueous phases [13]. Among them, enzymes attached to polymeric membranes are most welcome because of combining membrane selective separations with enzyme higher activities. Previous studies mainly investigated the effects of immobilization methods on enzyme activity and stability, etc. [71–77], while few publications referred to the product recovery from the reaction streams.

Battistel et al. [78] investigated the possibility of enzymatic dynamic resolution of racemic naproxen ethoxyethyl ester in an enzyme packed bioreactor and simultaneous recovery of product from the reaction stream. A schematic diagram used in this study is shown in Fig. 3, wherein the first column packed with the immobilized lipase was continuously fed with substrate and buffer and the second column packed with resin was used for the separation of the products from the reaction mixture. The buffer eluting from the second column was then recirculated onto the first column. In this way, the system was operated as continuous-flow closed-loop bioreactor. Another case was recently reported by Xin et al. [79], as shown in Fig. 4, in which the enzymatic resolution step was performed in an aqueous-organic biphasic system contained in a stirred tank reactor equipped with a hydrophilic dead-end membrane. Additionally, a silicone tube containing the solid/liquid mixture was immersed in the reactor. Chemical substrate racemization was favored in the silicone rubber. Because the hydrophobic, nonporous, dense, silicone rubber membrane was only permeable to nonpolar naproxen methyl ester, chemical catalytic racemization was separated from biocatalytic resolution processes. To overcome product inhibition and to facilitate product recovery, a hydrophilic porous semipermeable membrane was used in a stirred tank reactor. By control of transmembrane pressure, only aqueous phase products such as (S)-naproxen and methanol passed through the membrane and the nonpolar isooctane containing naproxen methyl ester was reserved. In operation, those two operation systems should be carefully controlled to make the lipase with high and stable activity. Additionally, how to economically separate/purify the product from the bioreactor needs much more attention, and more detailed laboratory-scale studies are necessary for considering practical applications.

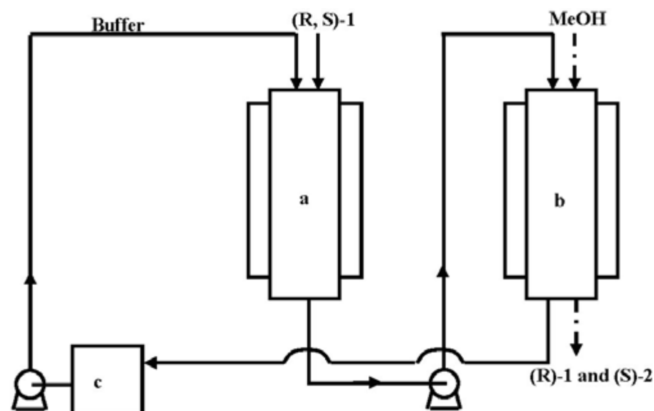

Fig. 3. Continuous enzymatic reactor. (a) enzymatic column, packed with amberlite XAD-7-immobilized lipase; (b) adsorption column, packed with amberlite XAD-4; (c) buffer reservoir.

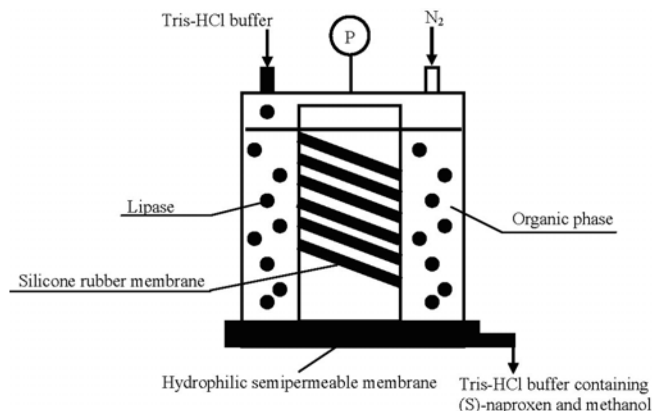

Fig. 4. Diagram of membrane bioreactor configuration and operation.

5.4. Improvement on mass transfer

Mass transfer of substrate through the enzyme-immobilized membrane is one of the concerned parameter that would determine its performance [80–82]. Additional mass transfer limitations due to the formation of enzyme layer on/into the membrane would be faced in BMR, in which it operates with two liquid phases in contact within the membrane, and then the reaction is occurring at the interface. The transport of product is produced by diffusion under a concentration gradient between the interface and the bulk aqueous phase where the product is more soluble. On the other hand, reactant (more soluble in the organic phase) is transported by diffusion from the bulk organic phase to the reaction site. Then, the mass transfer regulated by a membrane might limit the performance of the whole system [83–85]. The main side-effect for the poor mass transfer through the membrane would result in a large membrane area needed to achieve the specification required by the treatment process, leading to significant increases in the membrane capital costs, which in turn affects the economy of the whole process [86–89]. On recognizing the effect of mass transfer through the membrane on BMR performance, theoretical and experimental efforts have been made in the past to improve its performance [90–95]. Calabrò et al. [96] established a general model to analyze the phenomena of mass transport in a hollow fiber membrane reactor, which was based on the numerical solution of the dimensionless balance equations governing mass transfer within the regions that can be defined for this reacting system, namely the lumen of fiber, the layers of dense and sponge, in which the chemical reaction was supposed to take place only in the last two regions (skin and sponge) where the biocatalyst was supposed to be confined by entrapment. On screening gel layer effect on mass transfer of S- or R-

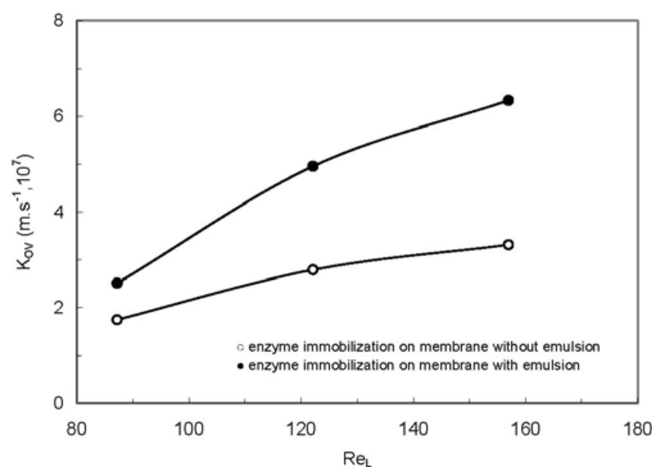

Fig. 5. Improvement on overall mass transfer coefficient (K_{ov}) for naproxen acid transport across the enzyme immobilization membrane from shell to lumen with and without addition of emulsion into lipase solution during immobilization (shell side Reynolds number (Re_S) =30.6).

glycidyl butyrate transport through membranes. Trusek-Holownia et al. [97] recently reported that the enzyme layer would have an important influence on total mass transfer coefficient. Xu et al. [98] proposed a method for determining diffusion and interfacial mass transfer rates through globular proteins immobilized on polymeric membranes based on microscopy FT-IR mapping technology. Our recent study [99], shown in Fig. 5, indicates that the overall mass transfer coefficient from shell to lumen, K_{ov}, for S-naproxen acid transport across the immobilized membrane with addition of emulsion during immobilization, has been improved compared with results obtained from experiments with immobilization of enzyme alone in the membrane. Comparing the permeate flux of enzyme-loaded membrane with the case of enzyme-emulsion-loaded membrane, an interesting result was also observed. The latter showed higher permeate flux than the former. One reason could be that the addition of emulsion to the lipase solution during immobilization created a more porous enzyme layer within the membrane matrix through which the transport could be improved.

5.5. Environmental considerations

It is noticed that an aqueous solution is usually necessary in BMR systems. Even if the product has been separated/purified from the aqueous solution (supposed the product preferred in aqueous), the remaining solution still contains some organics (e.g., products and other organic molecules) as well as traces of ions (such as Na^+) and enzymes (leakage from the membrane). In response to increasingly stringent requirements for effluent standards and the growing importance of wastewater reuse, it is impossible to discharge this wastewater into environment without further treatment. Membrane processes are often chosen since these applications achieve high removals of constituents such as dissolved solids, organics, inorganic ions, and regulated and unregulated organic compounds. In this area, reverse osmosis (RO), ultra-low pressure reverse osmosis (ULPRO), and nanofiltration (NF) are becoming increasingly widespread in water treatment and wastewater reclamation/reuse applications where a high product quality is desired [100–103]. However, as the wastewater from the BMR systems not only contains organics but also traces of inorganic ions and enzymes, the removal of these compounds in wastewater from BMR down streams is of great importance.

An understanding of the factors affecting permeation of solutes in pressure membrane systems is needed. Therefore, it is necessary to systematically study the membrane rejection mechanisms and factors affecting rejection of organic pollutants and traces of ions and enzymes under BMR reaction conditions. The following solute parameters, e.g., molecular weight, molecular size (length and width), charged and non-charged, and diffusion coefficients, can provide much information on the development of integrated systems related to BMR wastewater treatment if systematically investigated.

5.6. Integrated operation

As enzymes with higher activities and selectivities are compared to conventional chemical catalysts [104], their uses have been widely studied in different catalytic systems, e.g., hydrolysis [105–107], esterification [108], transesterification [109], enantioselective conversion of racemic mixtures [110], stereoselective oligomerization and regioselective conversion [111]. Particularly, BMRs offer advantages with respect to conversional enzyme reactors because of the membrane's ability to operate simultaneously as an enzyme support and selective barrier combining a reaction with a selective mass transfer through the membrane.

Simultaneous removal of products from the reaction site allows effective conversion to take place, even for product inhibited or thermodynamically unfavorable reactions [112]. As enzyme (lipase) and substrates (oils and fats) have limited solubility in water, multiphase enzyme reactors are used and enzyme (lipase) is known to act on an oil–water interface [113–116], which is ideal for such situations when the immobilized membrane forms the boundary between the two phases. Some of enzymes, such as lipases, are frequently utilized in kinetic resolutions of racemic mixtures because of their capability to discriminate between enantiomers. One of the most

important characteristics of lipases is activated by organic/aqueous biphasic interface (interface activation). Lipases are more active on water insoluble substrate compared to those on water soluble ester as substrate, and catalytic reactions are accelerated by adsorption of the lipase to the interface that contains the substrate, which are important in pharmaceutical and food industries [117], as well as the growth of such promising technologies with enzymes immobilized on the internal or the external surface of hollow fiber membranes [118,119]. Theoretically, if the product is preferred dissolution in an aqueous solution, the product will be continuously extracted (diffusion) into the aqueous while such process would be limited with a continuous reaction when recycling the aqueous phase in the system. This is mainly because of the decreasing impelling force between the active layer (reaction zone on enzyme) and aqueous phase. The choice is to real-time separate the product from the aqueous. However, it is impossible and uneconomical by traditional methods such as crystallization combing with filtration or centrifugation.

An alternative is a combination of membrane filtration and crystallization processes within a BMR system, wherein it would provide the following advantages: economical, continuous obtainable product, environmentally friendly (almost no waste disposal), as well as maintaining enzyme with higher activity and productivity, etc.

6. Conclusions

Immobilized enzyme membrane reactors, with their potential advantages over conventional chemical catalytic reaction systems with higher activity, mild operational conditions, and lower downstream separation/purification costs, will undoubtedly play an important role in many reaction systems, e.g., hydrolysis, esterification, transesterification, enantioselective resolution of racemic mixtures, stereoselective oligomerization and regioselective conversion, non-aqueous enzymology, and the development of novel biosensors for diagnostic purposes. Particularly, the recent trend focuses on environmentally friendly technologies where immobilized enzyme membrane reactors are very attractive because they can effectively reduce the formation of by-products and less waste disposal into the environment. Moreover, many methods are proven feasible to immobilize enzymes on various kinds of membranes in which adsorption methods are preferred because of their simple, cheap, and effective qualities.

However, in order to fully establish the use of biocatalytic membrane reactors, studies on the design of bioprocesses, particularly for large-scale production, the control of the reaction and kinetic mechanisms, and immobilization procedures need to continue. In the future, much concern should also be paid to improving enzyme stability with the development of genetic engineering, real-time to separate products from aqueous solutions, and effective methods to treat wastewater related to enzyme immobilized membrane reactors.

References

[1] B. Krajewska, Application of chitin- and chitosan-based materials for enzyme immobilizations: a review. Enzyme Microb. Technol. 35 (2004) 126–139.
[2] C. Bullock, Immobilised enzymes. Sci. Progr., 78 (1995)119–134.
[3] J.M. Woodley, Immobilized biocatalysts. Solid Supports Catal. Org. Synth., (1992) 254–271.
[4] F. van de Velde, N.D. Lourenço, H.M. Pinheiro and M. Bakker, Carrageenan: a food-grade and biocompatible support for immobilisation techniques. Adv. Synth. Catal., 344 (2002) 815–835.
[5] http://www.lsbu.ac.uk/biology/enztech/sources.html#tab2_1.
[6] S.G. Burton, Development of bioreactors for application of biocatalysts in biotransformations and bioremediation. Pure Appl. Chem., 73 (2001) 77–83.
[7] W.H. Scouten, J.H.T. Luong and R.S. Brown, Enzyme or protein immobilization techniques for applications in biosensor design. TIBTECH, 13 (1995) 178–185.
[8] W. Tischer and F. Wedekind, Immobilized enzymes: methods and applications. Top Curr. Chem., 200 (1999) 95–126.
[9] L. Giorno and E. Drioli, Biocatalytic membrane reactors: applications and perspective. TIBTECH, 18 (2000) 339–348.
[10] E. Drioli and L. Giorno, Biocatalytic Membrane Reactors—Applications in Biotechnology and the Pharmaceutical Industry, T.J. International, Padstow, UK, 1999, pp. 51–54.
[11] G. Carrea and S. Riva, Properties and synthetic applications of enzymes in organic solvents. Angew. Chem. Int. Ed., 39 (2000) 2226–2254.
[12] M. Ulbricht and A. Papra, Polyacrylonitrile enzyme ultrafiltration membranes prepared by adsorption, cross-linking, and covalent binding. Enzyme Microb. Technol., 20 (1997) 61–68.
[13] V.M. Balcão, A.L. Pavia and F.X. Malcata, Bioreactors with immobilized lipase state of the art. Enzyme Microb. Technol., 18 (1996) 392–416.
[14] http://infosys.korea.ac.kr/ippage/e/ipdata/2004/02/file/e200402-1001.pdf.
[15] A.P.V. Goncalves, J.M. Lopes, F. Lemos, F.R. Ribeiro and D.M.F. Prazeres, Effect of the immobilization support on the hydrolytic activity of a cutinase from *Fusarium solani pisi*. Enzyme Microb. Technol., 20 (1997) 93–101.
[16] D. Belhocine, H. Mokrane, H. Grib, H. Lounici, A. Pauss and N. Mameri, Optimization of enzymatic hydrolysis of haemoglobin in a continuous membrane bioreactor. Chem. Eng. J., 76 (2000) 189–196.
[17] E. Drioli and L. Giorno, Catalytic membrane reactors for bioconversion of low water soluble subtrate, in: Biocatalytic Membrane Reactors, Taylor Francis, 1999, pp. 153–187.
[18] K. Sakaki, L. Giorno and E. Erioli, Lipase-catalyzed optical resolution of racemic naproxen in biphasic enzyme membrane reactors. J. Membr. Sci., 184 (2001) 27–38.
[19] S.T. Bouwer, F.P. Cuperus and J.T.P. Derksen, The performance of enzyme-membrane reactors with immobilized lipase. Enzyme Microb. Technol., 21 (1997) 291–296.
[20] K. Sakaki, S. Hara and N. Itoh, Optical resolution of racemic 2-hydroxy octanoic acid using biphasic enzyme membrane reactor. Desalination, 149 (2002) 247–252.
[21] L. Giorno, N. Li and E. Drioli, Use of stable emulsion to improve stability, activity, and enantioselectivity of lipase immobilized in a membrane reactor. Biotechnol. Bioeng., 84 (2003) 677–685.
[22] L. Giorno, R. Molinari, E. Drioli, D. Bianchi and P. Cesti, Performance of a biphasic organic/aqueous hollow fiber reactor using immobilized lipase, J. Chem. Technol. Biotechnol. 64 (1995) 345–352.

[23] A. Gabelman and S.T. Hwang, Hollow fiber membrane contactors. J. Membr. Sci., 159 (1999) 61–106.
[24] M. Cheyran and M.A. Mehaia, in: Membrane Bioreactors, Membrane Separation in Biotechnology, W.C. McGregor, ed., Marcel Dekker, New York, 1986, pp. 255–301.
[25] Q. Gan, F. Baykara, H. Rahmat and L.R. Weatherley, Analysis of a direct contact membrane reactor for lipase catalysed oil hydrolysis in a dynamic emulsion system. Catal. Today, 56 (2000) 179–190.
[26] A. Mannheim and M. Cheyran, Continuous hydrolysis of milk protein in a membrane reactor. J. Food Sci., 55 (1990) 381–390.
[27] M.M. Hoq, T.Yamane, S. Shimizu, T. Funda and S. Ishida, Continuous hydrolysis of olive oil by lipase in microporous hydrophobic membrane bioreactor. J. Am. Oil Chem. Soc., 62 (1985) 1016–1021.
[28] Y. Pouliot, S.F. Gauthier and C. Bard, Fractionation of casein hydrolysates using polysulfone ultrafiltration hollow fiber membranes. J. Membr. Sci., 80 (1993) 257–264.
[29] S. Curcio, V. Calabro and G. Ioro, An experimental analysis of membrane bioreactor performances with immobilized chymosin. J. Membr. Sci., 173 (2000) 247–261.
[30] N. Cempel, J.M. Piot and D. Guillochon, Preparation of photodynamic hydrolysates from bovine haemoglobin. J. Agric. Food. Chem., 42 (1994) 2059–2063.
[31] P. Bressollier, J.M. Petit and R. Julien, Enzyme hydrolysis of plasma proteins in a CSTR ultrafiltration reactor: performance and modeling. Biotechnol. Bioeng., 31 (1988) 650–658.
[32] H. Tanigaki, Hydrolysis of soybean oil by lipase with a bioreactor having two different membranes. J. Ferment. Bioeng., 75 (1993) 53–57.
[33] L. Giorno, R. Molinari and E. Drioli, Experimental studies on enzyme membrane reactors in oil treatment, in: Advances in Oils and Fats, Antioxidants, and Oilsed By-products, Vol. 2, S.S. Koseoglu, et al., ed., AOCS Press, Champaign, IL, 1998, pp. 91–94.
[34] L. Giorno, R. Molinari, M. Natoli and E. Drioli, Hydrolysis and regioselective transesterification catalyzed by immobilized lipase in membrane bioreactors. J. Membr. Sci., 125 (1997) 177–187.
[35] A. Bhardwaj, J. Lee, S. Glauner, S. Ganapathi, D. Bhattacharyya and D.A. Butterfield, Biofunctional membranes: an EPR study of active site structure and stability of papain non-covalently immobilized on the surface of modified poly (ether) sulfone membranes through the avidin-biotin linkage. J. Membr. Sci., 119 (1996) 241–252.
[36] F.X. Yang, T.W. Weber, J.L. Gainer and G. Carta, Synthesis of lovastatin with immobilized *Candida rugosa* lipase in organic solvents: effects of reaction conditions on initial rates. Biotechnol. Bioeng., 56 (1997) 671–680.
[37] L. Giorno, E. Drioli, G. Carvoli, A. Cassano and L. Donato, Study of an enzyme membrane reactor with immobilized fumarase for production of L-malic acid. Biotechnol. Bioeng., 72 (2001) 77–84.
[38] H.A. Sousa, C.A.M. Afonso, J.P.B. Mota and J.G. Crespo, Modelling the enantioselective hydrolysis of a meso-diester using pig liver esterase in a two-phase hollow fibre reactor. Chem. Eng. Res. Des., 83 (2005) 285–294.
[39] M. Goto, Enzymatic resolution of racemic ibuprofen by surfactant-coated lipases in organic media. Biotechnol. Lett., 18 (1996) 839–844.
[40] S.C. Stinson, Chiral drug interactions. Chem. Eng. News, 77 (1999) 101.
[41] A.L. Demain, Small bugs, big business: The economic power of the microbe. Biotechnol. Adv., 18 (2000) 499–514.
[42] M. Arroyo, I. De La Mata, C. Acebal and M.P. Castillon, Biotechnological applications of penicillin acylases: state-of-the-art. Appl. Microbiol. Biot., 60 (2003) 507–514.
[43] C.J. Gray, J.S. Narang and S.A. Barker, Immobilization of lipase from *Candida cylindraceae* and its use in the synthesis of menthol esters by transesterification. Enzyme Microbiol. Technol., 12 (1990) 800–807.
[44] A. Mustranta, Use of lipase in the resolution of racemic ibuprofen. Appl. Microbiol. Biotechnol., 38 (1992) 175–180.
[45] A. Wiseman, Designer enzyme and cell applications in industry and in environmental monitoring. J. Chem. Tech. Biotechnol., 56 (1993) 3–13.
[46] G. Jönsson and L. Gorton, An amperometric glucose sensor made by modification of a graphite electrode surface with immobilized glucose oxidase and adsorbed mediator. Biosens., 1 (1985) 355–368.
[47] M. Demura, T. Asakura and T. Kuroo, Immobilization of biocatalysts with *Bombyx mori* silk fibroin by several kinds of physical treatment and its application to glucose sensors. Biosens., 4 (1989) 361–372.
[48] O. Fatibello-Filho, A. Suleiman and G.G. Guilbault, Enzyme electrode for the determination of aspartate. Biosens., 4 (1989) 313–321.
[49] Y.A. Cho, H.S. Lee, G.S. Cha and Y.T. Lee, Fabrication of butyrylcholinesterase sensor using polyurethane-based ion-selective membranes. Biosens. Bioelectron., 14 (1999) 435–438.
[50] D.C. Taek, A.J. Ran, K.K. Sun and C.K. Hee, Reproducible fabrication of miniaturized glucose sensors: preparation of sensing membranes for continuous monitoring. Biosens. Bioelectron., 16 (2001) 1079–1087.
[51] X.H. Chen, Y.B. Hu and G.S. Wilson, Glucose microbiosensor based on alumina sol–gel matrix/electropolymerized composite membrane. Biosens. Bioelectron., 17 (2002) 1005–1013.
[52] X.F. Yang, Z.D. Zhou, D. Xiao and M.M.F. Choi, A fluorescent glucose biosensor based on immobilized glucose oxidase on bamboo inner shell membrane. Biosens. Bioelectron., 21 (2006) 1613–1620.
[53] A.K. Basu, P. Chattopadhyay, U. Roychudhuri and R. Chakraborty, A biosensor based on co-immobilized l-glutamate oxidase and L-glutamate dehydrogenase for analysis of monosodium glutamate in food. Biosens. Bioelectron., 21 (2006) 1968–1972.
[54] S.Q. Liu and Y.M. Sun, Co-immobilization of glucose oxidase and hexokinase on silicate hybrid sol–gel membrane for glucose and ATP detections. Biosens. Bioelectron., 22 (2007) 905–911.
[55] S. S. Ordóñez and E. Fàbregas, New antibodies immobilization system into a graphite–polysulfone membrane for amperometric immunosensors. Biosens. Bioelectron., 22 (2007) 965–972.
[56] I. Chibata, Immobilized aspartase-containing microbial cells. Appl. Microbiol., 27 (1974) 878–885.
[57] M. Pastore and F. Morisi, Lactose reduction of milk by fiber-entrapped β-galactosidase. Pilot-plant experiments. Methods. Enzymol., 44 (1976) 822–830.
[58] E. Battistel, Enzyme resolution of (S)-(+)-naproxen in a continuous reactor. Biotechnol. Bioeng., 38 (1991) 659–644.
[59] H. Moueddeb, J. Sanchez, C. Bardot, M. Fick and H. Moueddeb, Membrane bioreactor for lactic acid production. J. Membr. Sci., 114 (1996) 59–71.
[60] S. Ganapathi-Desai, D.A. Butterfield and D. Bhattacharyya, Flat-sheet and hollow fiber membrane bioreactors: A study of the kinetics and active site conformational changes of immobilized papain including sorption studies of reaction constituents. J. Chem. Technol. Biot., 64 (1995) 157–164.
[61] S. Ganapathi-Desai, D.A. Butterfield and D. Bhattacharyya, Kinetics and active fraction determination of a protease enzyme immobilized on functionalized membranes: Mathematical modeling and experimental results. Biotechnol. Prog., 14 (1998) 865–873.
[62] D.A. Butterfield, D. Bhattacharyya, S. Daunert and L. Bachas, Catalytic biofunctional membranes containing site-specifically immobilized enzyme arrays: a review. J. Membr. Sci., 181 (2001) 29–37.
[63] D.A. Butterfield, J. Lee, S. Ganapathi and D. Bhattacharyya, Biofunctional membranes IV. Active site structure and stability of an immobilized enzyme, papain, on modified polysulfone membranes studies by electron paramagnetic resonance and kinetics. J. Membr. Sci., 91 (1994) 47–58.
[64] S. Ganapathi, D.A. Butterfield and D. Bhattacharyya, Flat sheet and hollow fiber membrane bioreactors: a study of the kinetics and active site conformational changes of immobilized papain including sorption studies of reaction constituents. J. Chem. Technol. Biotechnol., 64 (1995) 157–166.
[65] S. Ganapathi, D.A. Butterfield and D. Bhattacharyya, Kinetics and active fraction determination of a protease enzyme immobilized on functionalized membranes: mathematical modeling and experimental results. Biotechnol. Prog., 14 (1998) 865–877.
[66] P. Zhuang and D.A. Butterfield, Optimization of covalently

coupling enzymes to polymeric membranes: EPR studies of papain. J. Appl. Polym. Sci., 47 (1993) 1329–1338.
[67] P. Zhuang and D.A. Butterfield, Spin labeling and kinetic studies of a membrane-immobilized proteolytic enzyme. Biotechnol. Prog., 8 (1992) 204–219.
[68] P. Zhuang and D.A. Butterfield, Structural and enzymatic characterizations of papain immobilized onto vinyl alcohol/vinyl butyral copolymer membrane. J. Membr. Sci., 66 (1992) 247–257.
[69] S. Vishwanath, J. Wang, L.G. Bachas, D.A. Butterfield and D. Bhattacharyya, Site-directed and random immobilization of subtilisin on functionalized membranes: activity determination in aqueous and organic media. Biotech. Bioeng., 60 (1998) 608–616.
[70] Z.M. Liu, S. Tingry, C. Innocent, J. Durand, Z.K. Xu and P. Seta, Modification of microfiltration polypropylene membranes by allylamine plasma treatment-Influence of the attachment route on peroxidase immobilization and enzyme efficiency. Enzyme Microb. Technol., 39 (2006) 868–876.
[71] S. Vishwanath, D. Bhattacharyya, W. Huang and L.G. Bachas, Site-directed and random enzyme immobilization on functionalized membranes: kinetic studies and models. J. Membr. Sci., 108 (1995) 1–13.
[72] S.K. Vishwanath, C.R. Watson, W. Huang, L.G. Bachas and D. Bhattacharyya, Kinetic studies of site-specifically and randomly immobilized alkaline phosphatase on functionalized membranes. J. Chem. Technol. Biotechnol., 68 (1997) 294–302.
[73] D.A. Butterfield, R. Subramaniam, D. Bhattacharyya, S. Viswanath, W. Huang and L. Bachas, Biofunctional membranes: electron paramagnetic resonance studies of the active site structure of enzymes site-specifically immobilized onto polymeric supports through molecular recognition. Polym. Mat. Sci. Eng., 76 (1997) 602–603.
[74] W. Huang, J. Wang, D. Bhattacharyya and L.G. Bachas, Improved enzymatic activity by site-specific immobilization of subtilisin. Anal. Chem., 69 (1997) 4601–4607.
[75] H.K.W. Kallwass, W. Parris, E.L.A. McFarlane, M. Gold and J.B. Jones, Site-specific immobilization of an L-lactate dehydrogenase via an engineered surface cysteine residue. Biotechnol. Lett., 15 (1993) 29–44.
[76] S.J. Vigmond, M. Iwakura, F. Mizutani and T. Katsura, Site-specific immobilization of molecularly engineered dihydrofolate-reductase to gold surfaces. Langmuir, 10 (1994) 2860–2862.
[77] T.M. Spitznagel, J.W. Jacobs and D.S. Clark, Random and site-specific immobilization of catalytic antibodies. Enzyme Microb. Technol., 15 (1993) 916–921.
[78] E. Battistel, D. Bianchi, P. Cesti and C. Pina, Enzymatic resolution of (S)-(+)-naproxen in a continuous reactor. Biotechnol. Bioeng. 38 (1991) 659-664.
[79] J.Y. Xin, S.B. Li, Y. Xu, J.R. Chui and C.G. Xia, Dynamic enzymatic resolution of naproxen meyhyl ester in a membrane bioreactor. J. Chem. Biotechnol., 76 (2001) 579–585.
[80] M.J. Costello, A.G. Fane, P.A. Hogan and R.W. Schofield, The effect of shell-side hydrodynamics of axial flow hollow fibre modules. J. Membr. Sci., 80 (1993) 1–11.
[81] A. Guerra, G. Jonsson, A. Rasmussen, E. Waagner Nielsen and D. Edelsten, Low cross-flow velocity microfiltration of skim milk for removal of bacterial spores. Int. Diary J., 7 (1997) 849–861.
[82] U. Cocchini, C. Nicolella and A.G. Livingston, Braided silicone rubber membranes for organic extraction from aqueous solutions I. Mass transport studies. J. Membr. Sci., 199 (2002) 85–99.
[83] J.L. Lopez and S.L. Matson, A multiphase/extractive enzyme membrane reactor for production of diltiazem chiral intermediate. J. Membr. Sci., 125 (1997) 189–211.
[84] A.G. Livingston, J.P. Arcangeli, A.T. Boam, S. Zhang, M. Marangon and L.M. dos Santos, Extractive membrane bioreactors for detoxification of chemical industry wastes: process development. J. Membr. Sci., 151 (1998) 29–44.
[85] C. Nicolella, P. Pavasant and A.G. Livingston, Substrate counter diffusion and reaction in membrane-attached biofilms: mathematical analysis of rate limiting mechanisms. Chem. Eng. Sci., 55 (2000) 1385–1398.
[86] S.R. Wickramansinghe, M.J. Semmens and E.L. Cussler, Better hollow fiber contactors. J. Membr. Sci., 62 (1991) 371–388.
[87] L.M. Freitas dos Santos and G. Lo Biundo, Treatment of pharmaceutical industry process wastewater using the extractive membrane bioreactor. Environ. Prog., 18 (1999) 34–39.
[88] T.A.C. Oliveira, U. Cocchini, J.T. Scarpello and A.G. Livingston, Pervaporation mass transfer with liquid flow in the transition regime. J. Membr. Sci., 183 (2001) 119–133.
[89] V. Gekas amd B. Hallström, Mass transfer in the membrane concentration polarisation layer under turbulent cross flow. I. Critical literature review and adaptation of existing Sherwood correlations to membrane operations. J. Membr. Sci., 30 (1987) 153–168.
[90] M.J. Costello, A.G. Fane, P.A. Hogan and R.W. Schofield, The effect of shell-side hydrodynamics on the performance of axial flow hollow fibre modules. J. Membr. Sci., 80 (1993) 1–15.
[91] M.G. Parvatiyar, Mass transfer in a membrane tube with turbulent flow of Newtonian and non-Newtonian fluids. J. Membr. Sci., 148 (1998) 45–57.
[92] R.M.C. Viegas, M. Rodríguez, S. Luque, J.R. Alvarez, I.M. Coelhoso and J.P.S.G. Crespo, Mass transfer correlations in membrane extraction: analysis of Wilson-plot methodology. J. Membr. Sci., 145 (1998) 129–142.
[93] J. Wu and V. Chen, Shell-side mass transfer performance of randomly packed hollow fibre models, J. Membr. Sci., 172 (2000) 59–74.
[94] R. Gawronski and B. Wrzesinska, Kinetics of solvent extraction in hollow fibre contactors. J. Membr. Sci., 168 (2000) 213–222.
[95] F. Lipnizki and R.W. Field, Mass transfer performance for hollow fibre modules with shell-side axial feed flow: using an engineering approach to develop a framework. J. Membr. Sci., 193 (2001) 195– 208.
[96] V. Calabrò, S. Curcio and G. Iorio, A theoretical analysis of transport phenomena in a hollow fiber membrane bioreactor with immobilized biocatalyst. J. Membr. Sci., 206 (2002) 217–241.
[97] A.Trusek-Holownia and A. Noworyta, Mass transfer in the membrane phase contactor with an enzyme gel layer immobilized on a membrane. Desalination, 162 (2004) 335–342.
[98] T.W. Xu and R.Q. Fu, Determination of effective diffusion coefficient and interfacial mass transfer coefficient of bovine serum albumin (BSA) adsorption into porous polyethylene membrane by microscope FTIR-mapping study. Chem. Eng. Sci., 59 (2004) 4569– 4574.
[99] L. Giorno, J.C. Zhang and E. Drioli, Study of mass transfer performance of naproxen acid and ester through a multiphase enzyme-loaded membrane system. J. Membr. Sci., 276 (2006) 59–67.
[100] A. W. Mohammad, N. Ali. Understanding the steric and charge contributions in NF membranes using increasing MWCO polyamide membranes. Desalination 47(2002) 205-212.
[101] J. Drewes, M. Reinhard and P. Fox, Comparing microfiltration-reverse osmosis and soil-aquifer treatment for indirect potable reuse. Water Res., 37 (2003) 3612–3621.
[102] B.B. Levine, K. Madrireddi, V. Lazarova, M.K. Stenstrom and M. Suffet, Treatment of trace organic compounds by membrane processes: at the lake arrowhead water reuse pilot plant. Water Sci. Technol., 40 (1999) 293–302.
[103] H. Ozaki and H. Li, Rejection of organic compounds by ultra-low pressure reverse osmosis membrane. Water Res., 36 (2002) 123–130.
[104] F. Bjorkling, S.E. Godtfredsen and O. Kirk, The future impact of industrial lipases. TIBTECH, 9 (1991) 360–363.
[105] M. Rucka, B. Turkiewicz, S.J. Zuk, A. Krystynowicz and E. Galas, Hydrolysis of plant oils by means of lipase from *Rhizopus nigricans*. Bioprocess Eng., 7 (1991) 133–135.
[106] R. Molinari, M. E. Santoro and E. Drioli, Study and comparison of two enzyme membrane reactors for fatty and glycerol production. EC Res., 33 (1994) 2591–2599.
[107] E. Ruckenstein and X. Wang, Lipase immobilized on hydrophobic porous polymer supports prepared by concentrated emulsion polymerization and their activity in the hydrolysis of triacylglycerides. Biotech. Bioeng., 42 (1993) 821–828.
[108] H. Stamatic, A. Xenakis, U. Menge and F.N. Kolisis, Kinetic study of lipase catalyzed esterification reactions in water-in-oil microemulsions. Biotech. Bioeng., 42 (1993) 931–937.
[109] B. Cambou and A.M. Klibanov, Comparison of different

strategies for the lipase-catalyzed preparative resolution of racemic acids and alcohols: asymmetric hydrolysis, esterification, and transesterification. Biotech. Bioeng., 26 (1984) 1449–1454.
[110] P.E. Sonnet, Enzymes for chiral synthesis. Chemtech., 18 (1998) 94–98.
[111] J.O. Rich, B.A. Bedell and J.S. Dordick,l Controlling enzyme-catalyzed regioselectivity in sugar ester synthesis. Biotech. Bioeng., 45 (1995) 426–434.
[112] Y. Miyake, M. Ohkubo and M. Teramoto, Lipase-catalyzed hydrolysis of 2-naphtyl esters in biphasic system. Biotech. Bioeng., 38 (1991) 30–36.
[113] R. Molinari, M.E. Santoro and E. Drioli, Study and comparison of two enzyme membrane reactors for fatty acid and glycerol production, Ind. Eng. Chem. Res., 33 (1994) 2591–2599.
[114] L. Giorno, R. Molinari, M. Natoli and E. Drioli, Hydrolysis and regioselective transesterification catalyzed by immobilized lipases in membrane bioreactor. J. Membr. Sci., 125 (1997) 177–187.
[115] R.P.M. Guit, M. Kloosterman, G. W. Meindersma, M. Mayer and E.M. Meijer, Lipase kinetics: hydrolysis of triacetin by lipase from candida cylindracea in a hollow-fiber membrane reactor. Biotechnol. Bioeng., 38 (1991) 727–732.
[116] F. Carriere, K. Thirstup, E. Boel, R. Verger and L. Thim, Structure-function relationships in naturally occurring mutants of pancreatic lipase. Protein Eng., 7 (1994) 563–569.
[117] A.S. Michaels, Membranes, membrane processes, and their applications: needs, unsolved problems, and challenges of the 1990's. Desalination, 77 (1990) 5–34.
[118] M. Tanigaki, M. Sakata and H. Wada, Hydrolysis of soybean oil by lipase with a bioreactor having two different membranes. J. Ferm. Bioeng., 75 (1993) 53–57.
[119] L. Giorno, R. Molinari, E. Drioli, D. Bianchi and P. Cesti, Performance of a biphasic organic/aqueous hollow fiber reactor using immobilized lipase. J. Chem. Tech. Biotechnol., 64 (1995) 345–352.