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MICROBIAL LIPASES: AT THE INTERFACE OF AQUEOUS AND NON-AQUEOUS MEDIA

A REVIEW

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In recent times, biotechnological applications of microbial lipases in synthesis of many organic molecules have rapidly increased in non-aqueous media. Microbial lipases are the 'working horses' in biocatalysis and have been extensively studied when their exceptionally high stability in non-aqueous media has been discovered. Stability of lipases in organic solvents makes them commercially feasibile in the enzymatic esterification reactions. Their stability is affected by temperature, reaction medium, water concentration and by the biocatalyst's preparation. An optimization process for ester synthesis from pilot scale to industrial scale in the reaction medium is discussed. The water released during the esterification process can be controlled over a wide range and has a profound effect on the activity of the lipases. Approaches to lipase catalysis like protein engineering, directed evolution and metagenome approach were studied. This review reports the recent development in the field of non-aqueous microbial lipase catalysis and factors controlling the esterification/ transesterification processes in organic media.

Keywords: microbial lipase, nonaqueous media, lipase stability, lipase modifications, ester synthesis and advances in lipase catalysis

Introduction

Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) catalyzed esterification as well as transesterification reactions in nonaqueous media. The application of

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microbial lipases in ester synthesis is one of the most exciting facets of biotechnology industry in the present scenario [1–5]. At present, many esters are industrially manufactured by chemical methods. Chemical methods involve high temperature or high pressure; therefore it is difficult to esterify unstable substances, like ascorbic acid, polyols and polyunsaturated fatty acids (PUFAs). Further, the region-specific acylation of polyols requires protection and deprotection steps [6]. These steps cause a rise in manufacturing costs. The reagents used in synthesis of food-grade esters are limited. Therefore natural esters, such as those derived from sperm whale oil, carnauba wax and jojoba oil, were proposed. These oils are, however, expensive and not readily available in large amounts. Thus, it is economically important to develop more suitable biocatalytic methods for the production of such esters from cheaper and were readily available raw materials.

Lipase catalyzed condensations were used to overcome many of these drawbacks. They have advantages like mild reaction conditions, one step synthesis without protection and deprotection steps and easy application to food processing. A lipase catalyzes a reversible reaction and the direction and equilibrium of the reaction are determined by the activities of the substrates and products, temperature, and pressure. Although an enzyme-catalyzed reaction is usually performed in an aqueous medium, hydrolysis causes the production of desired product to fail when a lipase-catalyzed reaction is attempted in an aqueous solution. Thus, reduction of the amount of water in the reaction system would be desirable for the improvement of conversion through the condensation reaction. Some lipases have catalytic activity even in the presence of little or small amount of water [7]. For this reason, lipase-catalyzed condensation in non-aqueous medium and ionic liquids has attracted much attention [4, 8].

Lipase-catalyzed condensation in an organic solvent is useful for the syntheses of a variety of esters. Ester synthesis catalyzed by a lipase was first described by Inada et al. [9]. A variety of fatty acid esters are now being produced commercially using immobilized lipase in nonaqueous solvents [3, 5, 10, 11]. Fatty acid esters of carbohydrates, hydroxy acids and flavonoids have gained recent interest because of their broader use in biotechnological industry [12–17]. The interest in industrial processes employing lipase biocatalysts to synthesize more such esters is still growing due to their important and multiple applications in diverse technologies (Table I).

Lipase-catalyzed transesterification is another alternate process to synthesize esters [47, 48]. Transesterification involves the exchange of acyl groups between an ester and an acid (acidolysis), an ester and an alcohol (alcoholysis), two esters, and an ester (interesterifications or ester-ester interchange), or an ester and

an amine (aminolysis). The lipase catalysed transesterification in organic solvents is an emerging industrial application exploited in the production of a cocoa butter equivalent, human milk fat substitute "Betapol", pharmaceutically important polyunsaturated fatty acids rich/ low calorie lipids, designers fats or lipids and production of biodiesel from vegetable oils [5, 35]. The conversion in the transesterification using other esters, as substrate is also generally high compared to that in the condensation reaction. The problem of water encountered in direct esterification is eliminated in transesterification reactions [38, 49–55].

Lipase behavior/ properties in organic solvents

There are several media for lipase catalysed esterification reaction [56–58]. Nonaqueous media have many advantages (Table II) like better solubility of substrates and product; shifting of thermodynamic equilibria; simpler removal of solvent (most organic solvents have lower boiling point than water); reduction in water-dependent side reactions such as hydrolysis of acid anhydrides or polymerization of quinines; removal of enzyme after reaction since it is not dissolved; better thermal stability of enzymes since water is required to inactivate the enzymes at high temperatures; elimination of microbial contamination; potential of enzymes to be used directly in a chemical process [2, 44, 59–62].

Nature and characteristics of lipase structure in organic solvents

In nature, lipases are found to be active at the oil-water interface. *In vitro,* they are found to be active in aqueous as well as in anhydrous organic solvents. Lipases are hydrophobic proteins acting on carboxylic acid esters, such as glyceride lipids at the interface between an aqueous and an oil phase. Activation of lipases at interfaces [44, 63] manifests as pronounced activity increasing on substrate aggregation (i.e., over the substrate-critical micelle concentration/solubility limit). The interfacial activation was hypothesized to be due to a conformational change resulting from the adsorption of the lipases onto a hydrophobic interface [64]. X-ray crystallographic structural studies of several lipases have supported this hypothesis [65, 66].

The three-dimensional structures of lipases from various microbial sources like *Mucor miehei* [67], *Geotrichum candidum* [68], *Candida rugosa* [69] and *Chromobacterium viscosum* [70] were studied using x-ray crystallography. The

parts of lipases that are conserved may be important for their structural integrity and/or activity and/or specificity. Sequences of several lipases showed a large area of sequence identity in the proximity of the active-site serine residue [71]. The various available databases like Lipase Engineering Database (LED; http://www.led.uni-stuttgart.de) provide information on sequence-structure-function relationships of microbial lipases and homologous serine hydrolases [72].

The three-dimensional structures of microbial lipases were studied to understand the catalytic mechanism of lipase-mediated reactions. The main structural characteristics of microbial lipases include an α/β hydrolase fold, a catalytic triad consisting of a nucleophilic serine located in a highly conserved Gly-X-Ser-X-Gly pentapeptide, and an aspartate or glutamate residue that is hydrogen bonded to a histidine. Four substrate binding pockets were identified for triglycerides: an oxyanion hole and three pockets accommodating the fatty acids bound at positions *sn*-1, *sn*-2, and *sn*-3. The differences in size and the hydrophilicity/hydrophobicity of these pockets determine the enantiopreference of a lipase. The understanding of structure-function relationships will enable researchers to tailor new lipases for biotechnological applications [73].

Mechanism for lipase catalysis

The mechanism of lipase-catalyzed esterification involves two tetrahedral intermediates (Fig. 1). The first tetrahedral intermediate is formed by nucleophilic attack of active site serine residue of the catalytic triad on the substrate i.e., acid. The intermediate loses a water molecule to give an acyl-enzyme complex. Nucleophilic attack by an alcohol causes the addition of a hydroxyl group to the carboxyl group, producing another tetrahedral intermediate, which will rearrange, releasing the ester molecule and regenerating the active site serine of lipase. Both of the tetrahedral intermediates possess an oxyanion that is stabilized by hydrogen bonds to protein atoms of the oxyanion hole [45, 73, 74].

Lipase stability in organic media

The stability of lipases in organic solvents is strongly dependent and influenced by the solvent properties. Lipases, being activated by interfaces, however, show lower catalytic rates in homogeneous aqueous solutions than in the presence of interfaces e.g., water-organic solvent interface [75]. Typically, the lipase is dis-

solved in the aqueous phase and the substrate is dissolved in a water-immiscible organic solvent [59, 76]. Vigorous mixing of the two phases forms a suspension with a significantly large interfacial area.

Lipases are solubilized in discrete hydrated reverse micelles, formed by surfactants, within a continuous phase of a nonpolar organic solvent system. Under appropriate conditions, a reverse micellar solution is homogeneous, thermodynamically stable, and optically transparent [77, 78]. Solid enzyme preparations (lyophilized or adsorbed on an inert support) are suspended in organic solvent [79, 80]. Most lipases are known to be active and stable in anhydrous organic solvents. Several covalent modifications as well as noncovalent modification techniques have been developed for solubilization of lipases in organic solvents [81]. The biological origin of lipase, nature of the reaction to be carried out (hydrolysis or esterification), the substrates used, and so forth determine which solvent system will be the most suitable one. The cost of downstream processing is also a direct function of the solvent system and the impurities present.

Solubilization of lipases in organic solvents

Lipases are insoluble in organic solvents in their native form. Solubilized lipases have higher activities (as compared to the insoluble ones) as well as their optical transparency allows to perform theier structural characterization by spectroscopic techniques [82]. Two types of methods namely covalent and noncovalent modifications are in common use for lipase solubilization. Covalent modifications are achieved by using chemical modifiers like polyethylene glycol (PEG), poly *N*-vinylpyrrolidone, polystyrene and polymethyl methacrylate [83, 84]. Such chemical modifications greatly affect the activity, stability and selectivity of the enzyme [85, 86].

Noncovalent modification of lipases has essentially been restricted to coating the lipase molecule with different surfactants. Dissolution of the lipase and the surfactant in aqueous solution are the most widely applied techniques [87–90]. This enzyme-surfactant complex, which precipitates from the aqueous solution, can be dissolved in a variety of organic solvents. The solubilized lipase has shown greater catalytic activities than dry lipase preparations. Another related technique involves preparation of water-in-oil emulsions (or reverse micelles) containing lipase and then drying out the water from the emulsion phase that yields a lipase-surfactant complex, which is soluble and highly active in organic solvents [91, 92].

Molecular imprinting of lipase

One of the most successful strategies for enhancing lipase activity in organic solvents involves tuning the lipase active site by molecular imprinting with substrates or their analogues. Enzymes prepared from an aqueous/buffer solution via lyophilization results in undesirable changes in the protein's secondary structure, which could lead to denaturation of the active sites. The addition of specific small molecules (excipients) in the freeze-drying stage often improves catalytic activity of lipases. This formed the basis of molecular imprinting that involves formation of a complex between a macromolecule and a low-molecular-weight ligand in solution, followed by drying and washing with a selective solvent that effectively removes the ligand. The protein retains the ligand-induced conformation even after the removal of the ligand [93–97].

It is well known that lipases undergo interfacial activation, involving opening of a helical lid covering the active site when exposed to a water-oil interface [66, 98]. A helical lid covering the active site may remain predominantly closed in organic solvents thus contributing to lower enzymatic activity [75]. An exciting possibility of "trapping" the enzyme in an active form by lyophilizing the enzyme bound to an amphiphile-water interface has been demonstrated [99–101]. Such interfacial-activation-based molecular imprinting of lipases resulted in a catalyst that was significantly more active (up to 70-fold) than the non-imprinted counterpart.

Medium engineering of lipases in organic solvents

Lipases in nonaqueous systems can be active provided the essential water layer around them is not stripped off. Medium engineering for biocatalysis in nonaqueous media involves the modification of the immediate vicinity of the biocatalyst [102–104]. Nonpolar solvents are better than polar ones since they provide a better microenvironment for the lipase. If the enzyme's microenvironment favours high substrate and low product solubility, the reaction rates would be high. Several aspects have to be considered in choosing an appropriate solvent for a given reaction. These include compatibility with the selected reaction (substrates and products), inertness, low density to minimize mass transfer limitations, and other properties that are suitable (e.g. surface tension, toxicity, flammability, waste disposal and cost). Rates of enzymatic reactions in organic media are greatly dependent on the method of enzyme preparation [91, 105].

Lipase modifications in organic solvents

In view of the current high cost of lipases, the possibility of regenerating and reusing the enzyme would obviously be an attractive feature for large-scale syntheses. Immobilization tends to protect the enzyme to some extent from solvent denaturation as well as maintaining the enzyme's homogeneity in the reaction media since it avoids aggregation of enzyme particles and confinement.

Lipases have been immobilized by adsorption, entrapment in membrane network, cross-linking, adsorption followed by cross-linking or covalent attachment [106]. By taking advantage of the 'interfacial' hydrophobicity, immobilization of lipases has been performed by adsorption on hydrophobic adsorbents, including glass beads coated with hydrophobic materials, methylated silica, phenyl-Sepharose, *poly*-(ethylene glycol)-Sepharose, polypropylene particles, polypropylene hollow fibers and non-woven fabric, etc. Physical entrapment has been employed in many commercial carriers, e.g., controlled pore silica, natural/synthetic polymers, hollow fibber, activated charcoal, aluminum oxide and celite [107].

Because of the current high cost of some available commercial support matrixes, the possibility of using inexpensive supports for lipase immobilization such as rice husk [108], $CaCO_3$ powder [109], grafted hydrogels [110–113] and activated silica/celite [114] has also been considered (Table III). Although adsorption seems to be a promising technique for lipase recovery, the adsorbents are either expensive or not easily accessible. Desorption usually involves usage of a solution containing chaotropic agents or detergents, which leads to a complexity in subsequent processing steps and adds to environmental burden. Therefore, synthetic matrices of specific characteristics provide better lipase immobilization in a single step process.

A variety of hydrogels based upon starch, polyacrylamide, acrylic acid etc. are commercially available. These cross-linked hydrophilic polymers capable of imbibing large volumes of waters, and are yet insoluble because of their network structures, crystalline regions [131, 132]. Since they absorb large amounts of water that promotes the esterification reaction in forward direction, this makes them an interesting material for lipase immobilization [113, 133]. The search for newer inexpensive support(s) has also led to synthesis and selection of some synthetic/grafted polymer networks, which can be recognized as surface-active supports by lipases at the molecular level. Recent application of such surface-active matrices (hydrogel) has witnessed multifold usage of lipases.

Process optimization for esters synthesis

Biochemical processes were designed to transform reactant(s) into product(s) that satisfy a number of preset specifications (support material, solvent system and enzyme selectivity etc.) within limited time frames. However, due to either thermodynamic and/or kinetic limitations, the final yield and purity are often poor. The parameters like solvent and water are needed to regulate the lipase mediated catalysis in organic solvent medium.

Effect of solvents on ester synthesis

Solvent selection is a very important factor for the successful application of the lipase catalysis in esterification reactions. Lipase-catalyzed esterification reactions are not independent of nature of solvent [134–136]. The lipase activity, measured as the initial rate of the esterification reaction, is best correlated as a function of *n*-octanol-water partition coefficient (Log *P*); electron pair acceptance index or the polarizability [137, 138]. A good correlation was found between the ester mole fraction at equilibrium and log *P* of the solvent. The results have been attributed to the significant difference in polarity of the substrates and products, which often gave rise to dramatic differences in their relative thermodynamic activities in various solvents [139]. There has been much interest in the development of rules to predict the effects of various solvents on the biocatalyst [137]. When $\log P < 2$, distortion of water structure occurs; if $2 < \log P < 4$, the effect of solvent is unpredictable and if $\log P > 4$, water structure is intact.

Rates of esterification reaction catalyzed by lipases are greater in hydrophobic solvents than in hydrophilic ones [140–143]. The choice of solvent affects the thermodynamic activity coefficient of the substrate, or the ground-state free energy of the substrate. The activation energy barrier is bound to be different in various solvents due to the differences in the ground-state free energy [144]. The solvent greatly affects the hydration state of the enzyme and, in turn, its ability to interact with the substrate, or its intrinsic activity [145]. The rates of enzymatic reactions in anhydrous organic solvents could be increased significantly by the addition of denaturing cosolvents such as methyl sulfoxide or formamide [146].

Water-miscible solvents viz., acetone, acetonitrile, *t*-butyl alcohol and water-immiscible solvents viz., hexane, ethers, chloroform, and toluene have been used in lipase-catalyzed condensations. Water-miscible solvents can solubilize hydrophilic substrates such as saccharides to a certain degree without the addition

of solubilizing reagents thereby facilitating the esterification of the hydrophilic substrates [6, 147–149]. They may, however, remove water from a lipase molecule, which is essential for its catalytic activity [150], and this removal may deactivate the lipase. For this reason, lipases available for condensation in water-immiscible solvents are mostly commonly used compared to water miscible solvents.

Effect of substrate chemistry on ester synthesis

Lipases prefer straight-chain acids as substrates to branched ones [151, 152]. The position of the branching has an effect on the reaction rate(s). The effect of branching diminishes as the substituent is shifted further away from the carboxylic group. Unsaturated acids have shown lower enzymatic activity compared to the corresponding saturated acids with the α-double bond being more deleterious than a double bond at the β-position [152]. Similarly, primary alcohols are preferred over secondary ones, whereas tertiary alcohols are not accepted as substrates by the majority of lipases [56, 153].

Lipases show remarkable regioselectivity or selectivity based on molecular position of the functional group. It becomes extremely important in the synthesis of esters especially sugar and their derivatives. Sugars contain multiple hydroxyl groups, all of which are capable of being acylated by acidic moieties. Lipases are known to regio-selectively acylate monosaccharides as well as disaccharides in organic solvents [154–159]. The regioselectivity was found to be strongly dependent on the source of the lipase [160]; type of the substrate chemistry i.e., the acylating agents [161, 162]; solvent [163, 164], as well as level of hydration [165].

Effect of water produced in lipase catalysis esterification

The degree of solvent hydration has a profound effect on lipase catalysis in anhydrous organic solvents. Water activity affects the intrinsic activity of lipases as well as it acts as a competitive nucleophile [166, 167]. Water activity is a measureme of the energy status of the water in a system. It is defined as the vapor pressure of water divided by that of pure water at the same temperature Water activity values are obtained by either a capacitance or a dew point hygrometer. In esterification reactions, the water content affects the rate of reaction as well as the equilibrium position [168, 169]. The monolayer of water molecules around the en-

zyme molecule is essential for activity. Enzymatic activity increases very rapidly with the addition of small amounts of water to the organic media [79, 80].

The enzymatic activity in anhydrous solvents may be correlated to the thermodynamic activity *(aw)* rather than to the concentration of water [170, 171]. This is readily seen from the effect of water content on catalytic activity of lipase. The lipase showed a similar optimum at thermodynamic water activity of about 0.55 when used in solvents ranging from hexane to pentanone. The optimum varied widely in terms of total water content. Water activity fails to predict the critical hydration level for enzyme activity in polar organic solvents [172]. Thus, enzymatic activity can be correlated with water activity *(aw)* only for water-immiscible organic solvents. Lipases from different microbial sources show widely varying optimum aw ranging from 0.3 to 1.0 [166]. The optimum aw also depends on the type of support used for immobilization for a given lipase in a given solvent. The reaction rates for lipase catalysis in organic media show a similar dependence on water activity irrespective of the support used [173]. Thus, the optimum water requirement is dictated by the biological source of the enzyme, the organic solvent and the type of support used.

Water control in lipase catalysis esterification reaction

It is pertinent to perform lipase catalysis in organic solvents at an optimal hydration level. Salt hydrates, pervaporation, saturated salt solutions and molecular sieves check the control of water content during lipase catalysis in organic solvents.

Salt hydrates maintain the water activity *(aw)* of the organic systems used for reverse hydrolysis reactions by undergoing transition between their various states of hydration [174–176]. Similarly, other salt hydrate pairs can be chosen to maintain the thermodynamic water activity at the desired level.

The esterification reaction can be carried out in a membrane reactor with the reaction mixture on one side of the membrane and air, used as an extractive medium for water removal, on the other side [177]. Selective pervaporation of the reaction water through the membrane was achieved by using a suitable membrane [178]. The air re-circulated through the reactor condensed out the permeated water. Water-activity control can be achieved by controlling the temperature of the condenser. This method effectively esterified oleic acid with *n*-butanol in the presence of Lipozyme in isooctane using the cellulose acetate membrane [179]. A modification of this technique employed a hollow-fiber membrane reactor with

reaction mixture on the lumen side and controlled humidity air on the shell side [180].

Saturated salt solutions have a fixed thermodynamic activity at a given temperature. Water activity in an organic solvent can be adjusted by equilibrating with a saturated salt solution via vapor-phase equilibria [181, 182]. Water-producing or water-consuming reactions can be performed in chambers of controlled water activity using these saturated salt solutions [182]. Such systems have limitations on the rate of transfer of water and hence are not suitable for fast reactions. A method has been developed where water activity is controlled by circulation of a saturated salt solution through silicone tubing that is submerged in the reaction medium [183, 184]. The silicone membranes are permeable to water vapors and impermeable to ions. Thus, water and organic substances are only transferred through them. Mass-transfer properties for different types of silicone tubing have been characterized for a water-activity-controlling system in organic solvents using saturated salt solutions [185]. A modification of this technique-involved circulation of a saturated salt solution through microporous hollow-fiber polypropylene membranes while the reaction medium is circulated on the shell side [186].

Water formed by condensation exists in fluid and acts as a substrate for the hydrolysis reaction when the reaction is performed in a water miscible solvent [187, 188]. Therefore, elimination of water by desiccant/molecular sieves/ silica gel is quite effective for increasing the conversion in lipase-catalyzed condensation reaction [6, 189–191]. The quantitative relationship between the equilibrium conversion and the concentration of molecular sieves was discussed using adsorption isotherm of water onto molecular sieves, the apparent reaction constant, the solubility of a hydrophilic substrate in the solvent and the mass balance equation in terms of water were taken into consideration to obtain the criterion. Although the addition of a molecular sieve or silica gel usually improves the equilibrium conversion in many cases, little effect or negative effects were also reported such as the formation of diesters and the degradation of unstable substrates [188, 191]. Therefore, an appropriate combination of solvent and the amount of desiccant should exist for improvement of the equilibrium conversion in a lipase-catalyzed condensation.

Advances in catalysis by lipases in organic solvents

Protein engineering

Rational protein engineering through mutagenesis and directed evolution has provided a new and valuable tool for improving or adapting enzyme properties to the desired requirements. The upcoming trend to access a novel natural sequenced space, via the direct cloning of metagenomic DNA, is significantly contributing to the screening and identification of hitherto unexplored microbial consortia for valuable biocatalysts. Thus, the modern methods of genetic engineering combined with an increasing knowledge of structure and function are allowing further adaptation to industrial needs and exploration of novel applications.

Directed evolution of lipase

Directed evolution was used to lipase properties like the modification of stability, activity and specificity. Directed evolution in combination with appropriate screening systems will be used extensively as a novel approach to develop lipases with high stability and enantioselectivity. It was used for the creation of enantioselective catalysts for organic synthesis in the field of lipase research. *Pseudomonas aeruginosa* lipase was made to evolve towards a model substrate, 2-methyldecanoic acid p-NPP ester, to yield a lipase mutant showing >90% enantiomeric excess, as compared to the 2% of the wild-type lipase [192, 193]. *Bacillus subtilis* lipase developed to a catalyst of the asymmetric hydrolysis of meso-1, 4-diacetoxy-2-cyclopentene, with the formation of chiral alcohols [194]. Homologous lipases from *Candida antarctica, Hyphozyma* sp., *Cryptococcus tsukubaensis* become more stable and active toward 3-(3',4'-dichlorophenyl) glutarate [195–197]. Increased phospholipase activity was achieved through directed evolution in the cases of *Staphylococcus aureus* [198] and *Bacillus thermocatenulatus* lipases [199].

Metagenome approach for efficient lipase mediated catalysis

On average, the vast majority of microbes present in a single environmental niche are not culturable and less than 1% of them has ever been identified in the laboratory [200]. An alternative approach is to make use of the genetic diversity of

the microorganisms in a certain environment (the so-called "metagenome") to detect new or improved genes and gene products for biotechnological purposes [201]. Environmental DNA libraries were prepared from three different soil samples for genes conferring lipolytic activity on *E. coli* clones and identified four clones harboring hyper-lipase activities [201].

A PCR method suitable for the isolation of lipase genes directly from environmental DNA, using primers designed on the basis of lipase consensus sequences has been described [202]. Thus, today there is an ample need to develop production and downstream-processing systems that are cost-effective, simple and not time-consuming. The growing demand for lipases has shifted the trend towards prospecting for novel lipases, improving the properties of existing lipases for established technical applications and producing new enzymes tailored for entirely new areas of application. The number of novel microbial lipases being cloned and biochemically characterized is currently on the rise.

Current status of esters production using lipases

Esters have important industrial uses as well as biological and medical uses. Biocompatibility, biodegradability, and environmental acceptability of biotechnically produced polyesters are desired properties in agricultural and medical applications [203]. Many pharmaceutical companies are currently using microbial lipases worldwide for the preparation of optically active intermediates on a kilogram scale [5]. Sucrose esters are the most developed carbohydrate esters among carbohydrate fatty acid esters being produced at a rate of about 4000 Tm/year [204]. The fatty acid esters of sorbitol are the second largest class of carboxylic acid esters among fatty acid esters of sugars and sugar alcohols employed as surfactants [6].

Limited studies have been made on large-scale synthesis of esters through lipase catalysis synthesis in organic solvents [205, 206]. These studies would be helpful in potential commercialization of the enzymatic esterification processes. Scaled-up lipase-catalyzed synthesis of (Z)-3-hexen-1-yl acetate in hexane from laboratory scale (3–5 g) to pilot scale (1–5 kg) was reported [206]. A similar study for synthesis of ethyl stearate was also reported [205]. Investigation in large-scale production for oleyl oleate, a liquid wax ester was performed in batch mode of stirred tank reactor (STR) with a multi-bladed impeller [34].

Conclusions

Lipase catalysis in nonaqueous media significantly extends conventional aqueous-based biocatalysis. However, stability of lipase in organic media, its optimal solubilization in organic solvents, enhanced stability and control of water activity during bioconversion are the important parameters that govern the activity and performance of lipases in organic media. The phenomenal exponential increase in the use of lipases in biotechnology in comparison to other hydrolytic enzymes indicates the growing demand for and applications of lipase, especially in making fine chemicals. This review reported on the recent developments in the properties and applications of lipase catalysis in non-aqueous media. Recent examples of relevant applications are discussed that highlighted both the controlling variables of lipase catalysis in non-aqueous media as well as the potential of new technologies in biocatalysis.

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References

1. Sharma, R., Chisti, Y., Banerjee, U. C.: Production, purification, characterization and applications of lipases. Biotechnol Adv **19,** 627–662 (2001).
2. Bornscheuer, U. T., Bessler, C., Srinivas, R., Krishna, S. H.: Optimizing lipases and related enzymes for efficient application. Trends Biotechnol **20,** 433–437 (2002).
3. Gupta, R., Gupta, N., Rathi, P.: Bacterial lipases: An overview of production, purification and biochemical properties. Appl Microb Biotechnol **64,** 763–781 (2004).
4. Gupta, M. N., Roy, I.: Enzymes in organic media: Forms, functions and applications. Eur J Biochem **271,** 2575–2583 (2004).
5. Hasan, F., Shah, A. A., Hameed, A.: Industrial applications of microbial lipases. Enzyme Microb Technol **39,** 235–251 (2006).
6. Arcos, J. A., Bernabe, M., Otero, C.: Quantitative enzymatic production of 6-O-acylglucose esters. Biotechnol Bioeng **57,** 505–509 (1998).
7. Kichner, G., Scollar, M. P., Klibanov, A. M.: Resolution of racemic mixtures via lipase catalysis in organic solvents. J Am Chem Soc **107,** 7072–7076 (1985).

8. Sheldon, R.: Catalytic reactions in ionic liquids. Chem Commun 23, 2399–2407 (2001).
9. Inada, Y., Nishimura, H., Takahashi, K., Yoshimoto, T., Saha, A. R., Saito, Y.: Ester synthesis catalyzed by polyethylene glycol-modified lipase in benzene. Biochem Biophy Res Commun **122,** 845–890 (1984).
10. Macrae, A. R.: Tailored triacylglycerols and esters. Biochem Sot Trans **17,** 1146–l148 (1989).
11. Shimada, Y., Hirota, Y., Baba, T., Sughara, A., Moriyama, S., Tominaga, Y., Terai, T.: Enzymatic synthesis of steryl esters of polyunsaturated fatty acids. JAOCS **76,** 713–716 (1999).
12. Bauer, A. R., Garbe, D., Surgurg, H.: In Common Fragrance and flavour materials. 2nd revised edition, VCH, New York, pp. 67–81 (1990).
13. Gandhi, N. N., Sawant, S. B., Joshi, J. B.: Specificity of a lipase in ester synthesis: Effect of alcohol. Biotechnol Prog **11,** 282–287 (1995).
14. Guardia, T., Rotelli, E. A., Juarez, A. O., Pelzer, L. E.: Anti-inflammatory properties of plant flavonoids: Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. Farmaco **56,** 683–687 (2001).
15. Gao, C., Mayon, P., MacManus, D. A., Vulfson, E. N.: Novel enzymatic approach to the synthesis of flavonoid glycosides and their esters. Biotechnol Bioeng **71,** 235–243 (2001).
16. Tapiero, H., Tew, K. D., Nguyen, B. G., Mathe, G.: Polyphenols: Do they play a role in the prevention of human pathologies? Biomed Pharmaco **56,** 200–207 (2002).
17. Chang, S. W., Shaw, J. F., Yang, K. H., Shih, I. L., Hsieh, C. H., Shieh, C. J.: Optimal lipase-catalyzed formation of hexyl laurate. Green Chem **7,** 547–551 (2005).
18. Marshall, D. L., Bullerman, L. B.: Antimicrobial properties of sucrose esters. In: Akoh CC, Swanson BG, editors. Carbohydrate polyesters as fat substitutes. New York: Marcel Dekker; pp. 149–167 (1994).
19. Okabe, S., Saganuma, M., Tada, Y., Ochiai, Y., Sueoka, E., Kohya: Disaccharide esters screened for inhibition of tumor necrosis factor-α release are new anticancer agents. Jpn J Cancer Res **90,** 669–676 (1999).
20. Watanabe, Y., Miyawaki, Y., Adachi, S., Nakanishi, K., Matsuno, R.: Synthesis of lauroyl saccharides through lipase-catalyzed condensation in microaqueous water-miscible solvents. J Mol Catal B: Enzyme **10,** 241–247 (2000).
21. Devulapalle, K. S., Gomez de Segura, A., Ferrer, M., Alcalde, M., Moose, G., Plou, F. G.: Effect of carbohydrate fatty acid esters on *Streptococcus sobrinus* and glucosyltransferase activity. Carbohydr Res **339,** 1029–1034 (2004).
22. Song, Z., Li, S., Chen, X., Liu, L., Song, Z.: Synthesis of insecticidal sucrose esters. Forestry Stud China **8,** 26–29 (2006).
23. Angello, A. J., Vercellotti, J. R.: Phospholipids and fatty acid esters of alcohols. In: Charalambous, G., Doxastakis, G. (Eds.), Food Emulsifier Chemistry, Technology, Functional Properties and Applications. Elsevier Publications, Amsterdam (1989).
24. Gargouri, M., Drouet, P., Legoy, M. D.: Synthesis of a novel macrolactone by lipase-catalysed intra-esterification of hydroxy-fatty acid in organic media. J Biotechnol **92,** 259–266 (2002).
25. Foti, M., Piattelli, M., Tiziana, Baratta, M., Ruberto, G.: Flavonoids, coumarins, and cinnamic acids as antioxidants in a micellar system: Structure-activity relationships. J Agric Food Chem **44,** 497–501 (1996).
26. Cao, C., Sofic, E., Prior, R. L.: Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationships. Free Radic Biol Med **22,** 749–760 (1997).

27. Arora, A., Nair, M. G., Gale, M.: Structure activity relationships for antioxidant activities of a series of flavonoids in a liposomal system. Free Radic Biol Med **24,** 1355–1363 (1998).
28. Harborne, J. B., Williams, C. A.: Advances in flavonoid research since 1992. Phytochem **55,** 481–504 (2000).
29. Havsteen, B. H.: The biochemistry and medical significance of the flavonoids. Pharmacol Ther **96,** 67–202 (2002).
30. Tamura, M., Shoji, M., Nakatsuka, T., Kinomura, K., Okai, H., Fukui, S.: Methyl 2,3-di-(L-α-amimobutyryl)-α-D-glucopyranoside, a sweet substance and tastes of related compounds of neutral amino acids and d-glucose derivatives. Agric Biol Chem **49,** 2579–2586 (1985).
31. Kirk, O., Bjorkling, F., Godfredsen, S. E., Larsen, T. S.: Fatty acid specificity in lipase catalyzed synthesis of glucoside esters. Biocatalysis **6,** 127–134 (1992).
32. Zaks, A., Dodds, D. R.: Applications of biocatalysts and biotransformations to the synthesis of pharmaceuticals. Drug Dev Today **2,** 513–531 (1997).
33. Pandey, A., Benjamin, S., Soccol, C. R., Nigam, P., Krieger, N., Soccol, V. T.: The realm of microbial lipases in biotechnology. Biotechnol Appl Biochem **29,** 119–131 (1999).
34. Radzi, S. M., Basri, M., Salleh, A. B., Ariff, A., Mohammad, R., Rahman, M. B. A., Rahman, R. N. Z. R. A.: Large-scale production of liquid wax ester by immobilized lipase. J Oleo Sci **54,** 4203–4209 (2005).
35. Jaeger, K-E., Reetz, M. T.: Microbial lipases form versatile tools for biotechnology. Trends Biotechnol **16,** 396–403 (1998).
36. Jaeger, K-E., Eggert, T.: Lipases for biotechnology. Curr Opin Biotechnol **13,** 390–397 (2002).
37. Saxena, R. K., Gosh, P. K., Gupta, R., Davidson, W., Sheba, Bradoo, S., Gulati, R.: Microbial lipases: Potential biocatalysts for the future industry. Curr Sci **77,** 101–115 (1999).
38. Compton, D. L., Laszlo, J. A., Berhow, M. A.: Lipase-catalyzed synthesis of ferulate esters. J Am Oil Chem Soc **77,** 513–519 (2000).
39. KinChor, K., Yi, O. S.: Ferulic acid: pharmaceutical functions, preparation and applications in foods. J Sci Food Agric **84,** 1261–1269 (2004).
40. Tang, L. H., Zhang, H., Shehate, M. M., Sun, Y. F.: A kinetic study of the synthesis of ascorbate fatty acid esters catalysed by immobilized lipase in organic media. Biotechnol Appl Biochem **32,** 35–39 (2004).
41. Song, Q. X., Wei, D. Z., Zhou, W.Y., Xu, W. Q., Yang, S. L.: Enzymatic synthesis and biological effect of unsaturated fatty acid ester of L-ascorbic acid. Biotechnol Lett **26,** 1777–1780 (2004).
42. Villeneuve, P., Turon, F., Caro, Y., Escottier, R., Barea, B., Barouh, B., Lago, R., Plombo, G., Pina, M.: Lipase-catalyzed synthesis of canola phyosterols oleate esters as cholesterol lowering agents. Enzyme Microb Technol **37,** 150–155 (2005).
43. Lee, G. S., Widjaja, A., Ju, Y. H.: Enzymatic synthesis of cinnamic acid derivatives. Biotechnol Lett **28,** 81–85 (2006).
44. Gandhi, N. N., Patil, N. S., Sudhirprakash, B., Sawant, S. B., Joshi, J. B.: Lipase-catalyzed esterification. Catal Rev-Sci Eng **42,** 439–480 (2000).
45. Kanwar, S. S., Sharma, C., Verma, M. L., Chauhan, S., Chimni, S. S., Chauhan, G. S.: Short-chain ester synthesis by trans-esterification employing poly (MAc-*co*-DMA-*cl*-MBAm)-hydrogel-bound lipase of *Bacillus coagulans* MTCC-6375. J Appl Polym Sci DOI: 10.1002/app.25320 (2007b).

46. Linko, Y. Y., Lamsa, M., Wu, X., Uosukainen, E., Seppala, J., Linko, P.: Biodegradable products by lipase biocatalysis. J Biotechnol **66,** 41–50 (1998).
47. Kobayashi, T., Adachi, S.: Reaction equilibrium for lipase-catalyzed codensation in organic solvent system. Biotechnol Lett **26,** 1461–1468 (2004).
48. Pahujani S, Shukla SK, Bag BP, Kanwar SS, Gupta R (2008) Application of lipase immobilized on nylon-6 for the synthesis of butyl acetate by transesterification reaction in n-heptane. J Appl Polym Sci DOI 10.1002/app.25323.
49. Berger, B., Faber, K.: Immunization of lipase against acetaldehyde emerging in acyl transfer reactions from vinyl acetate. J Chem Soc Chem Commun 1198–1200 (1991).
50. Ferrer, M., Cruces, M. A., Bernabé, M., Ballesteros, A., Plou, F. J.: Lipase-catalyzed regioselective acylation of sucrose in two solvent mixtures. Biotechnol Bioeng **65,** 10–16 (1999).
51. Park, O. J., Kim, D. Y., Dordick, J. S.: Enzyme-catalyzed synthesis of sugar-containing monomers and linear polymers. Biotechnol Bioeng **70,** 208–216 (2000).
52. Maugard, T., Tudella, J., Legoy, M. D.: Study of vitamin ester synthesis by lipase-catalyzed transesterification in organic media. Biotechnol Prog **16,** 358–362 (2000).
53. Li, Y., Rethwisch, R. G.: Quantitative production of alpha-methyl glucoside acrylate using *Candida antarctica* lipase in acetone. Biotechnol Bioeng **79,** 15–22 (2002).
54. Hazarika, S., Goswami, P., Dutta, N. N.: Lipase catalyzed transesterification of 2-*o*-benzylglycerol with vinyl acetate: solvent effect. Chem Eng J **94,** 1–10 (2003).
55. Yadav, G. D., Trivedi, A. H.: Kinetic modeling of immobilized lipase catalyzed transesterification of *n*-octanol with vinyl acetate in non-aqueous media. Enzyme Microb Technol **32,** 783–789 (2003).
56. Borzeiz, F., Monot, F., Candecasteele, T. P.: Strategies for enzymatic esterification in organic solvents: Comparison of microaqueous, biphasic and micellar systems. Enzyme Microb Technol **14,** 791–797 (1992).
57. Yang, F., Russell, A. J.: A comparison of lipase-catalyzed ester hydrolysis in reverse micelles, organic solvents and biphasic system. Biotechnol Bioeng **47,** 60–67 (1995).
58. Krieger, N., Bhatnagar, T., Baratti, J. C., Baron, A. M., Lima, V. M. de., Mitchell, D.: Non-aqueous biocatalysis in heterogeneous solvent systems. Food Technol Biotechnol **42,** 279–286 (2004).
59. Halling, P. J.: Water activity in biphasic reaction systems. In: Laane, C., Tramper, J., Lilly, M. D., (Eds.), Biocatalysis in organic media. Elsevier, Amsterdam, pp. 125–132 (1987).
60. Dordick, J. S.: Enzymatic catalysis in monophasic organic solvents. Enzyme Microb Technol **11,** 194–211 (1989).
61. Arnold, F. H.: Engineering enzymes for non-aqueous solvents. Trends Biotechnol **8,** 244–249 (1990).
62. Klibanov, A. M.: Improving enzymes by using them in organic solvents. Nature **409,** 241–246 (2001).
63. Sarda, L., Desnuelle, P.: Inhibition of lipases by proteins: A kinetic study with dicarpin monolayers. Biochim Biophys Acta **30,** 513–521 (1958).
64. Desnuelle, P., Sarda, L., Ailhaud, G.: Inhibition of pancreatic lipase by diethyl-p-nitrophenyl phosphate in emulation. Biochim Biophys Acta **29,** 570–571 (1960).
65. Cambillau, C., van Tilbeurgh, H.: Structure of hydrolases-lipases and cellulases. Curr Opin Struct Biol **3,** 885–895 (1993).
66. Derewenda, Z. S., Sharp, A. M.: News from the interface: the molecular structures of triacylglyceride lipases. Trends Biochem Sci **18,** 20–25 (1993).

67. Brady, L., Andrzej, M., Brzozowski, Zygmunt, S., Derewenda, Dodson, E., Dodson, G., Tolley, S., Turkenburg, P., Christiansen, L., Huge-Jensen, B., Norskov, L., Thim, L., Menge, U.: A serine protease triad forms the catalytic centre of a triacylglycerol lipase. Nature **343,** 767–770 (1990).
68. Schrag, J. D., Cygler, M.: A refined structure of the lipase from *Geotrichum candidum*. J Mol Biol **230,** 575–591 (1993).
69. Grochulski, P., Li, Y., Schrag, J. D., Bouthillier, F., Smith, P., Harrison, D., Rubin, B., Cygler, M.: Insights into interfacial activation from an open structure of *Candida rugosa* lipase. J Biol Chem **15,** 12843–12847 (1993).
70. Lang, D., Hoffman, B., Haalack, L., Hecht, H-J., Spener, F., Schmid, R. D., Schomburg, D.: Crystal structure of a bacterial lipase from *Chromobacterium viscosum* ATCC 6918 refined at 1.6 Å resolution. J Mol Biol **259,** 704–717 (1996).
71. Anthonsen, H. W., Babtista, A., Drablos, F., Martel, P., Petersen, S. B., Sebastiao, M., Vaz, L.: in Biotechnology Annual Review, Vol. I (M. R. El-Gewely, ed.), Elsevier Science, Amsterdam pp. 315–371 (1995).
72. Pleiss, J., Fischer, M., Peiker, M., Thiele, C., Schmid, R. D.: Lipase engineering database: Understanding and exploiting sequence-structure-function relationships. J Mol Catal B: Enzym **10,** 491–508 (2000).
73. Jaeger, K-E., Dijkstra, B. W., Reetz, M. T.: Bacterial biocatalysts: Molecular biology, three dimensional structures, and biotechnological applications of lipase. Annu Rev Microbiol **53,** 315–351 (1999).
74. Grochulski, P., Li, Y., Schrag, J. D., Cygler, M.: Two conformational states of *Candida rugosa* lipase. Protein Sci **3,** 82–91 (1994).
75. Louwrier, A., Drtina, G. J., Klibanov, A. M.: On the issue of interfacial activation of lipase in nonaqueous media. Biotechnol Bioeng **50,** 1–5 (1996).
76. Antonini, E., Carrea, G., Cremonesi, P.: Enzyme catalysed reactions in water-organic solvent two-phase systems. Enzyme Microb Technol **3,** 291–296 (1981).
77. Shield, J. W., Ferguson, H. D., Bommarius, A. S., Hatton, T. A.: Enzymes in reversed micelles as catalysts for organic-phase synthesis reactions. Ind Eng Chem Fundam **25,** 603–612 (1986).
78. Klyachko, N. L., Levashov, A. V., Kabanov, A. V., Khmelnitsky, Y. L., Martinek, K.: Catalysis by enzymes entrapped in hydrated surfactant aggregates having various structures in organic solvents. In: Gratzel M, Kalyanasundaram K (eds.), Kinetics and catalysis in microheterogeneous systems. Surfactant Science Series, vol. 38. Marcel Dekker, New York (1991).
79. Zaks, A., Klibanov, A. M.: Enzymatic catalysis in nonaqueous solvents. J Biol Chem **263,** 3194–3201 (1988).
80. Zaks, A., Klibanov, A. M.: The effect of water on enzyme action in organic media. J Biol Chem **263,** 8017–8021 (1988).
81. Hernaiz, M. J., Sanchez-Montero, J. M., Sinisterra, J. V.: Influence of the nature of modifier in the enzymatic activity of chemical modified semipurified lipase from *Candida rugosa*. Biotechnol Bioeng **55,** 252–260 (1997).
82. Wangikar, P. P., Michels, D. S., Clark, D. S., Dordick, J. S.: Structure and function of subtilisin BPN¢ solubilized in organic solvents. J Am Chem Soc **119,** 70–76 (1997).
83. Ito, Y., Fujii, H., Imanishi, Y.: Modification of lipase with various synthetic polymers and their catalytic activities in organic solvent. Biotechnol Prog **10,** 398–402 (1994).

84. Inada, Y., Mahoto, F., Sasaki, H., Kodera, Y., Hiroto, M., Nishimura, H., Matsushima, A.: Biomedical and biotechnological applications of PEG and PM-modified proteins. Trends Biotechnol **13,** 86–91(1995).
85. Gu, Q-M., Sih, C. J.: Improving the enantioselectivity of *C. cylindracea* lipase by chemical modification. Biocatalysis **6,** 115–126 (1992).
86. Calvo, M. V., Plou, F. J., Pastor, E., Ballesteros, A.: Effect of chemical modification of isoenzymes A and B from *C. rugosa* on their activity and stability. Biotechnol Lett **17,** 171–176 (1995).
87. Goto, M., Kamiya, N., Miyata, M., Nakashio, F.: Enzymatic esterification by surfactant-coated HL in organic media. Biotechnol Prog **10,** 263–268 (1994).
88. Basheer, S., Nakajima, M., Cogan, U.: Sugar ester-modified lipase for the esterification of fatty acids and long-chain alcohols. J Am Oil Chem Soc **73,** 1475–1479 (1996).
89. Mogi, K-I., Nakajima, M.: Selection of surfactant-modified lipases for interesterification of triglyceride and fatty acid. J Am Oil Chem Soc **73,** 1505–1512 (1996).
90. Kamiya, N., Goto, M.: How is enzymatic selectivity of menthol esterification catalyzed by surfactant-coated lipase determined in organic media? Biotechnol Prog **13,** 488–492 (1997).
91. Paradkar, V. M., Dordick, J. S.: Extraction and solubilization of chymotrypsin into isooctane in the presence of low concentrations of aerosol OT in the absence of reverse micelles. Biotechnol Bioeng **43,** 529–540 (1994).
92. Okazaki, S., Kamiya, N., Goto, M., Nakashio, F.: Enantioselective esterification of glycidol by surfactant-lipase complexes in organic media. Biotechnol Lett **19,** 541–543 (1997).
93. Russell, A. J., Klibanov, A. M.: Inhibitor-induced enzyme activation in organic solvents. J Biol Chem **263,** 11624–11626 (1988).
94. Affleck, R., Haynes, C. A., Clark, D. S.: Solvent dielectric effects on protein dynamics. Proc Natl Acad Sci USA **89,** 5167–5170 (1992).
95. Hartsough, D. S., Merz, K. M. Jr.: Protein dynamics and solvation in aqueous and nonaqueous environments. J Am Chem Soc **115,** 6529–6537 (1993).
96. Desai, U. R., Klibanov, A. M.: Assessing the structural integrity of a lyophilized protein in organic solvents. J Am Chem Soc **117,** 3940–3945 (1995).
97. Mingarro, I., Abad, C., Braco, L.: Interfacial activation-based molecular bioimprinting of lipolytic enzymes. Proc Natl Acad Sci USA **92,** 3308–3312 (1995).
98. Cambillau, C., van Tilbeurgh, H.: Structure of hydrolases-lipases and cellulases. Curr Opin Struct Biol **3,** 885–895 (1993).
99. Braco, L., Mingarro, I.: In Engineering of/with lipases (X. Malcata, ed.), Kluwer, Dordrecht, pp. 229–242 (1996).
100. Mingarro, I., Gonzalez-Navarro, H., Braco, L.: Trapping of different lipase conformers in water-restricted environments. Biochem **35,** 9935–9945 (1996).
101. Yilmaz, E.: Orlistat induced molecular bio-imprinting of microbial lipase. World J Microbiol Biotechnol **19,** 161–165 (2003).
102. Tsai, S. W., Dordick, J. S.: Extraordinary enantiospecificity of lipase catalysis in organic media induced by purification and catalyst engineering. Biotechnol Bioeng **52,** 296–300 (1996).
103. Torres, C., Otero, C.: Influence of the organic solvents on the activity in water and the conformation of *Candida rugosa* lipase: Description of a lipase-activating pretreatment Enzyme Microb Technol **19,** 594–600 (1996).

104. Kim, J., Kim, B-G.: Effect of the hydration state of supports before lyophilization on subtilisin-A activity in organic media. Biotechnol Bioeng **50,** 687–692 (1996).
105. Triantafyllou, A. O., Wehtje, E., Adlercreutz, P., Mattiasson, B.: How do additives affect enzyme activity and stability in nonaqueous media? Biotechnol Bioeng **54,** 67–76 (1997).
106. Kanwar, S. S., Srivastava, M., Chimni, S. S., Ghazi, I. A., Kaushal, R., Joshi, G. K.: Properties of an immobilized lipase of *Bacillus coagulans* BTS-1. ACTA Microbiol et Immunol Hungarica **51,** 57–73 (2004).
107. Balcao, V. M., Paiva, A. L., Malcata, F. X.: Bioreactors with immobilized lipases: State of the art. Enzyme Microb Technol **18,** 392–416 (1996).
108. Tantrakulsiri, J., Jeyashoke, N., Krisanangkura, K.: Utilization of rice hull ash as support material for immobilization of *Candida cylindracea* lipase. J Am Oil Chem Soc **74,** 173–175 (1997).
109. Rosu, R., Iwasaki, Y., Shimizu, N., Doisaki, N., Yamane, T.: Intesification of lipase performance in a transesterification reaction by immobilization on $CaCO_3$ powder. J Biotechnol **66,** 51–59 (1998).
110. Basri, M., Harun, A., Ahmad, M. B., Razak, C. A. N., Salleh, A. B.: Immobilization of lipase on poly (N-vinyl-2-pyrrolodone-co-styrene) hydrogel. J Appl Polym Sci **82,** 1404–1409 (2001).
111. Harun, A., Basri, M., Ahmad, M. B., Salleh, A. B.: Enantioselective esterification reaction using immobilized *Candida rugosa* lipase on *poly* (*N*-vinyl-2-pyrrolidone-*co*-styrene) hydrogel. J Appl Polym Sci **92,** 3381–3386 (2004).
112. Kanwar, S. S., Kaushal, R. K., Verma, M. L., Kumar, Y., Chauhan, G. S., Gupta, R., Chimni, S. S.: Synthesis of ethyl laurate by hydrogel immobilized lipase of *Bacillus coagulans* MTCC-6375. Ind J Microbiol **45,** 187–193 (2005).
113. Kanwar, S. S., Verma, H. K., Kumar, Y., Chimni, S. S., Chauhan, G. S.: Effect of solvents and kinetic parameters on synthesis of ethyl propionate by *poly* (AAc-*co*-HPMA-*cl*-MBAm)-matrix immobilized lipase of *Pseudomonas aeruginosa* BTS-2. World J Microbiol Biotechnol **21,** 1037–1044 (2005).
114. Park, E. Y., Sato, M., Kojima, S.: Fatty acid methyl ester production using lipase-immobilizing silica particles with different particle sizes and different specific surface areas. Enzyme Microb Technol **39,** 889–896 (2006).
115. Hsu, C-H, Tsai, S-W.: Improvements of *Acinetobacter radioresistens* lipase adsorption on celite 535 by adding salts. Tamkang J Sci Eng **4,** 133–139 (2001).
116. Serri, N. A., Kamaruddin, A. H., Long, W. S.: Studies of reaction parameters on synthesis of citronellyl laurate ester via immobilized *Candida rugosa* lipase in organic media. Bioprocess Biosyst Eng **29,** 253–260 (2006).
117. Rahman, M. B. A., Tajudin, S. M., Hussein, M. Z., Rahman, R. N. Z. R. A., Salleh, A. B., Basri, M.: Application of natural kaolin as support for the immobilization of lipase from *Candida rugosa* as biocatalsyt for effective esterification. Appl Clay Sci **29,** 111–116 (2005).
118. Ghamgui, H., Karra-Chaabouni, M., Gargouri, Y.: 1-Butyl oleate synthesis by immobilized lipase from *Rhizopus oryzae:* a comparative study between *n*-hexane and solvent-free system. Enzyme Microb Technol **35,** 355–363 (2004).
119. Zaidi, A., Gainer, J. L., Carta, G., Mrani, A., Kadiri, T., Belarbi, Y., Mir, A.: Esterification of fatty acids using nylon-immobilized lipase in *n*-hexane: Kinetic parameters and chain-length effects. J Biotechnol **93,** 209–216 (2002).

120. Silva, J. S., Jesus, P. C.: Evaluation of the catalytic activity of lipases immobilized on chrysotile for esterification. Annals Brazilian Acad Sci **75,** 157–162 (2003).
121. Kanwar, S. S., Kaushal, R. K., Verma, M. L., Kumar, Y., Azmi, W., Gupta, R., Chimni, S. S., Chauhan, G. S.: Synthesis of ethyl oleate employing synthetic hydrogel-immobilized lipase of *Bacillus coagulans* MTCC-6375. Ind J Biotechnol **6,** 68–73 (2007).
122. Kanwar, S. S., Sharma, C., Verma, M. L., Chauhan, S., Chimni, S. S., Chauhan, G. S.: Short-chain ester synthesis by trans-esterification employing poly (MAc-*co*-DMA-*cl*-MBAm)-hydrogel-bound lipase of *Bacillus coagulans* MTCC-6375. J Appl Polym Sci DOI: 10.1002/app.25320 (2007).
123. Kanwar, S. S., Verma, M. L., Maheshwari, C., Chauhan, S., Chimni, S. S., Chauhan, G. S.: Properties of *poly* (AAc-*co*-HPMA-*cl*-EGDMA) hydrogel-bound lipase of *Pseudomonas aeruginosa* MTCC-4713 in synthesis of methyl acrylate. J Appl Polym Sci **104,** 183–191 (2007).
124. Kanwar, S. S., Pathak, S., Verma, H. K., Kumar, S., Chimni, S. S., Chauhan, G. S.: Characteristics of poly (AAc5-co-HPMA-cl-EGDMA) hydrogel-immobilized lipase of Pseudomonas aeruginosa BTS-2. J Appl Polym Sci **100,** 4636–4644(2006).
125. Kanwar, S. S., Verma, H. K., Kumar, Y., Chimni, S. S., Chauhan, G. S.: Catalytic potential of a *poly* (AAc-*co*-HPMA-*cl*-MBAm)-matrix-immobilized lipase from a thermotolerant *Pseudomonas aeruginosa* MTCC-4713. J Appl Polym Sci **100,** 4252–4259 (2006).
126. Kanwar, S. S., Verma, H. K., Kumar, Y., Chimni, S. S., Chauhan, G. S.: Enhancement of ethyl propionate synthesis by Hg^{2+} and NH^{4+} ions exposed *poly* (AAc-*co*-HPMA-*cl*-MBAm)-immobilized lipase of *Pseudomonas aeruginosa* MTCC-4713. Acta Microbiologica et Immunologica Hungarica **53,** 195–207 (2006).
127. Dave, R., Madamwar, D.: Esterification in organic solvents by lipase immobilized in polymer of PVA-alginate-boric acid. Process Biochem **41,** 951–955 (2006).
128. Yunus, W. M. Z. W., Salleh, A. B., Basri, M., Razak, C. A. N., Ampon, K.: Poly (methyl acrylate-methyl methacrylate-divinyl benzene) beads for immobilization of lipase. Ann NY Acad Sci **10,** 80–87 (2004).
129. Halliwell, C. M., Simon, E., Toh, C. S., Bartlett, P. N., Cass, A. E. G.: Immobilization of lactate dehydrogenase on poly (aniline)-poly (acrylate) and poly (aniline)-poly- (vinyl sulphonate) films for use in a lactate biosensor. Anal Chim Acta **453,** 191–200 (2002).
130. Reetz, M. T., Rüggeberg, C. J., Dröge, M. J., Quax, W. J.: Immobilization of chiral enzyme inhibitors on solid supports by amide-forming coupling and olefin metathesis. Tetrahedron **58,** 8465–8473 (2002).
131. Dave, A., Vaishnav, U., Desai, R., Shah, A., Ankleshwaria, B., Mehta, M.: On the assessment of hydrogels for fluid absorbency. J Appl Polym Sci **58,** 853–859 (1995).
132. Sen, M, Guven, ve. O.: Prediction of swelling behaviours of diprotic acid containing hydrogels. Polym **39,** 1165–1172 (1998).
133. Chauhan, G. S., Mahajan, S., Sddiqui, K. M., Gupta, R.: Immobilization of lipase on hydrogels: Structural aspects of polymeric matrices as determinants of enzyme activity in different physical environments. J Appl Polym Sci **92,** 3135–3143 (2004).
134. Janssen, A. E. M., Van der Padt, A., Van Sonsbeek, H. M., Van't, Riet.: The effect of organic solvents on the equilibrium position of enzymatic acylglycerol synthesis. Biotechnol Bioeng **41,** 95–103 (1993).
135. Janssen, A. E. M., Van der Padt, A., Van't Riet, K.: Solvent effects on lipase-catalysed esterification of glycerol and fatty acids. Biotechnol Bioeng **42,** 953–962 (1993).

136. Kuo, S-J., Parkin, K. L.: Solvent polarity Influences product selectivity of lipase-mediated micro aqueous media. J Am Oil Chem Soc **73,** 1427–1433 (1996).
137. Laane, C., Boeren, S., Vos, K., Veeger, C.: Roles for optimization of biocatalysis in organic-solvents. Biotechnol Bioeng **30,** 81–87 (1987).
138. Valivety, R. H., Johnston, G. A., Suckling, C. J., Halling, P. J.: Solvent effects on Biocatalysis in organic systems: Equilibrium position and rates of lipase catalyzed esterification. Biotechnol Bioeng **38,** 1137–1143 (1991).
139. Kobayashi, T., Furutani, W., Adachi, S., Matsuno, R.: Equilibrium constant for the lipase-catalyzed synthesis of fatty acid butyl ester in various organic solvents. J Mol Catal B: Enzym **24–25,** 61–66 (2003).
140. Yang, Z., Robb, D. A.: Partition coefficients of substrates and products and solvent selection for biocatalysis under nearly anhydrous conditions. Biotechnol Bioeng **43,** 365–370 (1994).
141. Martins, J. F., Correa de Sampaio, T., Borges de Carvalho, I., Barreiros, S.: Lipase catalyzed esterification of glycidol in nonaqueous solvents: solvent effects on enzymatic activity. Biotechnol Bioeng **44,** 119–124 (1994).
142. Van Tol, J. B. A., Stevens, R. M. M., Veldhuizen, W. J., Jongejan, J. A., Duine, J. A.: Do organic solvent affects the catalytic properties of lipase? Intrinsic kinetic parameters of lipase in ester hydrolysis and formation in various organic solvent. Biotechnol Bioeng **47,** 71–81(1995).
143. Vazquez Lima, F., Pyle, D. L., Asenjo, J. A.: Factors affecting the esterification of lauric acid using an immobilized biocatalyst: Enzyme characterization and studies in a well-mixed reactor. Biotechnol Bioeng **46,** 69–79 (1995).
144. Wangikar, P. P., Graycar, T. P., Estell, D. A., Clark, D. S., Dordick, J. S.: Protein and solvent engineering of subtilisin BPN in nearly anhydrous organic media. J Am Chem Soc **115,** 12231–12237 (1993).
145. Gorman, L. A. S., Dordick, J.: Organic solvents strip water of enzymes. Biotechnol Bioeng **39,** 392–397 (1992).
146. Almarsson, O., Klibanov, A. M.: Remarkable activation of enzymes in nonaqueous media by denaturing organic cosolvents. Biotechnol Bioeng **49,** 87–92 (1996).
147. Degn, P., Zimmermann, W.: Optimization of carbohydrate fatty acid ester synthesis in organic media by a lipase from *Candida antarctica*. Biotechnol Bioeng **74,** 483–491 (2001).
148. Otero, C., Arcos, J. A., Berrendero, M. A., Torres, C.: Emulsifiers from solid and liquid polyols: Different strategies for obtaining optimum conversions and selectivities. J Mol Catal B: Enzym **11,** 883–892 (2001).
149. Castillo, E., Pezzotti, F., Navarro, A., Lopez-Munguia, A.: Lipase catalysed synthesis of xylitol monoesters: Solvent engineering approach. J Biotechnol **102,** 251–259 (2003).
150. Krishna, S. H., Divakar, S., Prapulla, S. G., Karanth, N. G.: Enzymatic synthesis of isoamyl acetate using immobilized lipase from *Rhizomucor miehei*. J Biotechnol **87,** 193–201 (2001).
151. Gatfield, I. L.: The enzymatic synthesis of esters in nonaqueous systems. Annals New York Acad Sci **434,** 569–572 (1984).
152. Miller, C., Austin, H., Posorske, L., Gonzlez, J.: Characteristics of an immobilized lipase for the commercial synthesis of esters. J Am Chem Soc **65,** 927–932 (1988).
153. Claon, P. A., Akoh, C. C.: Enzymatic synthesis of geraniol and citronellol esters by direct esterification in n-hexane. Biotechnol Lett **15,** 1211–1216 (1993).

154. Therisod, M., Klibanov, A. M.: Regioselective acylation of secondary hydroxyl groups in sugars catalysed by lipases in organic solvents. J Am Chem Soc **109,** 3977–3981 (1987).
155. Ciuffreda, P., Colombo, D., Ronchetti, F., Toma, L.: Regioselective acylation of 6-deoxy-L- and -D-hexosides through lipase-catalyzed transesterification: Enhanced reactivity of the 4-hydroxy function in the L series. J Org Chem **55,** 4187–4190 (1990).
156. Colombo, D., Ronchetti, F., Toma, L.: Enzymic acylation of sugars: Rationale of the regioselective butyrylation of secondary hydroxy groups of D- and L-galacto and mannopyranosides. Tetrahedron **47,** 103–110 (1991).
157. Sin, Y. M., Cho, K. W., Lee, T. H.: Synthesis of fructose esters by *Pseudomonas* sp. lipase in anhydrous pyridine. Biotechnol Lett **20,** 91–94 (1998).
158. Otto, R. T., Bornscheuer, U. T., Scheib, H., Pleiss, J., Syldatk, C., Schmid, R. D.: Lipase catalyzed esterification of unusual substrates: Synthesis of glucuronic acid and ascorbic acid (vitamin C) esters. Biotechnol Lett **20,** 1091–1094 (1998).
159. Degn, P., Pedersen, L. H., Duus, J., Zimmermann, W.: Lipase-catalyzed synthesis of glucose fatty acid esters in tert-butanol. Biotechnol Lett **21,** 275–280 (1999).
160. Nicotra, F., Riva, S., Secundo, F., Zuchelli, L.: An interesting example of complementary regioselective acylation of secondary hydroxyl groups by different lipases. Tetrahedron Lett **30,** 1703–1704 (1989).
161. Rich, J. O., Bedell, B. A., Dordick, J. S.: Controlling enzyme catalyzed regioselectivity in sugar ester synthesis. Biotechnol Bioeng **45,** 426–434 (1995).
162. Woundenberg-van Oosterom, M., van Rantwijk, F., Sheldon, R. A.: Regioselective acylation of disaccharides in tert-butyl alcohol catalyzed by *Candida antarctica* lipase. Biotechnol Bioeng **49,** 328–333 (1996).
163. Rubio, E., Fernandes-Mayorales, A., Klibanov, A. M.: Effect of solvent on enzyme regioselectivity. J Am Chem Soc **113,** 695–696 (1991).
164. MacManus, D. A., Vulfson, E. N.: Reversal of regioselectivity in the enzymatic acylation of secondary hydroxyl groups mediated by organic solvents. Enzyme Microb Technol **20,** 225–228 (1997).
165. Kim, J. E., Han, J. J., Yoon, J. H., Rhee, J. S.: Effect of salt hydrate pair on lipase-catalyzed regioselective monoacylation of sucrose. Biotechnol Bioeng **57,** 121–125 (1998).
166. Valivety, R. H., Halling, P. J., Macrae, A. R.: Reaction rate with suspended lipase catalyst shows similar dependence on water activity in different organic solvents. Biochim Biophys Acta **1118,** 218–222 (1992).
167. Wangikar, P. P., Rich, J. O., Clark, D. S., Dordick, J. S.: Probing enzymic transition state hydrophobicities. Biochemistry **34,** 12302–12310 (1995).
168. Svensson, I., Wehtje, E., Adlercreutz, P., Mattiasson, B.: Effects of water activity on reaction rates and equilibrium positions in enzymatic esterification. Biotechnol Bioeng **44,** 549–556 (1994).
169. Dudal, Y., Lortie, R.: Influence of water activity on the synthesis of triolein catalyzed by immobilized *Mucor Miehei* lipase. Biotechnol Bioeng **45,** 129–134 (1995).
170. Halling, P. J.: Thermodynamic predictions for biocatalysis in non-conventional media: Theory, tests and recommendations for experimental design and analysis. Enzyme Microb Technol **16,** 178–206 (1994).
171. Wehtje, E., Adlercreutz, P.: Water activity and substrate concentration effects on lipase activity. Biotechnol Bioeng **55,** 798–806 (1997).

172. Bell, G., Janssen, A. E. M., Halling, P. J.: Water activity fails to predict critical hydration level for enzyme activity in polar organic solvents: Interconversion of water concentrations and activities Enzyme Microb Technol **20,** 471–477 (1997).
173. Oladepo, D. K., Halling, P. J., Larsen, V. F.: Reaction rates in organic media show similar dependence on water activity with lipase catalyst immobilised on different supports. Biocatalysis **8,** 283–287 (1994).
174. Berglund, P., Vorde, C., Hogberg, H. E.: Esterification of 2-methylalkanoic acids catalysed by lipase from candida rugosa: Enantioselectivity as a function of water activity and alcohol chain length. Biocatalysis **9,** 123–130 (1994).
175. Bin, K. S., Choi, C. Y.: Effect of solid salt hydrates on the asymmetric esterification of 2-chloropropionic acid: Control of water activity in organic solvent. Biotechnol Lett **17,** 1075–1076 (1995).
176. Zacharis, E., Omar, I. C., Partridge, J., Robb, D. A., Halling, P. J.: Selection of salt hydrate pairs for use in water control in enzyme catalysis in organic solvents. Biotechnol Bioeng **55,** 367–374 (1997).
177. Van der Padt, A., Sewalt, J. J. W., Van't Riet, K.: On-line water removal during enzymatic triacylglycerol synthesis by means of pervaporation. J Membr Sci **80,** 199–208 (1993).
178. Mulder, M.: Basic Principles of Membrane Technology, Kluwer Academic, Dordrecht (1991).
179. Kwon, S. J., Song, K. M., Hong, W. H., Rhee, J. S.: Removal of water produced from lipase catalyzed esterification in organic solvent by pervaporation. Biotechnol Bioeng **46,** 393–395 (1995).
180. Ujang, Z., Sharbati, N. A., Vaidya, A. M.: Organic-phase enzymatic esterification in a hollow fiber membrane reactor with in situ gas-phase water activity control. Biotechnol Prog **13,** 39–42 (1997).
181. Greenspan, L.: Humidity fixed points of binary saturated aqueous solutions. J Res Natl Bur Stand Phys Chem **81,** 89–96 (1977).
182. Goderis, H., Ampe, G., Feyten, M., Fouwe, B., Guffens, W. V., Cauwenbergh, S., Tobback, P.: Lipase-catalyzed ester exchange reactions in organic media with controlled humidity. Biotechnol Bioeng **30,** 258–266 (1987).
183. Wehtje, E., Kaur, J., Adlercreutz, P., Chand, S., Mattiasson, B.: Water activity control in enzymatic esterification processes. Enzyme Microb Technol **21,** 502–510 (1997).
184. Wehtje, E., Svensson, I., Adlercreutz, P., Mattiasson, B.: Continuous control of water activity during biocatalysis in organic media. Biotechnol Tech **7,** 873–878 (1993).
185. Kaur, J., Wehtje, E., Adlercreutz, P., Chand, S., Mattiasson, B.: Water activity control in enzymatic esterification processes. Enzyme Microb Technol **21,** 502–510 (1997).
186. Rosell, C. M., Vaidya, A. M., Halling, P. J.: Continuous *in-situ* water activity control for organic phase biocatalysis in a packed bed hollow fiber reactor. Biotechnol Bioeng **49,** 284–289 (1996).
187. He, X. L., Chen, B. Q., Tan, T. W.: Enzymatic synthesis of 2- ethylhexyl esters of fatty acids by immobilized lipase from *Candida* sp. J Mol Catal B: Enzym **18,** 333–339 (2002).
188. Sonwalkar, R. D., Chen, C. C., Ju, L. K.: Roles of silica gel in poly-condensation of lactic acid in organic solvent. Biores Technol **87,** 69–73 (2003).
189. Chamouleau, F., Coulon, D., Girardin, M., Ghoul, M.: Influence of water activity and water content on sugar esters lipase-catalyzed synthesis in organic media. J Mol Catal B: Enzym **11,** 949–954 (2001).

190. Giacometti, J., Giacometti, F., Milin, C., Racki, D. V.: Kinetic characterization of enzymatic esterification in a solvent system: Adsorptive control of water with molecular sieves. J Mol Catal B: Enzym **11,** 921–928 (2001).
191. Kuwabara, K., Watanabe, Y., Adachi, S., Nakanishi, K., Mutsuno, R.: Synthesis of 6-O-unsaturated acyl L-ascorbates by immobilized lipase in acetone in the presence of molecular sieve. Biochem Eng J **16,** 17–23 (2003).
192. Liebeton, K., Zonta, A., Schimossek, K., Nardini, M., Lange, D., Dijkstra, B. W., Reetz, M. T., Jaeger, K-E.: Directed evolution of an enantioselective lipase. Chem Biol **7,** 709–718 (2000).
193. Jaeger, K-E., Reetz, M. T.: Directed evolution of enantioselective enzymes for organic chemistry. Curr Opin Chem Biol **4,** 68–73 (2000).
194. Jaeger, K-E., Eggert, T., Eipper, A., Reetz, M. T.: Directed evolution and the creation of enantioselective biocatalysts. Appl Microbiol Biotechnol **55,** 519–530 (2001).
195. Zhang, N. Y., Suen, W. C., Windsor, W., Xiao, L., Madison, V., Zaks, A.: Improving tolerance of *Candida antarctica* lipase B towards irreversible thermal inactivation through directed evolution. Prot Eng **16,** 599–605 (2003).
196. Suen, W-C., Zhang, N., Xiao, L., Madison, V., Zaks, A.: Improved activity and thermostability of Candida antarctica lipase B by DNA family shuffling. Protein Eng Des Sel **17,** 133–140 (2004).
197. Chodorge, M., Fourage, L., Ullmann, C., Duvivier, V., Masson, J. M., Lefevre, F.: Rational strategies for directed evolution of biocatalysts-Application to *Candida antarctica* lipase B (CALB). Adv Synth Catal **347,** 1022–1026 (2005).
198. van Kampen, M. D., Egmond, M. R.: Directed evolution: From a staphylococcal lipase to a phospholipase. Eur J Lipid Sci Technol **102,** 717–726 (2000).
199. Kauffmann, I., Schmidt-Dannert, C.: Conversion of *Bacillus thermocatenulatus* lipase into an efficient phospholipase with increased activity toward long-chain fatty acyl substrates by directed evolution and rational design. Protein Eng **14,** 919–928 (2001).
200. Lorenz, P., Liebeton, K., Niehaus, F., Eck, J.: Screening for novel enzymes for biocatalytic processes: accessing the metagenome as a resource of novel functional sequence space. Curr Opin Biotechnol **13,** 572–577 (2002).
201. Henne, A., Schmitz, R. A., Bomeke, M., Gottschalk, G., Daniel, R.: Screening of environmental DNA libraries for the presence of genes conferring lipolytic activity on *Escherichia coli*. Appl Environ Microbiol **66,** 3113–3116 (2000).
202. Bell, P. J., Sunna, A., Gibbs, M. D., Curach, N. C., Nevalainen, H., Bergquist, P. L.: Prospecting for novel lipase genes using PCR. Microbiol **148,** 2283–2291(2002).
203. Evans, J. D., Sikdar, S. K.: Biodegradable plastics: an idea whose time has come? Chemtech **20,** 38–42 (1990).
204. Hill, K., Rhode, O.: Sugar-based surfactants for consumer products and technical applications. Fett-Lipid **101,** 25–33 (1999).
205. Bloomer, S., Adlercreutz, P., Mattiasson, B.: Kilogram-scale ester synthesis of acyl donor and its use in lipase-catalyzed interesterifications. J Am Oil Chem Soc **69,** 966–973 (1992).
206. Bourg-Garros, S., Razafindramboa, N., Pavia, A. A.: Large-scale preparation of (Z)-3-hexen-1-yl acetate using *Candida Antarctica* immobilized lipase in hexane. Biotechnol Bioeng **59,** 495–500 (1998).

Table I

Applications of esters synthesis by lipases in Biotechnology Industry

Ester type	Applications	References
Carbohydrates fatty acid esters	Antimicrobial, antitumorals, cosmetics, anticaries properties and insecticidals	[18–22]
Fatty acid esters of hydroxyl acids (lactic acid, citric acid, alkyl lactates)	Surfactants in food industry and cosmetics	[17, 23, 24]
Flavonoid esters, a group of polyphenolic compounds, found ubiquitously in fruits and vegetables	Broader applications like dietetic, nutritional, antioxidants, cosmetic and pharmacological	[14, 25–29]
Amino acyl esters of carbohydrates	Sweetening agents, surfactants, microcapsules in pharmaceutical preparations, active nucleoside amino acid esters, antibiotics and in the delivery of biologically active agents	[30–32]
Esters of long-chain acids with long-chain alcohols (12–20 carbon atoms)	Plasticizers and lubricants	[1, 13, 33, 34]
Esters of long chain fatty acids	Fuel (biodiesel) and waxes (oleo-chemical industries)	[1, 5, 34–36]
Fatty acid esters of sugars/sugar alcohol	Surfactants/emulsifiers used in food, detergent, cosmetic and pharmaceutical industries	[22, 37]
Medically important esters viz. ferulates, ascorbyl palmitate, estrogen palmitate, estrogen myristate, estrogen oleate, inositol hexanicinate, vitamin E acetate, Canola phytosterols oleate and L-Ascorbyl linoleate	Absorb the harmful ultraviolet light and are active ingredients in many sunscreens for anti-aging and anti-wrinkling, cholesterol lowering agents and inhibition of cancer	[38–43]
Fragrance esters viz butyl butyrate (pineapple), ethyl cinnamate (cinnamon), ethyl isovalerate (apple), ethyl lactate (butter, cream), geranyl acetate (geranium), geranyl butyrate (cherry), isopropyl acetate (fruity), methyl butyrate (pineapple, apple), methyl salicylate (root beer, wintergreen, Germolene™ and Ralgex™ ointments (UK)	Artificial flavorings and fragrances	[1, 44, 45]
Biodegradable polyesters like 1-butyl oleate, trimethylolpropane	Lubricants, applications in automobiles and petrochemical industry	[46]

Table II

Various solvent systems used in ester synthesis in lipase catalysis reaction

Solvent media	Salient features
Nonaqueous reaction media (water is not more than the absorptive capacity of the reaction medium)	Extremely reliable, versatile, simple, and easy to use. Water-immiscible solvents retain enzyme activity because they do not strip-off water from enzyme.
Anhydrous reaction media (water content is < 0.01% i.e., near the sensitivity limit of the classical Karl-Fischer method)	Use of lipase in anhydrous media has been limited.
Supercritical fluids (supercritical solvents like carbon dioxide used by compressing them at their critical temperatures)	Rapid mass transfer due to low viscosity, non-toxicity, non-flammability and simple down-stream processingby evaporation. Disadvantages include considerable initial investment for high-pressure equipment and enzyme deactivation due to depressurization step.
Reverse micellar systems (enzymes entrapped in hydrated reverse micelles of surfactants in organic solvents)	Thermodynamicaly stable, optically transparent and retention of lipolytic activity due to presence of interface.
Solvent-free systems (no use of organic solvent, particularly to the synthesis of food-grade flavors and additives)	Facilitates downstream processing since fewer components would be present at the end of the reaction thus minimizing the production cost. Also permits the use of high substrate concentrations. Problems arise when both substrates are solids and also when mass transfer limitations are associated due to the high viscosity of the substrates.
Ionic liquid or molten salts (composed entirely of ions)	Simple, inexpensive to manufacture and easy to recycle.

Table III

Lipase(s) immobilization on different support and their application(s)

Support	Direct enzymatic application	Reference(s)
Silica	A suitable cheap method for immobilizing lipase	[43, 114]
Celite	Improvement of lipase immobilization by adding salts	[115]
Amberlite	Synthesis of citronellyl laurate ester	[116]
Natural kaolin	Studies on effective esterification	[117]
$CaCO_3$	1-Butyl oleate synthesis	[118]
Nylon	Esterification of fatty acids	[119]
Chrysotile	Catalytic activity of immobilized lipases	[120]
Poly (AAc-*co*-HPMA-*cl*-EGDMA), *Poly* (AAc-*co*-HPMA-*cl*-MBAm), *Poly* (MAc-*co*-DMA-*cl*-MBAm)	Synthesis of methyl esters and various ethyl esters synthesis	[121–126]
Poly (PVA-alginate-boric acid)	Esterification studies	[127]
Poly (methyl acrylate-methyl methacrylate-divinyl benzene)	Studies on enhanced catalytic stability	[128]
Poly (aniline)-*poly* (acrylate) (PAN_i–PAA) or *poly* (aniline)-*poly* (vinyl sulphonate)	Biosensor development and biochemical fuel cell	[129]
Poly (N-vinyl-2-pyrrolidone-*co*-styrene)	Enantioselective esterification of *(R, S)*-2-(4-chlorophenoxy)-propanoic acid with *n*-tetradecanol	[111]
Poly (N-vinyl-2-pyrrolidone-*co*-styrene)	Butyl oleate	[110]
Tentagel (R) (polymer)	Olefin metathesis	[130]

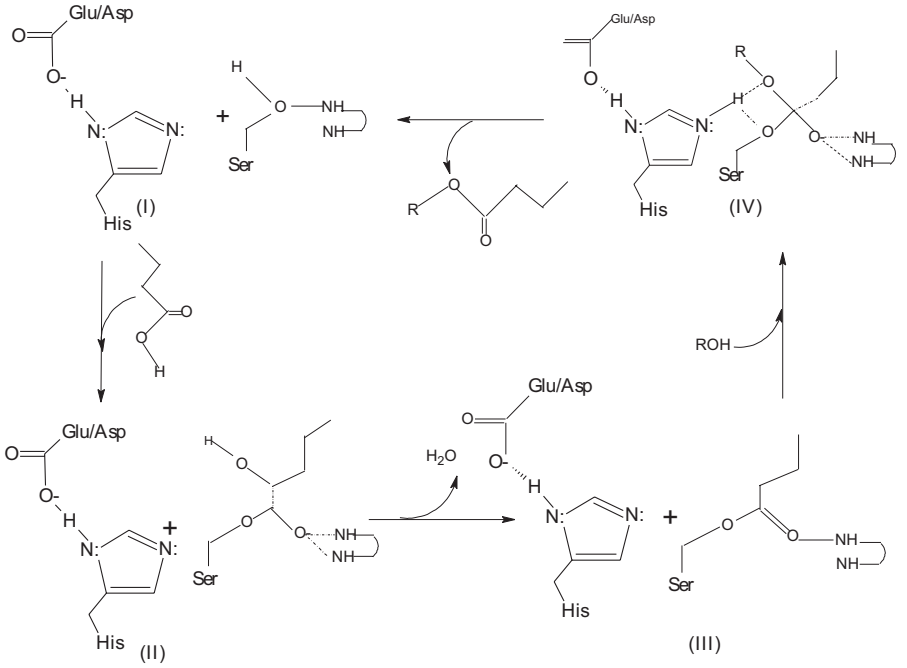

Figure 1. Mechanism of lipase-catalyzed esterification. (1) Binding of substrate, activation of nucleophilic serine residue by neighboring histidine and nucleophilic attack of the substrate's carbonyl carbon atom by Ser. (2) Transient tetrahedral intermediate that loses a water molecule to give an acyl enzyme complex. (3) An alcohol molecule attacks the acyl enzyme complex (nucleophilic attack) to give (4) another tetrahedral intermediate that releases an ester molecule to convert the lipase to its native form