Bioreactors with immobilized lipases: State of the art

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This review attempts to provide an updated compilation of studies reported in the literature pertaining to reactors containing lipases in immobilized forms, in a way that helps the reader direct a bibliographic search and develop an integrated perspective of the subject. Highlights are given to industrial applications of lipases (including control and economic considerations), as well as to methods of immobilization and configurations of reactors in which lipases are used. Features associated with immobilized lipase kinetics such as enzyme activities, adsorption properties, optimum operating conditions, and estimates of the lumped parameters in classical kinetic formulations (Michaelis–Menten model for enzyme action and first-order model for enzyme decay) are presented in the text in a systematic tabular form.

Keywords: Immobilized lipases; activity; stability; optimum operating conditions; reactors

Characteristics and applications of lipases

Lipases, also known as glycerol ester hydrolases (EC 3.1.1.3), belong to the hydrolase enzyme class and were originally employed for the hydrolysis of ester bonds. In addition to plants and animals, in which these enzymes are widespread, many microorganisms (natural or genetically engineered) are also capable of actively producing these enzymes both in endogenous and exogenous forms.

Lipases are unique enzymes in that they require interfacial activation for full catalytic performance, a fact that was initially established in 1958 by Sarda and Desnuelle.[1] (An interface is hereby considered as the imaginary surface that separates two physically distinct phases, which corresponds on the molecular level to a set of two adjacent layers of ordered molecules, one more hydrophobic and the other more hydrophilic in nature.) Although several theories have been postulated to explain the observed enhancement of lipase activity upon formation of an interface,[2] recent reports evolving from paneuropean projects on lipases sponsored by the BRIDGE (EC) and the Nordic (Scandinavian) protein engineering programs have shed, in a dramatic fashion, experimental light over such theoretical interpretations via determination of the three-dimensional structure of 10 lipases and characterization of the kinetic behavior and stereoselectivity of 25 different lipases. It is now well established that: 1) all lipases share primary sequence homologies including significant regions His-*X*-*Y*-Gly-*Z*-Ser-*W*-Gly or *Y*-Gly-His-Ser-*W*-Gly (where *X*, *Y*, *Z*, and *W* denote generic amino acid residues)[3]; and 2) the serine residue at the active site is protected by a flap (or α-helical lid), which opens upon contact of the lipase with an interface and thus leads to restructuring of the lipase by creating an electrophilic region (the oxyanion hole) around the aforementioned serine residue, by exposing hydrophobic residues, and by burying hydrophilic ones, all of which increase the affinity of the complex for lipid substrates and help stabilize the transition state intermediate during catalysis.[4–6] The requirement for an interface (irrespective of its nature) is critical; even if one uses a hydrophobic solvent, there are small local pools of water remaining within the folded structure of the lipase, and those water pools in the vicinity of the active site may provide the local interface necessary for enzyme activation.

Lipases catalyze a series of different reactions. In fact, although they were designed by nature to cleave ester bonds of triacylglycerols with the concomitant consumption of water molecules (hydrolysis), lipases are also able to catalyze the reverse reaction under microaqueous conditions,

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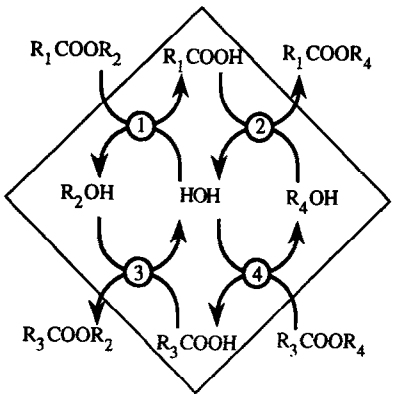
viz. the formation of ester bonds between alcohol and carboxylic acid moieties (ester synthesis). These two basic processes can then be combined in a sequential fashion to give rise to a set of reactions generally termed interesterifications. Depending on the particular starting point in terms of substrates, one may have acidolysis (where an acyl moiety is displaced between an acylglycerol and a carboxylic acid), alcoholysis (where an acyl moiety is displaced between an acylglycerol and an alcohol), and transesterification (where two acyl moieties are exchanged between two acylglycerols). All these processes are depicted in *Figure 1*.

Although lipase-catalyzed reactions can also be carried out using inorganic, metal-derived catalysts, the interest generated by these enzymes as biotechnological vectors for the achievement of various types of reactions in both macro- and microaqueous systems has expanded formidably during the last decade. Indeed, lipase-catalyzed reactions resemble closely the pathways designed by nature for the metabolism of live beings, and so the reaction mechanisms and processes associated therewith may be viewed as more environment friendly than bulk syntheses. Furthermore, lipases are capable of producing a wide variety of products with potential high purity (and consequently high added value) resulting from their substrate specificity and stereospecificity. Because of the low activation energies involved, further advantages of lipases include mild reaction conditions of temperature and pH, which lead to reduced energy consumption and less extensive thermal damage to reactants and products.

Although technical staff are well aware of their versatility and possible applications in industry, the use of lipases is not yet as significant as that associated with such other enzymes as proteases and carbohydrases. This may be because of, first, most detailed studies involving lipases are recent and the usual time lag prior to full commercial exploitation has not yet elapsed, and, second, their cost is still relatively high, a constraint that will eventually be overcome in the coming years as a result of evolution encompassing their extraction and purification, as well as their production via genetic engineering.



1 : Hydrolysis

2 : Ester synthesis

1 + 2 : Alcoholysis

1 + 3 : Acidolysis

1 + 2 + 3 + 4 : Transesterification

Figure 1 Schematic representation of the reactions catalyzed by lipases, where R_i (i = 1,2,3,4) denotes an acyl moiety

The manufacture of household detergents remains the biggest market for industrial enzymes in general, but only now is this market opening up for lipases after genetic design aiming at sufficient high stability and activity under the alkaline conditions prevailing in washing processes. Currently, one of the major application of lipases is in the dairy industry for the controlled hydrolysis of milk fat, a process which is useful for acceleration of cheese ripening, flavor enhancement of butter, manufacture of cheeselike products, and preparation of enzyme-modified cheeses for use as ingredients in dressings, soups, and sauces.

Lipases possess high potential for hydrolysis, glycerolysis, and alcoholysis of bulk fats and oils because of their high specificity and the relative purity of the products derived therefrom. The use of such enzymes avoids the need for the high temperatures required by nonenzymatic splitting, which leads to thermal degradation of the products (e.g., generation of off-flavors and off-colors) and so to the need for postprocessing purification and refining. The upgrading of fats by specific syntheses and the randomization of structured triglycerides is also possible by the use of appropriate lipases, thus allowing the tailoring of fats for desired functional and nutritional properties. Another application pertains to the production of ω3-polyunsaturated fatty acid (ω3-PUFA) concentrates from fish liver oils (which have been claimed to provide beneficial health effects via prevention of coronary heart diseases) for use as nutropharmaceutical food supplements, and sequential lipase-catalyzed chemical incorporation in triglycerides.

Another industrial application with a promising future is the lipase-mediated production of optically pure compounds (rather than racemic mixtures) for pharmaceutical and fine-chemical uses. Lipase-catalyzed resolution of racemic aqueous mixtures can occur through asymmetric hydrolysis of the corresponding esters, whereas in nonaqueous media, this approach can be extended to stereo- and regiospecific (trans)esterification reactions. The synthesis of optically active polymers can also be accomplished by this route.

Further applications of lipases in industry include (but are not limited to) the production of emulsifiers for personal care products (skin and suntan creams and bath oils), esters of fatty acids and fatty alcohols (wax esters), and oil waste treatment.

Immobilization of lipases

Lipases are spontaneously soluble in aqueous solutions (as a result of their globular protein nature), but their natural substrates (i.e., lipids) are not. Although use of a proper organic solvent or an emulsifier helps overcoming the problem of intimate contact between substrate and enzyme, the practical use of lipases in such pseudohomogeneous reaction systems poses technological difficulties (viz. contamination of the products with residual enzymatic activity) and economic difficulties (viz. use of the enzyme for a single

reactor pass). The former leads to constraints on the product level, because the final characteristics of the product depend on such postprocessing conditions as storage time and temperature. The latter leads to constraints on the process level, because the useful life of the enzyme is restricted to the space time of the reactor (on the assumption that the space time is small compared with the time scale associated with deactivation of the enzyme). In both cases, part of the overall potential enzymatic activity is lost. If the lipase is immobilized, then it becomes an independent phase within the reaction system, which may be easily retained in the reactor via mechanical means with concomitant advantages in preventing contamination of the products and extending its useful active life.

Immobilized lipases are considered hereafter as lipases which are localized in a defined region of space, which is enclosed by an imaginary or material barrier which allows for physical separation of the enzyme from the bulk reaction medium, and which is at the same time permeable to reactant and product molecules. Rending a lipase immobilized may therefore be achieved by engineering the enzyme microenvironment or macroenvironment. Examples of engineering on the level of the microenvironment of the enzyme encompass immobilization by attachment to a carrier (e.g., covalent attachment, hydrophobic and ion exchange adsorption, and cross-linking) and immobilization by containment in a barrier (e.g., microencapsulation using lipid vesicles, containment in reversed micelles, entrapment in polymeric matrices, and confinement in ultrafiltration hollow fibers). Engineering on the level of the macroenvironment of the enzyme may proceed via modification of the reaction medium (e.g., precipitation in an organic solvent).

In addition to the lipase, immobilization also requires an immobilizing agent (i.e., a force that keeps the enzyme as a separate phase from the bulk reaction medium). Such a force can be established between two enzyme molecules, E-E (e.g., cross-linking, intermolecular cystein and/or salt bridges, and intermolecular hydrogen bonds), or between one enzyme molecule and another catalytically inert molecule, E-C (e.g., covalent attachment, ion exchange, and hydrophobic interaction), or between two catalytically inert molecules, C-C (e.g., entrapment and mechanical containment). Virtually every immobilization protocol employed to date for lipases encompasses immobilizing agent(s) at one, two, or all three levels of interaction considered (i.e., E-E, E-C, and C-C, respectively). The immobilizing agent thus comprises: 1) covalent forces as in covalent attachment, cross-linking, entrapment via polymerization, containment within porous membranes of polymeric nature, and intermolecular cysteine bridges during precipitation; 2) ionic forces as in ion exchange, containment within porous membranes of mineral nature, and intermolecular salt bridges during precipitation in apolar solvents; 3) hydrogen bonds as in intermolecular interaction between hydrogen atoms and electronegative atoms during precipitation in apolar solvents; and 4) van der Waals forces as in hydrophobic adsorption, reversed micelles, microencapsulation, containment within porous membranes of monomeric and hydrophobic nature, and precipitation in polar solvents. The immobilizing agent may also include a material ligand; this is the case of multifunctional molecules in cross-linking and spacer molecules in covalent attachment following preliminary derivatization of the support.

The carrier (or the barrier) is an entity larger in size than the enzyme molecule (which may therefore be considered as a continuum) to which the enzyme is directly bound or within which the enzyme is confined, and has the role of helping in the creation of an immobilized, enzyme-rich phase. This support is absent in the case of cross-linking and plain precipitation of the enzyme in an organic solvent. The support may be a liquid, as in reversed micelles, or a solid, as in most of the commonly employed immobilization protocols.

When carriers (with lipase attached to them) or barriers (with lipase confined to them) are used to immobilize a lipase, considerations on three levels of structure are in the order: macroscopic level, microscopic level, and submicroscopic level. At the macroscopic level, there may be one dominant dimension (e.g., the length in the case of hollow fibers), two dominant dimensions (e.g., the surface in the case of flat-sheet membranes), or three dominant dimensions (e.g., the volume in the case of beads or dried micellia). The dominant dimension(s) are determined by the manufacture process and are directly related to the characteristics of the flow within the reactor (see below). In terms of microscopic characteristics, two factors are of relevance for solid supports: the thickness, on the one hand, and the

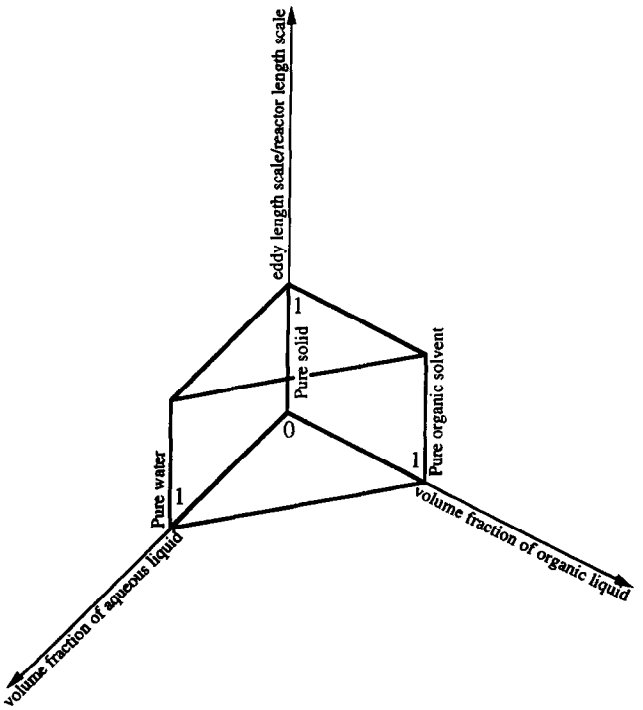


Figure 2 Immobilized lipase reactor pyramid enclosing the reactor configurations studied to date. The reactor space is characterized by an axis describing the ratio of length scales of the hydrodynamic eddies and the reactor itself, and two axes describing the volume fraction of the liquids in the reaction medium. The vertical sides of the pyramid correspond to a pure phase

porous structure, on the other. Small thicknesses and high porosities are usually sought to minimize diffusional limitations upon substrates and maximize available area for lipase attachment, but they also lead to mechanical weakness; hence, a compromise must be reached. The submicroscopic character is a direct result of the molecular characteristics of the carrier and may range from hydrophilic (i.e., essentially polar) to hydrophobic (i.e., essentially nonpolar). In the case of hydrophobic carriers, the immobilized enzyme is soaked in the organic liquid phase, whereas in hydrophilic carriers the immobilized enzyme is soaked in water; the former situation is useful if one wants deactivation reactions of the enzyme to be maintained as slow as possible, whereas the latter situation should be used when hydrolysis reactions are desired.

An overview of the various reactor configurations employing immobilized lipases in the light of the framework introduced above will be presented in the next section. Later, the characteristic physicochemical features of such reactors (activity, stability, and optimum operating conditions) will be listed whenever available followed by control and economic considerations.

Reactors with immobilized lipases

An immobilized lipase reactor, which, as previously explained, is a more cost-effective reactional alternative than a soluble lipase reactor, may be viewed as a portion of space in which the lipase macroscopic movement is restricted to its boundaries. Therefore, the ratio of the length scale associated with the hydrodynamic eddies of the reaction medium to the length scale associated with the reactor may range from zero (a situation that corresponds to pure molecular transport of the reacting species between the reaction medium and the enzyme) to unity (a situation that corresponds to pure, long-range convection of the reacting molecules through the reactor). This concept is illustrated in *Figure 2*.

Generation of hydrodynamic eddies may result solely from Brownian motion (as in reactors operated batchwise under virtually stagnant conditions), or from some extent of mechanical input to the reacting system via shaft work (as in solid rotational agitators) or via non–shaft work (as in bubbling of a gas within the liquid system or in deliberately disturbing the liquid stream-line pattern following the motionless mixer principle as in reactors operated continuously under essentially turbulent conditions). In general, contact of the reactants with the immobilized lipase is improved by convection relative to plain molecular transport; however, convection is also associated with higher shear rates and/or higher times of exposure to shear (in the case of rotational or motionless agitation), or with higher surface tension and/or higher interfacial areas (in the case of bubbling agitation), both of which promote disturbances of the elaborate three-dimensional shape of the lipase molecules that lead to faster deactivation.

Confinement of the lipase to a given portion of space within the reacting fluid defined by imaginary boundaries requires either an electromagnetic or a gravitational field able to function as a barrier to the movement of the enzyme molecules and unable to significantly affect the movement of the reacting molecules. Both configurations have, however, been seldom reported to date for the case of lipase reactors.

The other (more frequent) situation consists in the confinement of the lipase to a given portion of space within the reacting fluid defined by physical boundaries coinciding with interfaces; such a configuration requires the existence of at least two phases which are separable by such purely mechanical means as settling, centrifugation, or filtration. Various possibilities exist here for reactors containing immobilized lipases, but most situations fall within one of the following cases: 1) a lipase in solid form precipitated within an organic liquid phase; 2) a lipase in soluble form in an aqueous phase and confined by a solid ultrafiltration membrane; 3) a lipase contained in an aqueous phase and confined by a surfactant liquid membrane within an organic liquid phase; 4) a lipase entrapped within a three-dimensional polymeric matrix dispersed within an organic liquid phase, or dispersed within an aqueous phase; and 5) a lipase attached to a solid support and dispersed within an organic liquid phase, or dispersed within an aqueous phase. All these cases of technological feasibility and practical interest may be represented in the set of cartesian axes depicted in *Figure 2* in the portion of space corresponding to the triangular pyramid outlined in bold.

There are several reactor configurations described in the literature which have been used in studies of immobilized lipases, as are depicted in *Table 1* (where references are written in chronological order). Important characteristics as type of reaction, solvent (if any), substrate(s), reactor configuration, source of lipase, method of immobilization, immobilizing agent, and nature of carrier/barrier (if any) are also listed.

As discussed before, use of emulsifiers promotes formation of a pseudouniphase reaction system. However, emulsion systems have several drawbacks: relatively low productivity, need of high-speed stirring to prevent clumping, requirement for powerful centrifuging or solvent extraction for separation of product from emulsifier, virtual impossibility of reutilization of the enzyme, and difficult control of the water concentration. These difficulties can be circumvented by immobilizing the lipase, and enclosing it via one of several possibilities, namely, in batch stirred-tank reactors (BSTRs), packed-bed reactors (PBRs), fluidized-bed reactors (FBRs), or membrane reactors.

BSTRs are the reactors most commonly used; this reactor configuration accounts for more than two thirds of all reported uses (see *Table 1*). These reactors operate batchwise and consist of a vessel in which the reactant fluid mixture is stirred by some mechanical means (e.g., magnetic bars, submerged impellers, reciprocal oscillators, or end-over-end rotators) in a way that avoids the existence of temperature and concentration gradients. The immobilized enzyme is separated from the reaction medium at the end of the reaction by filtration or centrifugation. These reactors are easy to operate (e.g., heat, cool, clean, and maintain) and normally require a very limited set of auxiliary equipment; however, the upper average overall volumetric throughput

Table 1 Main features of reactors employing immobilized lipases

Reaction [solvent(s)]	Substrate(s)	Reactor configuration	Source of lipase	Method of immobilization	Binding agent(s)	Carrier or Barrier	Ref.
Hydrolysis	Olive oil	BSTR	*Candida rugosa*	Covalent binding	E-(cf)-E	PEG	21
Alcoholysis	β-substituted Ethanols (Cl-, Br-, MeO-, BuO-, Me_2N-, and Et_2N-CH_2CH_2OH) & Ethyl acetate	BSTR	*Candida cylindracea* & porcine pancreas	Precipitation	E-(vdW)-E		22
Hydrolysis	BFT	FTMR, PBR, CSTR	*Candida cylindracea*	Adsorption	C-(vdW)-E	Polypropylene	23
Interesterification	1-Stearyl 1,2-linolenyl, 3-oleylglycerol	BSTR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	24
Interesterification	1-Stearyl 1,2-linolenyl, 3-oleylglycerol	BSTR	*Candida deformans, Rhizopus arrhizus*	Adsorption	C-(vdW)-E	Celite	24
Alcoholysis (*n*-hexane, benzene, carbon tetrachloride, diisopropylether, acetonitrile)	Tributyrin & benzyl alcohol	BSTR	*Candida cylindracea, C. lipolytica, Pseudomonas fluorescens, Penicillium cyclopium, P. roqueforti,* porcine pancreas, *Humicola lanuginosa, Mucor javanicus, Rhizopus delemar, R. javanicus, R. niveus, Aspergillus niger*	Precipitation	E-(vdW)-E		25
Ester synthesis (hexane)	Oleic acid & mono-, dioleoylglycerol	BSTR	Oilseed rape	Precipitation & adsorption	E-(vdW)-E-(vdW)-C	Celite	26
Acidolysis (petroleum ether)	Olive oil & octanoic, decanoic, lauric acids	BSTR, CSTR	*Mucor miehei*	Precipitation & adsorption	E-(vdW)-E-(vdW)-C	Diatomaceous earth	27
Hydrolysis	Esters of triacylglycerols	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Duolite	28
Acidolysis (hexane)	Soybean oil & lauric, palmitic, oleic acids	BSTR, CSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	29
Acidolysis (hexane)	Soybean oil & lauric, palmitic, oleic acids	BSTR, CSTR	Porcine pancreas	Adsorption	C-(vdW)-E	Celite	29
Transesterification (isooctane)	Butterfat	BSTR	*Aspergillus niger*	Adsorption	C-(vdW)-E	Celite	30
Transesterification (isooctane)	Butterfat	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	30
Transesterification (isooctane)	Butterfat	BSTR	*Candida cylindracea*	Adsorption	C-(vdW)-E	Celite	31
Acidolysis (hexane)	Olive oil & methyl stearic acid, stearic acid, palmitic acid	CSTR	*Rhizopus chinensis*	Containment	C-(vdW)-C	CWD	32
Ester synthesis (hexane)	Oleic acid & oleoyl alcohol; octanoic acid & octanol; myristic acid & propanol	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	33
Transesterification	Coprah oil & methyl stearate	BSTR	*Candida deformans, Rhizopus arrhizus*	Adsorption	C-(vdW)-E	Celite	34
Transesterification	Coprah oil & methyl stearate	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	34
Ester synthesis (Benzene, chloroform, 1,1,1-trichloroethane, acetone, *N,N*-dimethyl sulfoxide)	1-Dodecanol & *n*-dodecanoic acid	BSTR	*Pseudomonas fragi*	Precipitation	E-(cf)-peg-(vdW)-E		35

Ester synthesis (cyclohexane)	(*R,S*)-1-Phenylethanol & heptanoic acid	BSTR	*Candida cylindracea*	Adsorption	C-(vdW)-E	Glass wool, porous glass, BE, phenyl-BE, C_{18}-BE, lichrosorb RP-18 gel	36
Ester synthesis	Geraniol & lauric acid	PBR	*Humicola lanuginosa*	Adsorption	C-(if)-E	Synthetic resin	37
Hydrolysis	Olive oil & water	BSTR	*Humicola lanuginosa*	Entrapment	C-(cf)-C	ENTP, polyurethane	38
Hydrolysis	Olive oil & water	BSTR	*Humicola lanuginosa*	Adsorption	C-(vdW)-E	Ca-alginate, ADCPG	38
Hydrolysis	Olive oil & water	BSTR	*Humicola lanuginosa*	Covalent binding	C-(cf)-E	Amberlite, diaion	38
Hydrolysis	Olive oil & water	BSTR	*Humicola lanuginosa*	Cross-linking	E-(cf)-ga-(cf)-E	Octyl-Sepharose	38
Interesterification	Cocoa butter stearine, palm stearine & coconut oil	PBR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	39
Hydrolysis	Soybean oil & water	HFMR	*Candida rugosa*	Adsorption	C-(vdW)-E	Cellulose	40
Acidolysis	Canola oil & lauric acid	BSTR	Porcine pancreas	Adsorption	C-(vdW)-E	Diatomaceous earth	41
Interesterification	Canola oil & trilaurin, fully hydrogenated HEAR stearin	BSTR	Porcine pancreas	Adsorption	C-(vdW)-E	Diatomaceous earth	41
Hydrolysis	Acylglycerol & water	BSTR	Human milk	Precipitation	E-(vdW)-E		42
Hydrolysis	Olive oil & water	BSTR	*Candida rugosa*	Precipitation	E-(vdW)-E		43
Ester synthesis	Butanol, ethanol & butyric acid	BSTR	*Candida cylindracea, Aspergillus niger,* porcine pancreas	Precipitation & adsorption	E-(vdW)-E-(vdW)-C	NA	44
Interesterification	Ethyl *D*-glucopyranoside & C_8–C_{18} fatty acids	BSTR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	45
Interesterification	Ethyl *D*-glucopyranoside & C_8–C_{18} fatty acids	BSTR	*Candida antarctica, Humicola* sp., *Candida cylindracea, Pseudomonas* sp.	NA	NA	NA	45
Interesterification	Castor oil & beef tallow	BSTR	*Chromobacterium viscosum*	Precipitation	E-(vdW)-E		46
Interesterification	Cod liver oil & EPA, DHA	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	47,48
Hydrolysis	1-Phenyl ethyl carboxylic esters & water	BSTR	*Candida cylindracea*	Precipitation	E-(vdW)-E		49
Ester synthesis (cyclohexane)	1-Phenyl ethanol & butyric acid	BSTR	*Candida cylindracea*	Precipitation	E-(vdW)-E		49
Transesterification	Cottonseed oil & beef fat	BSTR	Cotton seeds	Adsorption	C-(vdW)-C	CWD	50
Acidolysis (hexane)	Groundnut, coconut, mustard, soybean oils & lauric, oleic, stearic acids	BSTR	Porcine pancreas	Adsorption	C-(vdW)-E	Celite	51
Acidolysis (hexane)	Groundnut, coconut, mustard, soybean oils & lauric, oleic, stearic acids	BSTR, CSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	51
Acidolysis (hexane)	Groundnut, coconut, mustard, soybean oils & lauric, oleic, stearic acids	BSTR	*Rhizopus delemar*	Precipitation	E-(vdW)-E		51
Acidolysis (hexane)	Soybean oil & lauric acid	BSTR	Porcine pancreas	Adsorption	C-(vdW)-E	Celite	52
Transesterification (hexane, isooctane)	Butterfat	BSTR	*Pseudomonas fluorescens*	Adsorption	C-(vdW)-E	Celite	53–55
Hydrolysis (*n*-hexane, *n*-heptane, *n*-octane, *n*-nonane, *n*-decane, isopropylether, isooctane, cyclohexane)	Olive oil & water	BSTR	*Candida rugosa*	Adsorption	C-(vdW)-E	Sephadex	56

Table 1 (*continued*)

Reaction [solvent(s)]	Substrate(s)	Reactor configuration	Source of lipase	Method of immobilization	Binding agent(s)	Carrier or Barrier	Ref.
Hydrolysis (isooctane)	Olive oil & water	BSTR	*Candida rugosa*	Adsorption	C-(vdW)-E	Sephadex	57
Esterification	Palm olein	BSTR	*Pseudomonas* sp.	Adsorption	C-(vdW)-E	Celite	58
Acidolysis (hexane)	Refined sand eel oil & EPA, DHA	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	59
Transesterification	Palm oil & coprah, palmiste, colza, son de riz, soybean, bourrache oils	BSTR, PBR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	60,61
Ester synthesis	Acetone glycerol & oleic acid	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	62
Transesterification (isooctane)	Stearic acid & triolein, tripetroselinin, tri-α-linolenin; α-linolenic acid, γ-linolenic acid, & triolein; oleic acid & 1-γ-linoleoyl-2,3-dioleoylglycerol	BSTR	*Rhizopus delemar*	Containment	C-(vdW)-E	AOT-RM	63
Hydrolysis	Sunflower oil & water	HFMR	*Rhizopus* sp.	Cross-linking	E-(cf)-ga-(cf)-E	PTFE	64
Ester synthesis	Oleic acid & mono-, di-, trioleoylglycerol	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	65
Hydrolysis & transesterification	Palm oil & stearic acid	BSTR	*Rhizopus* sp.	Covalent binding	C-(cf)-E	TAS	66
Hydrolysis & transesterification	Palm oil & stearic acid	BSTR	*Rhizopus* sp.	Adsorption	C-(vdW)-E	Celite	66
Transesterification (*n*-heptane)	Palm oil mid fraction & ethyl stearate	BSTR	Porcine pancreas, *Rhizopus arrhizus, R. javanicus, R. delemar, R. niveus, Aspergillus niger, Candida cylindracea*	Adsorption	C-(vdW)-E	Celite	67
Transesterification (*n*-heptane)	Palm oil midfraction & ethyl stearate	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	67
Interesterification	Cottonseed oil & olive oil	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	68
Ester synthesis (*n*-hexane)	Oleic acid & ethanol	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	69
Hydrolysis (water)	High erucic acid rapeseed	BSTR	*Candida rugosa*	Precipitation	E-(vdW)-E		70
Ester synthesis	Heptanol & octanoic acid	BSTR	*Candida cylindracea*	Precipitation	E-(vdW)-E		71
Ester synthesis	Heptanol & oleic acid	BSTR	*Candida cylindracea*	Precipitation	E-(vdW)-E		72
Acidolysis	Butyl laurate & oleic acid	BSTR	*Candida cylindracea*	Precipitation	E-(vdW)-E		72
Alcoholysis	Butyl laurate, ethyl propionate & propanol, heptanol, nonanol	BSTR	*Candida cylindracea*	Precipitation	E-(vdW)-E		72
Alcoholysis	Menthol & tributyrin; menthol & triacetin	BSTR	*Candida cylindracea, Mucor miehei*	Cross-linking	E-(cf)-ga-(cf)-E	Cellulose, polyethylene	73
Alcoholysis	Menthol & tributyrin; menthol & triacetin	BSTR	*Candida cylindracea*	Adsorption	C-(vdW)-E	Polypropylene	73
Hydrolysis (isooctane)	Olive oil & water	BSTR	*Candida rugosa*	Containment	C-(vdW)-E	AOT-RM	74
Alcoholysis (hexane)	Evening primrose oil & butanol; Cod liver oil & butanol	BSTR	*Brassica napus Ceres*	Adsorption	C-(vdW)-E	Celite	75

Alcoholysis (hexane)	Evening primrose oil & butanol; Cod liver oil & butanol	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	75
Alcoholysis (water & cyclohexane)	1-Phenyl ethyl butyrate & 1-heptanol	BSTR	*Candida cylindracea*	Precipitation	E-(vdW)-E		76
Transesterification	NA	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	77
Interesterification	Butterfat	BSTR	*Pseudomonas fluorescens*	Adsorption	C-(vdW)-E	Celite	78
Interesterification	Butterfat	BSTR	*Pseudomonas fluorescens*	Adsorption	C-(vdW)-E	Celite	79
Hydrolysis (isooctane)	Olive oil & water	BSTR	Porcine pancreas	Adsorption	C-(vdW)-E	Trityl cellulose	80
Hydrolysis (isooctane)	Olive oil & water	BSTR	Porcine pancreas	Covalent binding	C-(cf)-E	Cellulose	80
Hydrolysis	Olive oil & water	PBR	Porcine pancreas	Covalent binding	C-(cf)-E	EPSPS	81
Ester synthesis	Oleic acid & *n*-butanol	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	82
Ester synthesis (decane)	Hexanol & oleic, heptanoic, and lauric acids	BSTR	*Rhizopus delemar*	Precipitation	E-(vdW)-E		83
Ester synthesis (heptane)	Acetic, propionic, butyric, valeric, and caproic acids & methanol, ethanol, butanol, 1-pentanol, hexanol, citronellol, and geraniol	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	84
Ester synthesis (heptane)	Acetic, propionic, butyric, valeric, and caproic acids & methanol, ethanol, butanol, 1-pentanol, hexanol, citronellol, and geraniol	BSTR	*Rhizopus arrhizus*	Containment	C-(vdW)-C	CWD	84
Ester synthesis	Oleic acid & *n*-butanol	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	85
Hydrolysis (isooctane)	Olive oil & water	BSTR	*Candida cylindracea, Pseudomonas fluorescens, Rhizopus arrhizus, R. niveus*	Containment	C-(vdW)-E	AOT-RM	86
Ester synthesis	Oleic acid & methanol, ethanol, propanol, butanol, pentanol, hexanol, decanol	BSTR	Achromobacter sp., *Alcaligenes* sp., *Aspergillus niger, Candida cylindracea, C. lipolytica, Chromobacterium viscosum, Geotrichum candidum, Humicola lanuginosa, Mucor javanicus, M. miehei, Penicillium cyclopium, P. roqueforti, Pseudomonas fluorescens, Rhizopus arrhizus, R. delemar, R. japonicus, R. niveus*	Precipitation	E-(vdW)-E		87
Interesterification	Trilaurin and myristic acid	PBR	*Rhizopus arrhizus*	Covalent binding	C-(cf)-ga-(cf)-E	AMDG	88
Hydrolysis (acetonitrile)	Tributyrin & water	BSTR	*Candida cylindracea*	Covalent binding	C-(cf)-E	Sepharose	89
Hydrolysis (acetonitrile)	Tributyrin & water	BSTR	*Mucor miehei, Candida antarctica*	Adsorption	C-(if)-E	Synthetic resin	89
Hydrolysis (acetonitrile)	Tributyrin & water	BSTR	*Candida cylindracea,* Porcine pancreas, *Rhizopus arrhizus, Mucor miehei, Candida antarctica*	Precipitation & adsorption	E-(vdW)-E-(vdW)-C	Celite	90
Alcoholysis	Glycerol & ethyl butyrate	BSTR	*Candida cylindracea,* porcine pancreas, *Rhizopus arrhizus, Mucor miehei, Candida antarctica*	Precipitation & adsorption	E-(vdW)-E-(vdW)-C	Celite	90

Table 1 (*continued*)

Reaction [solvent(s)]	Substrate(s)	Reactor configuration	Source of lipase	Method of immobilization	Binding agent(s)	Carrier or Barrier	Ref.
Ester synthesis (hexadecane)	Decanoic acid & glycerol	HFMR	*Candida rugosa*	Adsorption	C-(vdW)-E	Cellulose	91
Ester synthesis (toluene)	3-Benzoylthio-2-methyl propanoic acid & methanol	BSTR	*Candida cylindracea, Rhizopus* sp., *Mucor* sp., *Penicillium* sp., *Pseudomonas* sp., *Aspergillus niger, Humicola lanuginosa*	Adsorption	C-(if)-E	Synthetic resin	92
Acidolysis	Olive oil & octanoic acid	BSTR	*Pseudomonas cepacia*	Precipitation & adsorption	E-(vdW)-E-(vdW)-C	Polypropylene	93
Hydrolysis	Sunflower oil & water	FSMR	*Rhizopus* sp.	Entrapment	C-(cf)-C	PVC	94
Hydrolysis	Sunflower oil & water	FSMR	*Rhizopus* sp.	Adsorption	C-(vdW)-E	PVC	94
Hydrolysis	Sunflower oil & water	FSMR	*Candida cylindracea*	Cross-linking	E-(cf)-ga-(cf)-E	PTFE, Polyethylene, collagen, cellulose acetate, PVC	95
Hydrolysis	Olive oil	PBR	*Candida rugosa*	Covalent binding	E-(cf)-eda-(cf)-C	PVC	96
Hydrolysis	Olive oil	PBR	*Candida rugosa*	Covalent binding	E-(cf)-hmda-(cf)-C	PVC	96
Hydrolysis	Olive oil	PBR	*Candida rugosa*	Covalent binding	E-(cf)-dda-(cf)-C	PVC	96
Hydrolysis	Olive oil	PBR	*Candida rugosa*	Covalent binding	E-(cf)-aha-(cf)-C	Chitin	96
Hydrolysis	Olive oil	PBR	*Candida rugosa*	Covalent binding	E-(cf)-dda-(cf)-C	Agarose	96
Hydrolysis	Olive oil	PBR	*Candida rugosa*	Covalent binding	E-(cf)-C	Chitosan	96
Hydrolysis	Olive oil	PBR	*Candida rugosa*	Covalent binding	E-(cf)-C	Sepharose	96
Hydrolysis	Olive oil	PBR	*Candida rugosa*	Covalent binding	E-(cf)-C	Trisacryl	96
Acidolysis (toluene)	Phosphatidylcholine & heptadecanoic acid	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	97
Acidolysis (toluene)	Phosphatidylcholine & heptadecanoic acid	BSTR	*Rhizopus arrhizus,* potato tubers, *Candida cylindracea,* porcine pancreas	Adsorption	C-(vdW)-E	Celite	97
Hydrolysis	Olive oil, beef tallow, & water	FSMR	*Thermomyces lanuginosus*	Adsorption	C-(vdW)-E	NA	98
Esterification (methylene, hexane, octane, decane)	Butanol, ethanol, i-pentanol, butyric acid, & acetic acid	BSTR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	99
Ester synthesis (benzene)	15-Hydroxypentadecanoic acid	BSTR	*Pseudomonas fluorescens*	Adsorption	C-(vdW)-E	Celite	100
Ester synthesis (*n*-hexane, *n*-heptane, *n*-dodecane)	Butyric acid & ethanol	BSTR	*Candida rugosa*	Entrapment	C-(cf)-C	Polyurethane	101
Ester synthesis (t-amylic alcohol)	Glycerol & oleic acid	PBR	*Rhizopus arrhizus*	Containment	C-(vdW)-C	CWD	102
Hydrolysis	Butterfat & water	BSTR	*Aspergillus niger*	Precipitation	E-(vdW)-E		103
Acidolysis	Cod liver oil & ω3-PUFA	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	104
Transesterification (isooctane)	Trioctanoylglycerol & trihexadecanoylglycerol	BSTR	*Pseudomonas fluorescens*	Adsorption	C-(vdW)-E	Celite	105
Ester synthesis	Capric acid & glycerol	BSTR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	106
Transesterification (hexane)	Olive oil & methyl stearate	PBR	*Rhizopus chinensis*	Containment	C-(vdW)-E	BSP	107
Hydrolysis	Butterfat & water	FSMR	*Aspergillus niger*	Adsorption	C-(vdW)-E	Polypropylene	11
Ester synthesis	Propionic, butyric, hexanoic acids & ethyl, hexyl alcohols	BSTR	*Mucor miehei*	Adsorption	C-(vdW)-E	Celite	108

Ester synthesis	Propionic, butyric, hexanoic acids, & ethyl, hexyl alcohols	BSTR	*Mucor miehei*	Covalent binding	C-(cf)-E	Nylon	108
Alcoholysis	Glycerol & beef tallow, lard, milk fat, palm oil, palm olein, palm stearin, coconut oil, rapeseed oil, olive oil, corn oil, hydrogenated tallow, hydrogenated lard	BSTR	*Pseudomonas fluorescens*	Precipitation	E-(vdW)-E		109
Alcoholysis	Glycerol & palm oil, stearin	BSTR	*Pseudomonas fluorescens, Chromobacterium viscosum, Mucor miehei*	Precipitation	E-(vdW)-E		109
Ester synthesis (cyclohexane)	Dilaurin & lauric acid	BSTR	*Candida cylindracea*	Precipitation	E-(vdW)-E		110
Ester synthesis (hexadecane)	Decanoic acid, glycerol	HFMR	NA	Adsorption	C-(vdW)-E	Cellulose	111
Acidolysis (hexane)	Sunflower, safflower, soy bean, linseed oils & oleic acid	BSTR	*Candida cylindracea*	Adsorption	C-(vdW)-E	Celite	112
Transesterification (hexane)	Sunflower, safflower, soy bean, linseed oils & methyl oleate	BSTR	*Candida cylindracea*	Adsorption	C-(vdW)-E	Celite	112
Acidolysis (hexane)	Sunflower, safflower, soy bean, linseed oils & oleic acid	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	112
Transesterification (hexane)	Sunflower, safflower, soy bean, linseed oils & methyl oleate	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	112
Transesterification (hexane)	Sal, kokum, mahua, dhupa, mango oils, & methyl palmitate, stearate	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	113
Hydrolysis	Edible tallow & water	FSMR	*Thermomyces lanuginosus*	Adsorption	C-(vdW)-E	Acrylic	114
Acidolysis	Oleic acid & oleic acid stearyl ester	BSTR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	115
Hydrolysis (isooctane)	Olive oil & water	BSTR	*Candida rugosa*	Containment	C-(vdW)-E	AOT-RM	116
Acidolysis (petroleum ether)	Triolein & lauric acid	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	117
Acidolysis (*n*-hexane, diethyl ether, *t*-BuOMe)	1-Monovalerin & valeric acid; 1-Monoolein & oleic acid	BSTR	*Rhizopus delemar, Chromobacterium viscosum*	Precipitation	E-(vdW)-E		118
Transesterification (*n*-hexane, diethyl ether, *t*-BuOMe)	1-Monocaprylin & vinyl caprylate	BSTR	*Rhizopus delemar*	Precipitation	E-(vdW)-E		118
Transesterification (*n*-hexane, diethyl ether, *t*-BuOMe)	1-Monolaurin & vinyl laurate	BSTR	*Rhizomucor miehei*	Adsorption	C-(if)-E	Synthetic resin	118
Acidolysis (*n*-hexane, diethyl ether, *t*-BuOMe)	1,3-Divalerin & valeric acid; 1,3-Diolein & oleic acid	BSTR	*Rhizopus delemar; Chromobacterium viscosum*	Precipitation	E-(vdW)-E		119

Table 1 (*continued*)

Reaction [solvent(s)]	Substrate(s)	Reactor configuration	Source of lipase	Method of immobilization	Binding agent(s)	Carrier or Barrier	Ref.
Transesterification (*n*-hexane, diethyl ether, *t*-BuOMe)	1,3-Dicaprylin & vinyl caprylate	BSTR	*Rhizopus delemar*	Precipitation	E-(vdW)-E		119
Transesterification (*n*-hexane, diethyl ether, *t*-BuOMe)	1,3-Dicaprin & vinyl caprinate; 1,3-Dilaurin & vinyl laurate; 1,3-Dimyristin & vinyl myristate; 1,3-Dipalmitin & vinyl palmitate; 1,3-Distearin & vinyl stearate	BSTR	*Rhizomucor miehei*	Adsorption	C-(if)-E	Synthetic resin	119
Acidolysis (*n*-hexane, diethyl ether, *t*-BuOMe)	1,3-Dipentadecanoin & pentadecanoic acid	BSTR	*Rhizomucor miehei*	Adsorption	C-(if)-E	Synthetic resin	119
Ester synthesis (benzene)	Dodecanoic alcohol & dodecanoic acid	BSTR	Porcine pancreas	Adsorption	C-(vdW)-E	Glass, Kieselguhr, Al_2O_3, agar	120–121
Transesterification (benzene)	Butyl octadycylate & dodecanoic alcohol	BSTR	Porcine pancreas	Adsorption	C-(vdW)-E	Glass, Kieselguhr, Al_2O_3, agar	120–121
Hydrolysis	Olive oil, milk fat, & water	CSTR	*Rhizopus delemar*	Containment	C-(vdW)-E	BSP, polyurethane	122
Hydrolysis	Milk fat & water	BSTR	*Candida cylindracea, Rhizopus arrhizus*	Entrapment	C-(cf)-C	ENT, ENTP	123
Hydrolysis	Olive oil	RDRMR	*Candida rugosa*	Containment	C-(vdW)-E	AOT-RM	124
Alcoholysis (*n*-hexane)	Geraniol & methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *tert*-butyl, amyl, and octyl acetates	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	125
Acidolysis	Stearic acid & palm olein	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	126
Interesterification	Milk fat	BSTR	NA	Precipitation & adsorption	E-(vdW)-E-(vdW)-C	Celite	127
Deacidification	Hyperacid acid oil	BSTR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	128
Transesterification	Tallow & rapeseed oil	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	129
Hydrolysis	Milk fat & water	FSMR	*Candida rugosa*	Adsorption	C-(vdW)-E	Polypropylene	130
Hydrolysis	Castor oil & water	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	131
Acidolysis	Calf fat & isostearic acid	BSTR	*Chromobacterium viscosum, Rhizopus arrhizus, Candida cylindracea*	Adsorption	C-(vdW)-E	Polypropylene	132
Transesterification	Beef tallow & castor oil	BSTR	*Chromobacterium viscosum, Rhizopus arrhizus, Candida cylindracea*	Adsorption	C-(vdW)-E	Polypropylene, Duolite	132
Acidolysis	Calf fat & isostearic acid	BSTR	*Rhizopus arrhizus*	Adsorption	C-(vdW)-E	Polypropylene, Duolite	132
Transesterification	Beef tallow & castor oil	BSTR	*Rhizopus arrhizus*	Adsorption	C-(vdW)-E	Polypropylene, Duolite	132
Acidolysis	Calf fat & isostearic acid	BSTR	*Geotrichum candidum*	Adsorption	C-(if)-E	Synthetic resin	132
Transesterification	Beef tallow & castor oil	BSTR	*Geotrichum candidum*	Adsorption	C-(if)-E	Synthetic resin	132
Ester synthesis	Cod liver fatty acids & glycerol	BSTR	*Mucor miehei, Candida antarctica*	Adsorption	C-(if)-E	Synthetic resin	133
Transesterification	Cod liver oil ethyl esters & tributyrin	BSTR	*Mucor miehei, Candida antarctica*	Adsorption	C-(if)-E	Synthetic resin	133
Ester synthesis (isooctane)	Ethylene glycol & lauric acid	BSTR	*Rhizopus delemar*	Containment	C-(vdW)-E	AOT-RM	134

Ester synthesis (*n*-hexane)	Butanoic acid & *n*-butanol	BSTR	*Candida cylindracea*	Entrapment	C-(cf)-C	Sodium alginate	135
Alcoholysis (*n*-hexane)	Octyl-2-bromopropanoate & ethanol; ethyl-2-bromopropanoate & octanol	BSTR	*Candida cylindracea*	Entrapment	C-(cf)-C	Sodium alginate	135
Hydrolysis (*n*-hexane)	Butyl butanoate & water	BSTR	*Candida cylindracea*	Entrapment	C-(cf)-C	Sodium alginate	135
Interesterification (*n*-hexane)	Olive oil & methyl stearate	BSTR	*Rhizopus chinensis*	Containment	C-(vdW)-E	BSP	18
Ester synthesis (hexane)	Octanol & acids from C_6 to C_{18}	BSTR	*Candida antarctica*	NA	NA	NA	136
Interesterification	High free fatty acid rice bran oil	BSTR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	137
Hydrolysis	Butterfat & water	HFMR	*Aspergillus niger*	Adsorption	C-(vdW)-E	Polypropylene	138–141
Transesterification	Olive oil & methyl stearate	BSTR	*Rhizopus chinensis*	Containment	C-(vdW)-E	BSP	142
Ester synthesis	Glycerol & decanoic acid	HFMR	*Candida rugosa*	Adsorption	C-(vdW)-E	Cellulose	143
Alcoholysis (nitrogen)	Methyl propionate, ethyl propionate & butanol, pentanol, hexanol	PBR	Porcine pancreas & *Fusarium solani*	Precipitation	C-(vdW)-E		144
Ester synthesis	1,2-Isopropylidene glycerol & oleic acid	BSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	145
Hydrolysis (isooctane)	Olive oil & water	CSTR	*Chromobacterium viscosum*	Containment	C-(vdW)-E	AOT-RM	146
Ester synthesis (*n*-hexane)	Ethanol & acetic acid	CSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	147
Hydrolysis	Tallow & water	FSMR	*Thermomyces lanuginosus*	Adsorption	C-(vdW)-E	NA	148
Ester synthesis (hexane)	Dodecanol & decanoic acid	BSTR	*Rhizomucor miehei, Rhizopus niveus, Humicola* sp., *Candida rugosa, Ps. cepacia*	Adsorption	C-(vdW)-E	Polypropylene	149
Ester synthesis (hexane)	Dodecanol & decanoic acid	BSTR	*Rhizomucor miehei, Rhizopus niveus, Humicola* sp., *Candida rugosa, Ps. cepacia*	Ion exchange	C-(if)-E	Duolite	149
Alcoholysis (hexane)	Nonanol & ethyl acetate	CSTR	*Mucor miehei*	Adsorption	C-(if)-E	Synthetic resin	150
Transesterification (*S*-ethyl thiooctanoate)	Racemic alcohol [(*S*)-2-octanol] & *S*-ethyl thiooctanoate	BSTR	*Candida antarctica*	Ion exchange	C-(if)-E	NA	151
Esterification (*n*-butanol)	Lauric acid & *n*-butanol	BSTR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	152
Transesterification	Butterfat	BSTR	*Pseudomonas fluorescens*	Adsorption	C-(vdW)-E	Celite	154–15
Esterification (pentane, hexane, heptane, isooctane, decane, benzene, acetone, chloroform)	Glycerol & highly polyunsaturated ω-3 fatty acids concentrate from cod liver oil	BSTR	*Candida cylindracea, Geotrichum candidum, Pseudomonas* sp., *Penicillium* sp., porcine pancreas, *Mucor miehei, Mucor* sp.	Precipitation	E-(vdW)-E		157
Hydrolysis	Butterfat & water	HFMR	*Aspergillus niger*	Adsorption	C-(vdW)-E	Polypropylene	158,15
Interesterification (hexane)	Triolein & tripalmitin	BSTR	*Rhizopus arrhizus*	Containment	C-(vdW)-E	AIL-RM	160
Acidolysis (hexane)	Triolein & lauric acid	BSTR	*Candida cylindracea, Aspergillus niger, Pseudomonas fluorescens*	Adsorption	C-(vdW)-E	Diatomaceous earth	161
Acidolysis (hexane)	Triolein & lauric acid	BSTR	*Candida cylindracea, Aspergillus niger, Pseudomonas fluorescens*	Adsorption	C-(if)-E	Resin	161

Table 1 *(continued)*

Reaction [solvent(s)]	Substrate(s)	Reactor configuration	Source of lipase	Method of immobilization	Binding agent(s)	Carrier or Barrier	Ref.
Ester synthesis (hexane)	Lauric acid & methanol, ethanol, amyl alcohol	BSTR	*Candida cylindracea, Aspergillus niger, Pseudomonas fluorescens*	Adsorption	C-(vdW)-E	Diatomaceous earth	161
Ester synthesis (hexane)	Lauric acid & methanol, ethanol, amyl alcohol	BSTR	*Candida cylindracea, Aspergillus niger, Pseudomonas fluorescens*	Adsorption	C-(if)-E	Resin	161
Esterification	Octanol & octanoic acid	BSTR	*Rhizomucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	162
Hydrolysis	Triolein	BSTR	*Rhizomucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	162
Interesterification (hexane)	Butteroil & polyunsaturated fatty acid concentrate with 39% EPA and 28% DHA	BSTR	*Rhizomucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	163
Interesterification (*n*-hexane)	ω-3 PUFA concentrate from cod liver oil & corn oil, soybean oil, rape oil, olive oil, peanut oil, sunflower oil	BSTR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	164
Interesterification (*n*-hexane)	Palm oil midfraction & stearic acid	BSTR	*Rhizopus arrhizus*	Adsorption	C-(vdW)-E	Celite	165
Interesterification	Canola oil & palm oil Palm oil & soybean oil	BSTR	*Rhizopus delemar*	Adsorption	C-(vdW)-E	Celite	166
Interesterification	Tallow and butterfat & high-oleic sunflower oil and soybean oil	BSTR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	167
Interesterification	Triolein & stearic acid	BSTR	*Mucor miehei, Aspergillus niger, Humicola lanuginosa, Rhizopus delemar, Rhizopus javanicus, Rhizopus niveus, Penicillium roquefortii*	Cross-linking	E-(cf)-ga-(cf)-E	Florisil mesh	168
Transesterification	Rapeseed oil & lauric acid	PBR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	169
Hydrolysis (isooctane)	Lesquerella fendleri oil	BSTR	*Rhizopus arrizus*	Containment	C-(vdW)-E	AOT-RM	170
			Rhizomucor miehei	Ion exchange & containment	C-(if)-E & C-(vdW)-E	Synthetic resin inside AOT-RM	
			Rhizomucor miehei	Ion exchange	C-(if)-E	Synthetic resin	
			Rhizomucor miehei	Precipitation	E-(vdW)-E		
Alcoholysis	Castor oil & *n*-butanol Coconut oil & *n*-butanol	BSTR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	171
Acidolysis	Cod liver oil & ω-3 PUFA-enriched free fatty acid	PBR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	172
Hydrolysis	Butterfat	HFMR	*Aspergillus niger*	Adsorption	C-(vdW)-E	Polypropylene	173
Esterification	Glycerol & EPA, DHA	BSTR	*Candida antarctica*	NA	NA	NA	174
Interesterification	Tributyrin & EPA, DHA	BSTR					
Hydrolysis	Olive oil, (*R,S*)-cyanomethyl ester of 2-(4-isobutylphenyl)propionic acid	HFMR	*Candida cylindracea*	Containment	C-(vdW)-E	Polyamide	175
Hydrolysis	Decylchloroacetate	BSTR	*Pseudomonas fluorescens*	Adsorption	C-(vdW)-E	Decylchloroacetate emulsion	176
Transesterification	Methyl hexanoate & rac-1-phenyl ethanol	BSTR	*Rhizomucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	178
Transesterification	Lecithin & ethanol, 2-propanol, 1-butanol	BSTR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	179

Transesterification	Phosphatidylcholine dipalmitoyl & ethanol	BSTR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	179
Transesterification	Soybean lecithin & ethanol	PBR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	179
Interesterification	Olive oil & lauric acid	PBR	*Rhizomucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	180
Interesterification	Milkfat	BSTR	*Candida rugosa*	Adsorption	C-(vdW)-E	Silica gel	181
Hydrolysis	Tuna oil	BSTR	*Candida cylindracea*	Precipitation	E-(vdW)-E		183
Interesterification	Trioleoylglycerol & linoleic acid, linolenic acid, DHA, tridocosahexaenoyl glycerol, & oleic acid, linoleic acid, linolenic acid	BSTR	*Chromobacterium viscosum*	Precipitation & adsorption	E-(vdW)-E-(vdW)-C	Celite	183
Esterification (1,1,1-trichloroethane)	Farnesylacetic acid & geraniol	BSTR	*Pseudomonas fragii*	Precipitation	E-(cf)-peg-(vdW)-E		184
Esterification	Rice bran oil & glycerol	FBR	*Rhizomucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	185
Esterification (hexane)	Propylene glycol & ethyl laurate, ethyl myristate, ethyl palmitate, ethyl stearate, ethyl oleate, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, lauric anhydride, myristic anhydride, palmitic anhydride, stearic anhydride, oleic anhydride	BSTR	*Pseudomonas* sp.	Precipitation	E-(vdW)-E		186
Esterification (*n*-hexane)	Decanol & 9(10),12(13)-dihydroxyoctadecanoic acid methyl ester	BSTR	*Rhizomucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	187
Hydrolysis (hexane)	Anchovy oil, menhaden oil	BSTR	*Pseudomonas fluorescens*	Precipitation	E-(vdW)-E		188
Interesterification (*n*-hexane)	Whale oil, sardine oil, cod liver oil, skipjack oil	BSTR	*Chromobacterium viscosum, Pseudomonas fluorescens*	Precipitation	E-(vdW)-E		189
Hydrolysis (2-hexanone, octanone, hexane, isooctane, decane)	Phosphatidylcholine	BSTR	*Candida rugosa, Humicola lanuginosa, Rhizopus delemar, Rhizopus niveus, Rhizopus javanicus, Penicillium cyclopium, Pseudomonas* sp., calf pregastric	Adsorption	C-(vdW)-E	Celite, polypropylene, glass, amberlite	190
Esterification (*n*-hexane)	Fatty acids of borage seed oil or evening primrose oil & *n*-butanol	BSTR	*Rhizomucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	191
Hydrolysis	Borage seed oil or evening primrose oil	BSTR	*Candida cylindracea*	Precipitation	E-(vdW)-E		192
Esterification (*n*-hexane)	Geraniol & acetic acid	BSTR	*Candida antarctica*	Ion exchange	C-(if)-E	Synthetic resin	193
Acidolysis	Babassu fat & palmitic acid	BSTR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	194
Esterification	Glycerol & oleic acid, stearic acid	BSTR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	195
Glycerolysis	Glycerol & hydrogenated beef tallow	BSTR	*Pseudomonas* sp.	Precipitation	E-(vdW)-E		196
Interesterification (isooctane)	Milkfat & oleic acid	BSTR	*Rhizopus oryzae*	Adsorption	C-(vdW)-E	Glass	197
Interesterification	Milkfat & octanoic acid, linolenic acid	BSTR	*Pseudomonas cepacia*	Precipitation & adsorption	E-(vdW)-E-(vdW)-C	Polypropylene	198

Table 1 *(continued)*

Reaction [solvent(s)]	Substrate(s)	Reactor configuration	Source of lipase	Method of immobilization	Binding agent(s)	Carrier or Barrier	Ref.
Transesterification (benzene/pyridine)	Methyl glucoside, methyl galactoside, octyl glucoside, & methyl oleate, EPA, DHA	BSTR	*Candida antarctica, Mucor miehei, Candida cylindracea*	NA	NA	NA	199
Hydrolysis (isooctane, *n*-heptane, *n*-pentane, isopropanol, ethyl ether)	Beef tallow, inedible pork lard, Edible pork lard, olive oil	BSTR	*Candida rugosa*	Adsorption	C-(vdW)-E	Polypropylene	200
Alcoholysis (methyl-*tert*-butyl ether, diisopropyl ether, hexane, isooctane, methyl isobutyl ketone, toluene)	Tripalmitin & ethanol; triolein, trilaurin & ethanol	BSTR	*Rhizopus arrhizus*	Adsorption	C-(vdW)-E	Celite	201
Esterification (supercritical carbon dioxide)	Glycidol & butyric acid	CSTR	Porcine pancreas	Adsorption	C-(vdW)-E	Celite, aminopropyl glass beads, silanized glass beads, acid-washed glass beads	202
Alcoholysis	Mutton tallow & butanol, methanol, ethanol, 1-propanol, 2-propanol, 1-dodecanol, cyclohexyl methanol, butane-1,4-diol, 2-fluoroethanol; butanol & beef tallow, butterfat, borage seed oil, hoki liver oil, olive oil, corn oil, palm oil, peanut oil, tripalmitin	BSTR, PBR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	203
Esterification (toluene, benzene)	16-hydroxyhexadecanoic acid (juniperic acid)	BSTR, PBR	*Candida antarctica*	NA	NA	NA	204
Esterification (*n*-hexane, petroleum ether, isooctane, cyclohexane, pentane, toluene, benzene, diethyl ether, tetrahydrofuran, acetonitrile)	Geraniol, citronellol, & acetic acid	BSTR	*Candida antarctica*	Ion exchange	C-(if)-E	Acrylic resin	205
Esterification	Ethanol & acetic acid	PBR	*Mucor miehei*	Ion exchange	C-(if)-E	Synthetic resin	206

NA, Not available

is relatively low because of dead times taken up by the operations of emptying, cleaning, and filling, and so economic considerations constrain their large scale use.

Because of their high efficiency, low cost and ease of construction, operation and maintenance, PBRs, also known as fixed-bed reactors, have traditionally been used for most large-scale catalytic reactions. In these reactors, the granules of immobilized enzyme are usually packed within a jacketed thermostatted pipe or column, thus providing a large surface area per unit volume of reaction. In the presence of a single phase, pumping may be made upward (to avoid extensive bypassing) or downward (to take advantage of gravitational forces), whereas in a biphase situation the two phases may be pumped in opposite directions (countercurrent flows) with the most dense flowing downward, or in the same direction (cocurrent flows). Great pressure drops are normally associated with this kind of reactor, especially if diffusional limitations are to be alleviated by decreasing the mesh size of the bed granules.

In CSTRs, no gradients of temperature or concentration exist, because of the efficient stirring which promotes intimate contact of the enzyme with the reaction mixture. The immobilized enzyme is retained within the reactor by means of a filter at the outlet. Low costs of construction are normally associated with these reactors, but to achieve similar degrees of conversion, a CSTR must be larger than a PBR, or a cascade should be used instead of a single unit (with concomitant problems of layout space requirements).

An FBR is, in a sense, a hybrid of a CSTR and a PBR, for which the upward linear velocity of reacting fluid is above the terminal velocity of the bed of enzyme. The usually high pump size and power requirement nevertheless prevent extensive use of this type of reactor.

Membrane or diaphragm reactors (which are in essence the linear counterpart of a PBR) may be operated with one or two liquid phases. In these reactors, the enzyme is immobilized onto the membrane, which may take either a flat sheet (FSMR) or hollow fiber (HFMR) form. Because of the role of the membrane in the segregation of two immiscible fluids, membrane reactors are commonly employed for biphasic liquid systems. Prevention and elimination of membrane pore plugging is considerably more difficult than packed-bed plugging in a PBR, but the lower pressure drops for a given specific area of reaction usually compensate for such drawback.

In what concerns the flow of the reacting fluid from the point of view of the solid carrier or barrier, a combination of two or more of the following four extreme situations arises: 1) tangential flow or 2) normal flow (which occur usually with membranes in either FSMRs or HFMRs), and 3) contour flow and 4) random flow (which are typical of particulate solids as in PBRs). If two liquid phases are flown tangentially, their relative direction of motion may also be classified as cocurrent or countercurrent. The tangential flow occurs in membrane reactors operated continuously, or operated batchwise under total recycle conditions; the normal flow corresponds to bulk flow of the reaction system through the carrier or the barrier, and again is typical of membrane reactors; the random flow encompasses BSTRs and CSTRs, as well as FBRs; and the contour flow is found in PBRs with or without recycle. The pumping requirements increase usually in the order of: tangential flow, normal flow, contour flow, and random flow; preselection of one of these types of flow usually results from a compromise between the high activity of immobilized enzyme (available surface area for immobilization is considerably different for the various types of flows described) and low pressure drops.

An inspection of *Table 1* indicates that among all immobilization protocols, adsorption has been clearly elected by most researchers. 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 realization. Examples of physicochemical parameters able to describe adsorbed lipases are given in *Tables 2* and *3*. The model entertained there is the classical Langmuir adsorption isotherm, $C_{lip,ads} = C_{lip,ads,max}K_{lip}C_{lip,free}/(1 + K_{lip}C_{lip,free})$, where $C_{lip,ads}$ is the concentration of lipase adsorbed, $C_{lip,free}$ is the concentration of lipase in the bulk of the supernatant solution, K_{lip} is an adsorption equilibrium constant, and $C_{lip,ads,max}$ is the maximum concentration of lipase that can ever be adsorbed. It has been shown[7] that this model provides accurate (empiric) fittings to adsorption data of several enzymes even though the theoretical assumptions underlying it are not at all met by proteins. The temperature dependence of K_{lip} can be expressed via van't Hoff's relationship in view of its (tentative) nature of an equilibrium constant, viz. $K_{lip} = \beta\exp\{-\Delta h_{lip,ads}/RT\}$, where β is a preexponential constant, R is the ideal gas constant, T denotes the absolute temperature, and $\Delta h_{lip,ads}$ represents the standard enthalpy change accompanying the adsorption of lipase onto the membrane surface. The values suggested for $\Delta h_{lip,ads}$[7] were 5.3 kJ mol^{-1} (for the overall protein) and 20.5 kJ mol^{-1} (for the lipase), both of which

Table 2 Maximum coverage for adsorption of lipase

Saturation coverage

Values	Units	Temp. (°C)	pH	Ref.
38,000	$Å^2_{molecule}{}^{-1}$	35	7.0	11
50	$g_{support}\ g_{protein}{}^{-1}$	~20	NA	26
4.643	$m^2_{support}\ g_{protein}{}^{-1}$	30	NA	40
111	$m^2_{support}\ g_{protein}{}^{-1}$	30	7.0	57
82–103	$m^2_{support}\ g_{protein}{}^{-1}$	37	8.0	64
2.5	$g_{support}\ g_{protein}{}^{-1}$	NA	NA	79
50	$g_{support}\ g_{protein}{}^{-1}$	25	9.0	80
5	$g_{support}\ g_{protein}{}^{-1}$	~15	7.2	89
294.1–357.1	$g_{support}\ g_{protein}{}^{-1}$	NA	8.0	94
3.95–41.7	$m^2_{support}\ g_{protein}{}^{-1}$	NA	8.0	95
10	$g_{support}\ g_{protein}{}^{-1}$	NA	8.0	97
250	$m^2_{support}\ g_{protein}{}^{-1}$	25	NA	111
20,030	$Å^2_{molecule}{}^{-1}$	45	7.0	130
60,000–80,000	$Å^2_{molecule}{}^{-1}$	20–45	7.0	139
1,700–2,100	$Å^2_{molecule}{}^{-1}$	25	8.0	176

NA, Not available

Table 3 Equilibrium constant for adsorption of lipase

K_{eq} (M)	Temp. (°C)	pH	Ref.
0.00000070	35	7.0	11
0.00000103–0.00000122	20–45	7.0	139

compare well with data obtained for other proteins,[8] whereas the values found for β were $8.17 \cdot 10^5$ and $9.70 \cdot 10^5$ $dm^3\ mol^{-1}$, respectively, also similar to the values found for hog pancreas lipase immobilized on Sepharose[9] and on siliconized glass.[10] Although there is no special prerequisite for selecting a particular functional form to describe the variation of the saturation coverage parameter ($C_{lip,ads,max}$) with temperature, Malcata *et al.*[7] tested three alternative empirical approaches for correlating this parameter and concluded that $C_{lip,ads,max} = \alpha\exp\{-\Delta h_{lip,sit}/RT\}$, where α and $\Delta h_{lip,sit}$ are constants, provided the best fit. The results in *Tables 2* and *3* are useful for the prediction of the amount of lipase that can be adsorbed onto a given support, which in principle correlates with the lipolytic activity that will be available.

The principle of reversibility in adsorption of lipase has been questioned based on the observation that desorption of the lipase into plain aqueous buffer does not occur significantly. It has been claimed[11] that during the adsorption process, the protein molecules which are essentially folded in the beginning and thus able to contact the surface at only one site (or, at most, at a very limited number of sites) begin slowly to unfold upon contact, thus allowing more of their surface to come into contact with the support and consequently creating a multiplicity of binding sites; although breakage of contact at a single site is quite feasible, desorption via simultaneous detachment of the protein segments from all binding sites is an extremely improbable event. This pseudoadsorption process is assumed to basically consist of a temporary reversible alteration of the lipase conformation upon change of the nature of the microenvironment with concomitant lowering of the total Gibbs' free energy of the system (as required for a spontaneous process), which eventually becomes irreversible; the forward process (that is, adsorption) is expected to be largely favored when compared with the reverse process (that is, desorption) in the case of lipases, as these enzymes are especially hydrophobic. However, proteins may desorb readily upon addition of a component possessing larger free energy of adsorption (e.g., another protein), which will compete for binding sites with the already bound protein, or of a solvent possessing both hydrophilic and hydrophobic character (e.g., ethanol).

In terms of the design of immobilized lipase reactors, consideration of the time scales associated with the physicochemical processes in question should be made. Three major types of processes are to be considered: 1) transport of the reactant (product) molecules of reactants from (to) the bulk of the reaction system to (from) the vicinity of the immobilized lipase, 2) transformation of the reactant molecules by chemical reaction catalyzed by the immobilized lipase, and 3) deactivation of the immobilized lipase. The order of magnitude analyses, based on the assumptions that these processes are essentially diffusion-driven (with diffusivity D and molecular path L), and described by Michaelis–Menten kinetics (with maximum rate v_{max}) and first-order decay (with rate constant k_d), respectively, indicate that good estimates of the time scales associated therewith are given by L^2/D, C_0/v_{max}, and $1/k_d$, respectively, where C_0 is the initial concentration of limiting substrate. If $L^2/D \ll C_0/v_{max} \ll 1/k_d$, then the process is kinetically controlled; if $C_0/v_{max} \ll L^2/D \ll 1/k_d$, then the process is diffusion controlled; if $1/k_d \ll C_0/v_{max}$, L^2/D, then the process is deactivation controlled. Knowledge of the controlling factor al-

Table 4 Total number of independent chemical reactions naturally catalyzed by lipases (first row) and type of such reactions (second row)[a]

	Glycerol	Monoglyceride	Diglyceride	Triglyceride
Water		3 N (hydrolysis)	6 N^2 (hydrolysis)	3 N^3 (hydrolysis)
Fatty acid	3 N (ester synthesis)	6 N^2 (acidolysis)	3 N^3 (acidolysis)	3 N^4 – 3 N^3 (acidolysis)
Glycerol		6 N (alcoholysis)	18 N^2 – 6 N (alcoholysis)	9 N^3 (alcoholysis)
Monoglyceride		15 N^2 (alcoholysis)	45 N^3 – 45 N^2 + 78 N (alcoholysis)	18 N^4 – 12 N (alcoholysis)
		36 N^2 – 6 N (transesterification)	99 N^3 + 9 N^2 – 36 N (transesterification)	18 N^4 + 21 N^3 – 72 N^2 + 66 N (transesterification)
Diglyceride			24 N^4 – 24 N^2 – 4 N – 14 (alcoholysis)	9 N^5 – 12 N (alcoholysis)
			72 N^4 + 44 N^2 – 236 N (transesterification)	36 N^5 – 108 N^2 (transesterification)
Triglyceride				(18 N^8 – 18 N^7 + 81 N^4 – 215 N^2 – 150 N – 16)/4 (transesterification)

[a] N denotes the total number of different fatty acid, or acyl, moieties (either in free or bound form) in the reaction system under consideration. (An independent chemical reaction is assumed to consist on the chemical displacement of one acyl moiety or the chemical exchange of two acyl moieties; any molecular event that leads to the formation of products which are analogous to the reactants is not considered to be an independent chemical reaction.)

Table 5 Half-lives for reactions catalyzed by immobilized lipases

$t_{1/2}$ (h)	Temp. (°C)	pH	Ref.
477	35	7.0	11
7.0	45	NA	21
110–5,680	35–40	7.0	23
912	NA	NA	37
552	60	NA	39
1,032	30	6.0	40
240–480	40	NA	62
26.4–180	37	8.0	64
0.12	50	9.0	80
33–138	20–70	NA	82
120–768	37	8.0	94
400	30	7.0	96
1,200	40	NA	107
1,200	40	NA	143
480	40	NA	153

NA, Not available

lows one to devise ways to improve the kinetic performance of the reactor in an educated fashion. Data and discussion pertaining to these parameters and to the rate expressions containing them which describe reactions catalyzed by immobilized lipases are presented in the next section.

Activity, stability, optimum operating conditions, and economic assessments

The number of reactions catalyzed by lipases increases rapidly as the number of existing fatty acid moieties increases (remember that oils and fats of natural origin are complex feedstocks with several fatty acid residues). If one considers only the reactions naturally catalyzed by lipases, i.e., the breakdown of tri-, di-, and monoglycerides by hydrolysis; the esterification of glycerol, mono-, and diglycerides with fatty acids; and the various combinations of these reactions falling under the denomination of interesterifications, and if the different fatty acid moieties susceptible to lipase catalytic action number up to N, then the total number of possible independent chemical reactions is shown in *Table 4*. Such a number is paramount for as few moieties as 10. For this reason, most attempts to develop accurate rate expressions have been based either on experimental data encompassing virtually pure reactants coupled with (complex) rate expressions, or on lumped experimental data obtained for natural (complex) systems coupled with simplified lumped rate expressions. Detailed kinetic mechanisms and associated rate expressions depart from the scope of this communication, where only parameter estimates based on such simplifications as the assumption of first-order behavior for the lipase deactivation reactions and the assumption of Michaelis–Menten behavior for the lipase-catalyzed reactions are considered. Lists of the parameters associated with those two mechanisms for immobilized lipases are given in *Tables 5* and *6*.

The activation energies associated with v_{max} and k_d are listed in *Table 7* under the assumption that both parameters follow the (empirical) Arrhenius law; as expected, the activation energies for the deactivation of lipases are in general considerably higher than those for lipase-catalyzed reactions, but within the usual range for hydrolases (from 65.7 to 615.0 kJ · mol^{-1} [12,13]). When one compares the values of

Table 6 (Apparent) values for v_{max} and K_m for reactions catalyzed by immobilized lipases

v_{max}					
Values	Units	K_m (M)	Temp. (°C)	pH	Ref.
0.06476	$\mu mol_{product}\ (min \cdot cm^2_{support})^{-1}$	0.141	35	7.0	11
2.0–25.0	$\mu mol_{product}\ (min \cdot mg_{protein})^{-1}$	0.05–0.033	25	NA	35
0.005–2.29	$g_{product}\ (l \cdot h)^{-1}$	NA	30	8.0	44
0.04	$g_{product}\ (l \cdot h)^{-1}$	NA	30	7.0	44
0.68	$g_{product}\ (l \cdot h)^{-1}$	NA	30	6.0	44
0.001–15.2	$g_{product}\ (l \cdot h)^{-1}$	NA	50	8.0	44
0.003	$g_{product}\ (l \cdot h)^{-1}$	NA	50	7.0	44
0.006–0.034	$g_{product}\ (l \cdot h)^{-1}$	NA	50	6.0	44
0.305–0.420	$\mu mol_{product}\ (min \cdot mg_{protein})^{-1}$	0.000032–0.00004	30	8.0	52
0.380–0.570	$\mu mol_{product}\ (min \cdot mg_{protein})^{-1}$	0.00004–0.000048	40	8.0	52
5.7	$\mu mol_{product}\ (min \cdot mg_{protein})^{-1}$	0.120	40	NA	69
3–182	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	0.092–0.420	30	NA	99
2.95–52.0	$\mu mol_{product}\ min^{-1}$	0.000117–0.00034	40	7.0	103
67.1	$\mu mol_{product}\ (min \cdot mg_{protein})^{-1}$	0.717	37	7.1	116
8,000,000	$\mu mol_{product}\ (min \cdot mg_{protein})^{-1}$	NA	20	NA	120
0.0142–0.0615	$\mu mol_{product}\ (min \cdot mg_{protein})^{-1}$	NA	40	NA	120
0.0248–0.167	$\mu mol_{product}\ (min \cdot mg_{protein})^{-1}$	NA	40	NA	121
67.1	$\mu mol_{product}\ (min \cdot mg_{protein})^{-1}$	0.717	37	7.1	124
5.37–7.47	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	0.058–0.128	40	NA	125
1.92–618	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	NA	NA	NA	149

NA, Not available

Table 7 Activation energies for lipase-catalyzed reactions and lipase thermal deactivation

Activation energy (kJ mol^{-1})				
Enzyme reaction	Enzyme deactivation	Temp. (°C)	pH	Ref.
15.9	NA	10–50	7.0	57
0.97	NA	0–130	NA	82
NA	114–144	40–55	8.0	96
33.5	NA	25–60	7.0	122
NA	136	45–60	NA	130
34.5	27.2	40–60	7.0	142
20.82–32.41	NA	10–70	NA	146
8.67	NA	30–70	NA	152
24.3	NA	10–65	NA	182

NA, Not available

the activation energies for lipase-catalyzed reactions with those for other immobilized hydrolases, the former seem to be somewhat lower; for lipases, activation energies range from 0.97 to 34.5 kJ mol^{-1}, whereas for other enzymes they range from 14.33 to 49.0 kJ mol^{-1}.[14,15] (It may be argued that the Michaelis–Menten mechanism, on the one hand, and the unimolecular deactivation mechanism, on the other, provide simplistic views of the reaction schemes in question; however, parameters v_{max} and K_m, on the one hand, and k_d [or, equivalently, $k_{1/2} = ln(2)/k_d$], on the other, provide consistent frameworks for the assessment of activities and stabilities irrespective of the enzyme under consideration, and have so been used for comparative kinetic assessments since the beginning of this century.)

The effect of immobilization upon the intrinsic activity of lipases is depicted in *Tables 8* and *9*. Immobilization seems either to inhibit the action of lipases or dramatically

Table 8 Catalytic activity retained upon immobilization

Activity after immobilization				
Values	Units	Temp. (°C)	pH	Ref.
0.0723	$\mu mol_{product}\ (min \cdot cm^2_{support})^{-1}$	35	NA	11
1.2–28	Specific transesterification activity	NA	NA	24
10,000–10,500	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	40	NA	27
34.2	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	60	NA	33
0.55–3850	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	50	NA	34
0.19–69.8	Specific transesterification activity	50	NA	34
0.05–2.2	$\mu mol_{product}\ (min \cdot g_{enzyme})^{-1}$	24	NA	36
4,800–5,000	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	50	NA	37
25,000,000–181,000,000	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	70	NA	45
128	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	30	NA	57
2,950	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	40	NA	62
0.1472	$\mu mol_{product}\ (min \cdot cm^2_{support})^{-1}$	25	8	64
0.1283	$\mu mol_{product}\ (min \cdot cm^2_{support})^{-1}$	25	10.0	64
0.1263	$\mu mol_{product}\ (min \cdot cm^2_{support})^{-1}$	37	8.0	64
0.1253	$\mu mol_{product}\ (min \cdot cm^2_{support})^{-1}$	37	10.0	64
0.1390	$\mu mol_{product}\ (min \cdot cm^2_{support})^{-1}$	60	8.0	64
0.1716	$\mu mol_{product}\ (min \cdot cm^2_{support})^{-1}$	60	10.0	64
200	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	40	NA	69
8,000	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	40	NA	81
23	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	20–70	NA	82
23	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	20–70	NA	85
0.021	$\mu mol_{product}\ (min \cdot cm^2_{support})^{-1}$	25	NA	91
1,000–1,700	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	37	8.0	95
2.29–21.79	$\mu mol_{product}\ (min \cdot ml_{gel})^{-1}$	NA	7.0	96
0.00000389–0.0000705	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	40	NA	97
75–200	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	30	NA	102
31–400	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	40	NA	106
78–2,100	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	40	NA	108
0–24.8	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	40	NA	121
10–2,200	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	60	NA	132
27,300	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	80	NA	136
30	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	NA	NA	137
23	$\mu mol_{product}\ (min \cdot g_{support})^{-1}$	10–70	NA	146
25	BIU g^{-1}	30	NA	152
1,150–4,500	BIU g^{-1}	40	NA	153
500–650	$\mu mol_{product}\ (min \cdot g_{enzyme})^{-1}$	20	NA	162
4,530	BIU g^{-1}	40	NA	172

NA, Not available

Table 9 Fractional activity retained by lipases upon immobilization

Fractional activity retained (%)	Temp. (°C)	pH	Ref.
14.5	35	NA	11
9–107	42	NA	23
92.3–560	NA	NA	24
280–3,489,900	50	NA	34
0.9–90.7	45	7.0	38
14	30	6.0	40
132	40	NA	108
3–226	42	NA	190

NA, Not available

enhance it. However, care should be exercised to use the same basis for the reference of activity; if amount of protein is used as a basis, the selectivity of the immobilization process toward lipase when compared with other proteins in crude lipase preparations may partially account for some of the enhancement factors reported.[7]

Because lipases are proteins, their conformation is affected by both pH and temperature, and so their catalytic power is expected to exhibit optima for these two operating parameters as happens with most enzymes; this assertion is apparent from an inspection of *Tables 10* and *11*. Upon immobilization, the optimum pH for reactions catalyzed by lipases is slightly shifted toward more alkaline values. In view of the established serine-based nucleophilic attack aided by a histidine residue working as a base, this observation is expected because the (at least) partial opening of the lid upon immobilization is likely to expose the His at the active site more directly to solution hydrogen ions, and so only less acidic conditions yield the unprotonated form of

Table 10 Optimum pH for reactions catalyzed by immobilized lipases compared with those for the corresponding free lipases

Optimum pH			
Before	After	Temp. (°C)	Ref.
NA	6.62	35	11
4.0	5.0	40	23
NA	7.0	NA	28
7.0	6.8–8.0	45	38
NA	7.5–8.5	45–60	50
NA	7.0	30	56
8.0	10.0	25–60	64
10.0	10.0	37	94
7.5	8.5	37	96
5.0–7.0	5.0–5.5	35	122
NA	6.46	45	130
7.0	7.0	50–60	132
NA	7.0	40	158
NA	7.0	40	159
NA	7.0	30–50	161
8	8	25	175
NA	10	30	186

NA, Not available

Table 11 Optimum operating temperature for reactions catalyzed by immobilized lipases compared with those for the corresponding free lipases

Optimum temp. (°C)			
Before	After	pH	Ref.
51	51–61	7.0	38
NA	60	NA	41
NA	50–55	5.0–8.5	50
NA	30–35	7.0	57
37	60	8.0–10.0	64
NA	10–90	NA	82
NA	37	10.0	94
35	43–55	8.0	96
NA	50–60	NA	106
NA	40–50	NA	108
30	40	NA	122
NA	60	NA	128
37	60	NA	132
NA	55	NA	145
NA	30–60	NA	152
NA	40	7.0	158
NA	60	NA	167
NA	37	NA	168
37.5	40	8	175
NA	37	NA	182
NA	10–50	NA	186
NA	35–40	NA	193
NA	48–60	NA	196

NA, Not available

the imidazolium ring required for a basic behavior. After immobilization one can also notice a shift toward higher optimum temperature values. Increasing temperature generally increases the rate of a lipase-catalyzed reaction per unit amount of active enzyme; however, increasing temperature also leads to a higher thermal deactivation rate of the lipase itself, thus yielding decreasing amounts of active enzyme. Because immobilization provides a more rigid external backbone for lipase molecules, the effect of higher temperatures in breaking the interactions that are responsible for the proper globular, catalytically active structure becomes less notorious, and so temperature optima are expected to increase.

The optimum water levels (*Table 12*) for lipase-catalyzed reactions vary from as low as 0.042 (%v/v) to as high as 50 (%v/v); such discrepancies are related to whether net esterification or net hydrolysis is sought, respectively. Nil values are not suitable, because stripping of catalytic water from the vicinity of the lipase would not permit enzyme action; on the other hand, water levels above stoichiometry ratios do not favor the conversion extent, and also help in increasing the deactivation rate of the lipase.

A chemical catalyst such as metallic sodium, which is used to promote acyl migration among glyceride molecules, distributes the acyl residues randomly among them. By exploiting the specificity of lipases, however, it is possible to produce more useful glyceride mixtures by selective interesterification. In view of the effect of water in determining the dominant direction of a lipase-catalyzed process, maximum productivity of triglyceride and low concentration of

Table 12 Optimum water concentration for reactions catalyzed by immobilized lipases

Optimum water concentration					
Values	Units	Type of reaction	Temp. (°C)	pH	Ref.
7,100	ppm	Esterification	30	NA	37
40–50	% v/v	Hydrolysis	45	4.0–9.0	38
0–1	% v/v	Esterification	40	NA	62
10,000	ppm	Esterification	27–55	NA	69
12.5–23.3	mol%	Esterification	20–70	NA	85
448	mM	Acidolysis	40	NA	93
0.042–0.098	% v/v	Esterification	40	NA	100
100	ppm	Transesterification	40	NA	107
≤1	% v/v	Esterification	10–70	NA	108
0–10	% v/v	Esterification	40	NA	121
3	µl	Alcoholysis	40	NA	125
~100	ppm	Transesterification	40	NA	142
2.5	% v/v	Esterification	40	NA	147
0.32–0.97	a_w	Esterification	30	NA	149
100,000	ppm	Interesterification	37	NA	165
0.25	a_w	Interesterification	30–60	NA	168
350,000–400,000	ppm	Hydrolysis	23	NA	170
0.84	a_w	Alcoholysis	25	NA	177
0.002	% v/v	Hydrolysis	37	NA	182
1000	ppm	Esterification	30	NA	186
0.43	a_w	Acidolysis	25–45	NA	194
200,000–250,000	ppm	Esterification	31.1	NA	202

NA, Not available

other by-products such as diglycerides (which disturb the polymorphic state of fat crystals by softening them,[16] and therefore produce lower quality additives for confectionary products) requires accurate control of the water content in the reaction medium, and such control also leads to more predictable rates of deactivation of the lipase.[17] A feedback control system (with a hygrometer as sensor) coupled with two regulatory control systems (a feedforward/feedback controller with an on-line enzyme activity estimator and a state estimator based on a Kalman filter) was successfully employed[18] to master the glyceride composition of fats and oils during continuous interesterification. Numerical models which deal with the dynamic behavior of the water concentration are important when evaluating dynamic characteristics, optimizing outlet conversion, and designing control systems. If such models are capable of providing good representations of dynamic behaviors, then they will serve as useful aids in the development of more accurate and selective reaction systems involving immobilized lipase reactors.

Although a great many lipase-catalyzed reactions studied in the past were carried out in emulsion systems, there is an impetus to avoid use of emulsifiers because of several drawbacks for industrial processes, viz. 1) emulsions are sometimes difficult to break, and 2) replacing oil–bulk water interfaces by bound water–oil or bound water–organic solvent interfaces leads to lower deactivation rates. This statement is backed up by a careful inspection of *Table 1,* in which only few references exist encompassing the use of immobilized lipases in emulsion systems.

The discovery that lipases successfully catalyze reactions under almost anhydrous conditions in organic media (i.e., when water is eliminated except for an essential thin layer of water more or less tightly bound to the enzyme and required for maintenance of its integrity) has made the range of possible chemical reactions widen far beyond hydrolysis. However, an overview of *Table 1* indicates an increasing trend for the use of solvent-free systems to accomplish reactions catalyzed by immobilized lipases, although it is known that use of solvents lowers the viscosity of the reaction system and thus facilitates such reactions in terms of mechanical processing. It is a fact that when reactions are carried out in a solvent, the reaction product(s) has to be cleaned and deodorized for safety of human use, and these processes are likely to destroy fine flavors, as in the case of lipolyzed butterfat. However, use of apolar (hydrophobic) solvents to liquefy substrates retards deactivation of the lipase, because most water is kept away from the enzyme folded structure (except for bound water needed for maintaining the integrity of the lipase's globular structure), thus promoting a more rigid lipase conformation. When the substrates for lipase-catalyzed reactions are solid at room temperature (e.g., butterfat and tallow), the absence of solvents to liquefy them by solubilization can be replaced by increasing the temperature (to values usually ranging from 30 to 60°C) to liquefy them by melting, and this increase in temperature is expected to accelerate deactivation of the immobilized lipase. From these arguments, it is obvious that decision of whether to select a solvent to carry out lipase-catalyzed reactions must carefully take several considerations into account and balance process and product constraints.

Despite all possible applications of immobilized lipases, it seems that hydrolysis and interesterification reactions (i.e., the most ancient and classical applications of lipases) are still the ones for which lipase reactors are most commonly employed; in addition, although applications of li-

pases for low-molecular-weight acid and alcohol handling have been reported, preferred substrates are still long-chain organic compounds (see *Table 1*).

Search for lower operating costs (which depend heavily on the enzyme cost itself) has led, in the last few years, to an increase in the use of lipase reactors working on a continuous fashion although, on a laboratory scale, BSTRs are still the most appropriate choice for testing and comparing results obtained with other tentative configurations. A comparative assessment of the merits of an HFMR operated continuously for the controlled accelerated lipolysis of butterfat relative to the conventional free enzyme process[19] indicates that the membrane reactor provides a very attractive alternative to traditional batch reactor technology. The costs arising from addition of emulsifier, homogenization and stirring of butterfat, and thermal processing of the lipolyzed butterfat are nil for the immobilized lipase, whereas the costs resulting from addition of glycerol, and use of ethanol and hexane during the cleaning and regeneration step (when spent absorbed enzyme is removed and replaced with fresh lipase) are nil for the traditional process. The incremental costs associated with the immobilized lipase continuous process are more than 20 times smaller than the ones associated with the traditional one, arising primarily from the cost of the relatively large amount of lipase required by the latter process[20]; therefore, the use of lipase in an immobilized form leads to a much greater productivity. A major advantage here results from using adsorption to immobilize the lipase, because its low cost and feasibility of regeneration of the lipase activity (and consequent reuse of the support) provide clear economic benefits to the reactor technology in question.

Final remarks

The increasing availability of lipases from genetically engineered microbial sources, their intrinsic capacity to act as catalysts in both macro- and microaqueous systems coupled with their activation by oil–water interfaces, and the recent advances in the knowledge of structure–function relationships for their enzymes have contributed to a situation where lipases have generated the interest of a great number of both fundamental and applied researchers. However, although their range of potential features is very wide, their use in industrial scale is not yet very common, mainly because of the high cost normally associated with them or with supports used in immobilization procedures which are not reusable. Hence, major efforts in the near future should focus both on techniques on the genetic expression side to increase the amounts and improve the stability and activity of lipases, and on techniques on the material science side to improve the mechanical, diffusional, kinetic, and regeneration characteristics of the support.

It is expected that in the near future, the interchange of experiences and knowledge between crystallographers, biochemists, geneticists, and enzyme kineticists on the one hand, and food, chemical, and biochemical engineers on the other will create synergisms by allowing the former to learn what are the practical aims and constraints associated with industrial applications of lipases (and thus be able to design lipases to address these issues), and by allowing the latter to learn how advantage can be taken from the structural knowledge of lipases and their metabolic genesis to better design media, processes, and products in terms of biochemical, technical, and economic feasibility (and concomitantly optimize them in terms of volumetric efficiency). It is hoped that the present report will help achieve this goal.

Abbreviations

ADCPG	alkylamine derivative of controlled pore glass
AHA	aminohexanoic acid
AMDG	alkylamine derivative of glass
AOT-RM	sodium *bis*(2-ethylhexyl) sulphosuccinate reverse micelles
BE	bleaching earth
BFT	bleachable fancy tallow
BIU	batch interesterification unit
BSP	biomass support particles
BSTR	batch stirred-tank reactor
C	carrier
cf	covalent forces
CSTR	continuous flow stirred-tank reactor
CWD	cell wall debris
DDA	1,12-diaminododecane
DHA	C22:6ω3(*cis*-4,7,10,13,16,19 docosahexanoic acid)
E	enzyme
EDA	ethylene diamine
ENT	cross-linkable resin prepolymer containing polyethylene glycol
ENTP	cross-linkable resin prepolymer containing polypropylene glycol
EPA	C20:5ω3(*cis*-5,8,11,14,17 eicosapentaenoic acid)
EPSPS	epoxypropylsilanized PartiSphere-5
FBR	fluidized-bed reactor
FTMR	flow-through membrane reactor
FSMR	flat-sheet membrane reactor
ga	glutaraldehyde
HDPE	high density polyethylene
HEAR	high erucid acid rapeseed oil
HFMR	hollow-fiber membrane reactor
HMDA	hexamethylenediamine
if	ionic forces
PBR	packed-bed reactor
peg	polyethylene glycol
PTFE	polytetrafluoroethylene
PUFA	polyunsaturated fatty acids
PVC	polyvinylchloride
RDRMR	recycle dialysis reversed micellar reactor
TAS	tresyl-activated silica
vdW	van der Waals forces

Acknowledgments

The authors are grateful to JNICT (Portugal) and Institut CANDIA (France) for fellowship support and PRAXIS XXI (Portugal, Project Extractive Biocatalysis) for financial support.

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