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Review

Lipase catalysis in ionic liquids/supercritical carbon dioxide and its applications

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ABSTRACT

Ionic liquids (ILs) and supercritical carbon dioxide (SC-CO_2) have been accepted as solvents facilitating green processing by lots of scholars and researchers in recent decades. The combination of these two solvents owns advantages, such as improving the stability of the enzymes, less or no pollutants. Enzymatic catalysis has been thought as one of the potent methods to replace the chemical catalysis, which has some drawbacks, such as using chemical catalysts caused hard to recycle and to pollute the environment, because the enzymes are easy to be degraded. In the enzymes, lipases are important and potent in industrial application. Therefore, lipase catalysis in IL/SC-CO_2 is green and benign to the environment. In this paper, advantages, mass transfer, characteristics and applications of lipase catalysis in IL/SC-CO_2 are reviewed.

Contents

1. Introduction

Lipases are widely available in nature and broadly used in chemical reactions, such as ester hydrolysis, esterification, transesterification, amidation, and so forth. Furthermore, lipases accept a wide variety of substrates while maintaining their regioselectivity and stereoselectivity. Applications of lipases are very wide, including food additives, chiral intermediates in pharmaceutical products and pesticide products [1].

Because of the solubility of substrates and absence of water eliminates the competing hydrolysis reaction; lipase-catalyzed esterification and transesterification in anhydrous media, such as organic solvents, supercritical fluids and ionic liquids, have been an area of major research activities in the past decades [1–3]. Among the supercritical fluids, SC-CO_2 owns the benefits of an environmentally benign nature, such as non-flammability, high availability, low toxicity, and an ambient critical temperature [4]. Recently, ionic liquids (ILs) are considered as an alternative to organic solvents for biocatalysis and biotransformations in view of sustainable and ultimately "green" processes, not only because enzymes displayed high level of activity and stereoselectivity of many different chemical transformations (esterifications, transesterifications, kinetic resolution, and so forth), but mainly because

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of an overstabilization effect on biocatalysts (up 2300-folds half-life time respect to classical organic solvents) [5]. Moreover, recent researches have verified the possibility to carry out integral green biocatalytic processes by combining these two completely different neoteric solvents (ILs and SC-CO_2) with enzymes, because their different miscibilities produce two-phase systems that show an exceptional ability to perform both the biotransformation and the products extraction steps simultaneously, even under extremely extreme conditions (such as 150 °C and 100 bar) [6–8]. In this review, the characteristics and applications of lipase catalysis in ILs/SC-CO_2 have been presented.

2. Lipase catalysis and applications

Lipases catalyze hydrolysis of triglycerides to the corresponding fatty acids and glycerol in nature. Other reactions, such as esterification, amidation and transesterification, are also catalyzed by lipases. Because of their ready availability, low cost of production, and enormous utilities in organic syntheses, lipases are used widely in industry and in laboratory. Now, lipases from a number of different sources, especially from microorganisms, are commercially available. Each of these lipases owns distinct substrate specificity, regioselectivity and stereoselectivity. However, the substrate specificity of lipases is known to be less rigorous compared to other enzymes [1]. The commercially available lipases have provided biocatalysts that have broad substrate specificity, even accepting substrates that are structurally quite dissimilar to the natural substrates, while maintaining a high degree of regioselectivity and stereoselectivity for individual substrates. For a specific application, one can choose the most suitable lipase from the commercially available ones. Furthermore, lipases have the usual advantages owned by enzymes, such as mild operating conditions, biodegradability, and so forth. Therefore, lipases have tremendous potential as industrial catalysts [1].

The currently commercialized as well as the potential application of lipases include [1]: (1) Synthesis of chiral compounds. Lipase-mediated optical resolution processes typically involve enantioselective hydrolysis of a racemic mixture of an ester or enantioselective esterification/transesterification of a racemic mixture of an acid/ester followed by separating of the acid from the ester. For example, kinetic resolution of 1-phenylethanol integrated with separation of substrates and products by a supported ionic liquid membrane was reported recently by Hernández-Fernández et al. [9]. (2) Carbohydrate ester synthesis. Monoesters of carbohydrates are surfactants of good biodegradability and low toxicity and therefore, have applications in detergents, food products (i.e., as food emulsifier), and so on. Regioselective monoacylation of sugars can be catalyzed by lipases, which was performed chemically. (3) Polyunsaturated fatty acid purification/enrichment. Polyunsaturated fatty acids, such as docosahexaenoic acid, eicosapentaenioc acid, and γ-linolenic acid, need to be selectively extracted/enriched from natural oils in order to use them in medical applications and food additives. Processes have been developed for the purification polyunsaturated fatty acids by utilizing substrate selectivity of lipases. (4) Synthesis of biologically active compounds. Lipase-catalyzed synthesis of various biologically active compounds, such as alkaloids, antibiotics, terpenoids, pheromones, and so on, was completed. (5) Ester synthesis for perfumes and flavors. Short-chain fatty acid esters synthesized with lipases are useful in making fruity flavors. (6) Synthesis of structured lipids. (7) Synthesis of organic carbonates. Organic carbonates can be synthesized via lipase-catalyzed transesterification involving carbonates and alcohols in a water-restricted environment.

3. Ionic liquids and SC-CO_2

3.1. Ionic liquids

Ionic liquids (ILs) are organic salts with melting points below 373 K. ILs include cations and anions. The cations and anions in ILs applied in biocatalysis found in the literature are shown in Fig. 1. From the 26 cations and 20 anions yielding 520 combinations, only

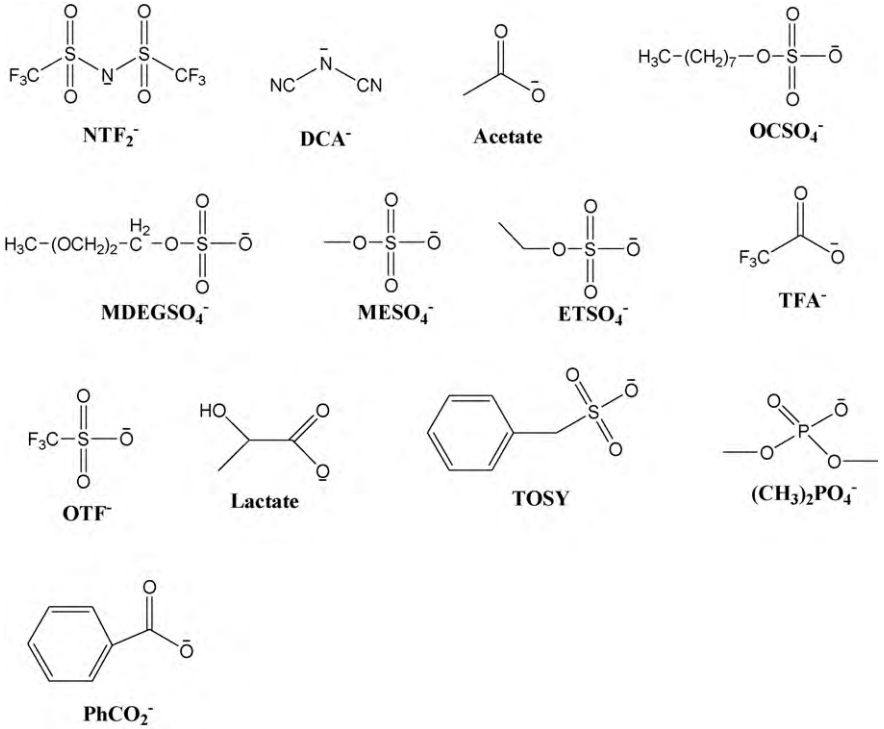


Fig. 1. Structures and abbreviations of IL cations and anions applied in biocatalysis.

11% have been applied. From the commercial availability, [BMIM]-based ILs have gained the main attention with [BF_4] and [PF_6] and recently [NTF_2] and [OTF] as anions [2].

Unlike molecular liquids, ILs usually have negligible vapor pressures [10]. They also own thermal stability [11], and tunable properties with regard to polarity, hydrophobicity and solvent miscibility behavior through appropriate modification of the cation and anion [12]. The behavior of ILs is similar to organic solvents in contact with enzymes, and ILs could replace organic solvents as solvents facilitating green processing in many enzymatic processes [12]. Enzymatic activities in ILs are generally comparable with or higher than those observed in conventional organic solvents [6–8,12–15]. Furthermore, enhanced thermal [8,13] and operational stabilities [16,17], regioselectivities and stereoselectivities [6–8,12–24] of enzymes were discovered.

3.2. SC-CO_2

Supercritical fluids have the unique properties to present a grand opportunity to discover range of novel chemical processes. Among many these fluids, SC-CO_2 has the added benefits of an environmental benign nature, low toxicity, non-flammability, high availability and ambient critical temperature (T_c = 31.0 °C) [4]. Supercritical fluids are different from ordinary solvents in owning gas-like low viscosities, high diffusivities and their liquid-like solubilizing power. Furthermore, these properties are tunable by manipulating the pressure and temperature. Small changes in pressure or temperature can lead to significant changes in density and density-dependant solvent properties, such as the dielectric coefficient. When solvent effects on the reaction are determined with supercritical fluids, it can be done without changing the kind of solvent. Moreover, the solvent properties can be changed continuously by varying the pressure and temperature, so continuous change in a reaction can be expected.

The first report on the lipase-catalyzed reactions in supercritical fluids was in 1985 by Randolph et al. [25]. From then on, using supercritical fluids, especially SC-CO_2, as the reaction media in lipase-catalyzed reactions has been one of the hot research points in recent decades. The benefits [25,26] of using supercritical fluids for lipase-catalyzed reactions are high reaction rates, good control of selectivities [13,27–33], including regioselectivities and stereoselectivities, etc.

4. Lipase catalysis in the ionic liquids/SC-CO_2

Since the first report about the combination of ILs and SC-CO_2 [34,35], the system has widely been used in biocatalysis, especially lipase catalysis [6–8,22,37–47].

4.1. Advantages of using ionic liquids/SC-CO_2 biphasic system in lipase-catalyzed reactions

ILs have obvious potential as reaction media, and one important challenge is to use their unique solvent properties to develop efficient methods for product separation and IL recycling [46]. SC-CO_2 has adverse effects on enzyme activity due to the chemical modification of the free amino groups, local pH changes caused by CO_2, or conformational changes produced during the pressurization/depressurization steps, making it necessary to develop new stabilization strategies for enzyme [48,49]. The combination of ILs and SC-CO_2 could solve the problems aroused by SC-CO_2 alone.

The first advantage of lipase catalysis in IL/SC-CO_2 system is that the system could have a stabilizing effect against enzyme deactivation caused by SC-CO_2 as the reaction medium alone. This own to ILs, which is helpful to stabilize the activity of lipase. Some ILs have been proved to be by far the best nonaqueous media for enzyme-catalyzed reactions, not only because the enzymes display a high level of activity and stereoselectivity for many different chemical transformations, e.g., aliphatic ester synthesis [13,50], the kinetic resolution of racemic alcohols [51], carbohydrate ester synthesis [52], polymer synthesis [53], etc., but also because of an overstabilization effect on the biocatalysts (over 2,300-fold half-life time with respect to classical organic solvents) [16,54,55]. In the system, lipases are mainly suspended in the IL phase, not in SC-CO_2 phase. Thus, lipases can keep stable in the catalysis, even the activity of lipases being strengthened. Lozano et al. [8] reported that lipases remain active even under extremely harsh conditions (e.g., 150 °C and 10 MPa). However, it has also been reported that the activity of lipases in ILs/SC-CO_2 systems was 10 times lower than that observed without SC-CO_2 [6].

The second advantage of lipase catalysis in ILs/SC-CO_2 system is that the combination of ILs and SC-CO_2 is helpful to solve the problems of ILs used alone, which caused product separation and IL recycling. Extraction with solvents immiscible with the ILs, giving biphasic systems, is one of the simplest methods to separate the products from the IL phase. Drawbacks are the extraction of small amounts of the IL and eventually of the catalyst, if a catalytic reaction is involved [56]. Also, the partitioning of the solutes between the phases limits the extent of solute extraction, and the use of VOSs is obviously a breakdown-point for the integral green design of the process [46,57,58]. Alternatively, supercritical fluids, such as SC-CO_2, are environmentally benign solvents that enable efficient isolation of organic products. SC-CO_2 has been described as an excellent solvent for the transport of hydrophobic compounds, because its solvent properties can be adjusted by changing either the pressure or the temperature, and it is employed in a wide range of industrially extractive clean processes [59]. Brennecke et al. discovered that SC-CO_2 can dissolve over 0.6 mole fraction in 1-butyl-3-methylimidiazolium hexafluorophosphate ([BMIM][PF_6]) while no IL is detected in the vapor phase [36,58]. They have also reported that the exceptional ability of SC-CO_2 to extract a wide variety of hydrophobic compounds from the ILs. Therefore, there is a continuing interest in developing new systems for biphasic or phase-separable catalysis, whereby a homogeneous catalyst is immobilized in the IL phase and reactants and/or products reside largely in the SC-CO_2 phase [6,7,60].

So the employment of these neoteric solvents (ILs/SC-CO_2) as novel media for enzyme-catalyzed transformations has attracted considerable interest.

4.2. Mass transfer of lipase catalysis in the ionic liquids/SC-CO_2

Extraction of solutes from the ILs with SC-CO_2 in lipase catalysis is one of the most important advantages of the biphasic systems. The applicability of SC-CO_2 for extraction of solutes from IL heavily relies on the phase behavior of the binary IL/SC-CO_2 system. CO_2 dissolution in the IL-rich phase not only is necessary for contact with the desired solute but decreases the viscosity of the IL. CO_2 readily dissolves in the liquid phase of all the ILs tested, while the IL remains insoluble in the CO_2 vapor phase [57]. This improves the mass transfer of the IL/SC-CO_2 system.

The mass transfer of lipase catalysis in the ionic liquids/SC-CO_2 is shown in Fig. 2. Lipases are immobilized on the support in the IL phase. The substrates are dissolved in SC-CO_2 and diffused into IL phase, then to the active center of lipases. The products of the lipase-catalyzed reaction are released to IL phase, and then extracted by SC-CO_2.

Lozano et al. [44] investigated the mass transfer in bioreactors based on monolith-supported ionic liquid phase for enzyme catalysis in supercritical carbon dioxide (Fig. 3). The catalytic system is based on CALB immobilized by non-covalent attachment onto

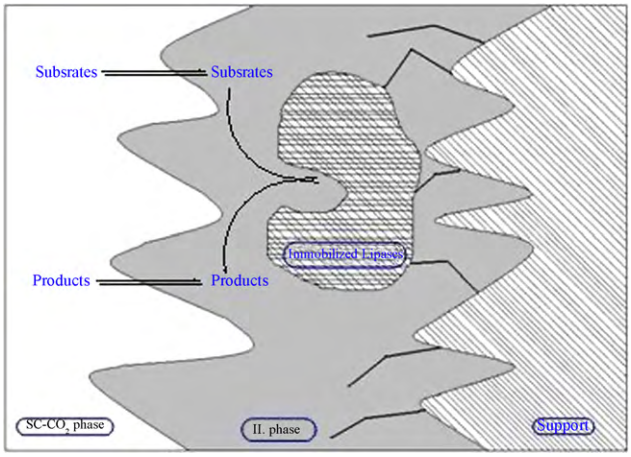


Fig. 2. Mass transfer of lipase catalysis in IL/SC-CO_2 biphasic systems.

macroporous monolith-supported ionic liquid phase, which were applied for a continuous transesterification process in SC-CO_2. In the research, the large pore distribution of monoliths, together with the high efficiency of SC-CO_2 to transport hydrophobic compounds, ensures very fast mass transfer through the pores directly. On the other hand, ILs have been described as liquid immobilization supports because multipoint enzyme–IL interactions (ionic, hydrogen bonds, van der Waals, etc.) may occur, resulting in a supermolecular network able to maintain the protein conformation activity. In this case, monoliths containing covalently attached ILs not only provide an adequate microenvironment for lipase action, but improve the mass-transfer phenomena of hydrophobic substrates and products from the SC-CO_2 phase, leading to a highly selective and stable immobilized enzyme even at high temperatures and pressures. The higher hydrophilicity and the different morphologies lead to higher back pressures, resulting in a clear decrease in the CALB's synthetic activity. Clearly hydrophilic support, like silica gel, displays the worst activity level for the whole temperature range under study.

Recently, de los Ríos et al. described the mass transfer in a recirculating enzymatic membrane reactor for green ester synthesis in

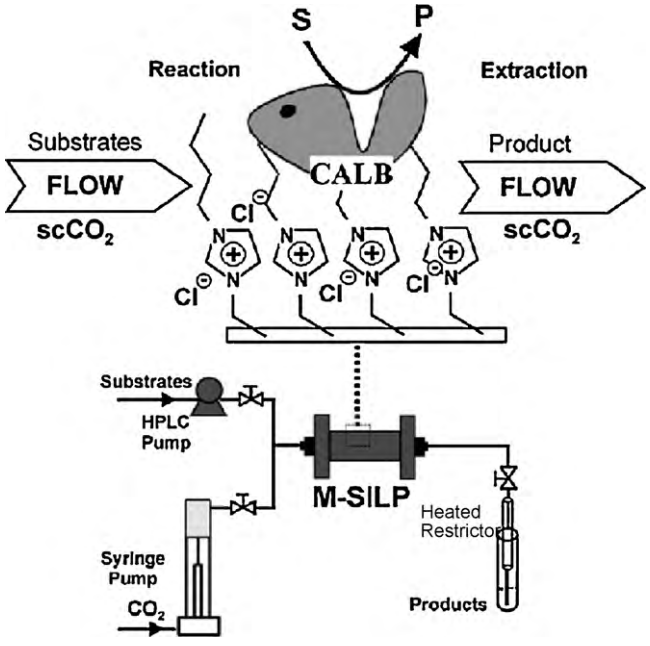


Fig. 3. Mass transfer of CALB catalysis in bioreactors based on the monolith-supported ionic liquid phase in SC-CO_2 [44].

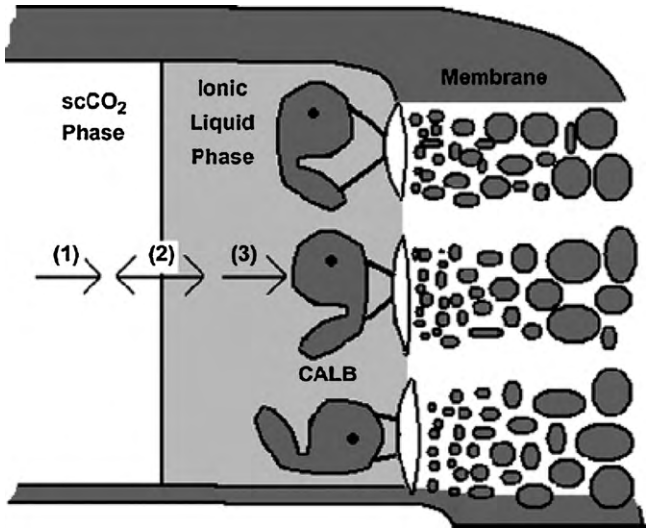


Fig. 4. Mechanism of substrate transport from SC-CO_2 phase to the immobilized enzyme in IL/SC-CO_2 biphasic system [49].

IL/SC-CO_2 biphasic systems (Fig. 4) [49]. The mechanism of substrate transport involves three consecutive steps: diffusion of the substrates through the diffusion layer from the bulk of the SC-CO_2 phase to the IL/SC-CO_2 interface (step 1); partitioning of the substrates between the SC-CO_2 and the IL phase (step 2), and diffusion into the IL phase towards the immobilized enzyme (step 3). However, the observed decrease of enzymatic activity in IL/SC-CO_2 biphasic systems could be due to limitations in the mass-transfer phenomena across the IL-layer around the biocatalyst, rather than to an enzyme deactivation phenomenon. In the literature, the protective effect of ILs on enzymes against both nonaqueous environments and SC-CO_2 in extreme conditions has been reported [8,16]. It might be concluded that the activity shown by CALB immobilized in the IL/SC-CO_2 biphasic systems depends on two factors: the specific enzyme–IL interactions and the mass-transfer limitation between ILs and SC-CO_2. Thus, the appropriate selection of the IL can greatly improve the enzymatic activity and the mass-transfer phenomena, permitting better design of IL/SC-CO_2 biphasic systems.

4.3. Characteristics of the lipase catalysis in IL/SC-CO_2

The operation stability of CALB in IL/SC-CO_2 was investigated by Lozano et al. [8]. At 10 MPa and at 120 and 150 °C, both free and immobilized CALB were able to catalyze specifically the synthesis of (*R*)-1-phenylethyl propionate in all cases. The increase of temperature only produced a slight decrease in the synthetic activity, the maximum level of enantiomeric excess for product purity being maintained (ee > 99.9%). The protective effects of ILs, including [EMIM][Tf_2N] and [BMIM][Tf_2N], against denaturation by CO_2 and heat were very important. For free CALB, [EMIM][Tf_2N] appeared as the best IL to protect the enzyme, showing a half-life time of 15 cycles. In addition, for the worst IL case, it should be noted how a free biological macromolecule designed to work under room conditions can maintain its catalytic activity for several daily cycles ($t_{1/2}$ = 2 cycles) of continuous operation in anhydrous conditions at 150 °C and 10 MPa. The results could be explained by a double role on the part of the ILs nonmiscible with water. First, IL acts as a solvent, providing an adequate microenvironment for the catalytic action of the enzyme (mass transfer phenomena and active catalytic conformation). Second, IL could also be regarded as a liquid immobilization support, because multipoint enzyme IL interactions (ionic, hydrogen, van der Waals, etc.) may occur,

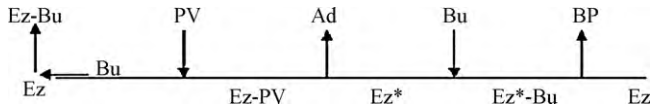


Fig. 5. Ping-Pong Bi–Bi with competitive alcohol inhibition reaction mechanism, where Ez is free enzyme, Ez-PV enzyme-vinyl propionate complex, Ez* enzyme-acyl complex, Ad acetaldehyde, Bu 1-butanol, Ez*-Bu binary complex of acyl enzyme and 1-butanol, BP butyl propionate and Ez-Bu enzyme-1-butanol complex [39].

resulting in a flexible supermolecular net able to maintain the active protein conformation in these high denaturative conditions. In this way, enzyme immobilization with multipoint attachment onto the solid support is a classical strategy to stabilize proteins toward non-conventional media, as a result of a rigidification of the protein structure. Furthermore, it has been demonstrated that enzymes enhance their thermal stability in both aqueous and non-aqueous media containing polyols as a consequence of the increase in interactions of hydrogen bond. Therefore, both the solvophobic interactions essential to maintain the native structure and the water shell around the protein molecule are preserved by the "inclusion" of the aqueous solution of free enzyme into the IL net, resulting in a clear enhancement of the enzyme stability. In the case of immobilized enzyme (Novozym 435), no loss of activity could be observed at 120 °C, and the resulting half-life time at 150 °C was twice that observed for the free enzyme. Then, the enzyme-support interactions combined with the influence of IL improving the enzyme stability greatly.

Kinetic model for butyl propionate synthesis catalyzed by CALB in IL/SC-CO_2 biphasic systems was studied by Hernández et al. [40]. The initial substrate concentration ranges were 75–200 mM for substrates (1-butanol and vinyl propionate). In the ranges, strong alcohol inhibition was observed. Alcohol inhibition is generally observed during lipase esterification and transesterification reactions. As some researchers have reported [61,62], in both conventional and non-conventional media, the kinetic mechanism for these reactions was a Ping-Pong Bi-Bi with competitive alcohol inhibition. In these reactions, the lipase may react with 1-butanol to yield a dead-end enzyme-1-butanol complex or with vinyl propionate to yield the lipase-vinyl alcohol, which tautomerizes to acetaldehyde. This is followed by the interaction of the enzyme-acyl intermediate with 1-butanol to form another binary complex, which then yields the propyl butyrate and free lipase (Fig. 5).

In the experiments, a recirculating membrane reactor was employed with CALB immobilized on the surface of the membrane. As the recirculation rate is high enough, the conversion per pass is low, so the system acts as an ordinary batch reactor, which could be represented by the following continuity equation, with time corrected through the ratio S/V_{total}:

$$\frac{S}{V_{total}}dt = \frac{dC_a}{-r_a} \quad (1)$$

where S is the membrane internal surface (cm^2), V_{total} the reactor internal volume (mL), t the reaction time (min), C_a the product concentration in the reactor (mol/L) and r_a the reaction rate ($mmol\,min^{-1}\,cm^{-2}$).

The initial reaction rate, referring to the effective surface of the membrane, was obtained from the initial rate of change of the concentration as follows:

$$V_0 = \left(\frac{V_{total}}{S}\right)\left(\frac{dC_a}{dt}\right) \quad (2)$$

As mentioned above, a Ping-Pong Bi–Bi mechanism with competitive alcohol inhibition could be expected for this reaction. The corresponding kinetic equation for this mechanism was used to fit the experimental data, determining the kinetic parameters by the following expression:

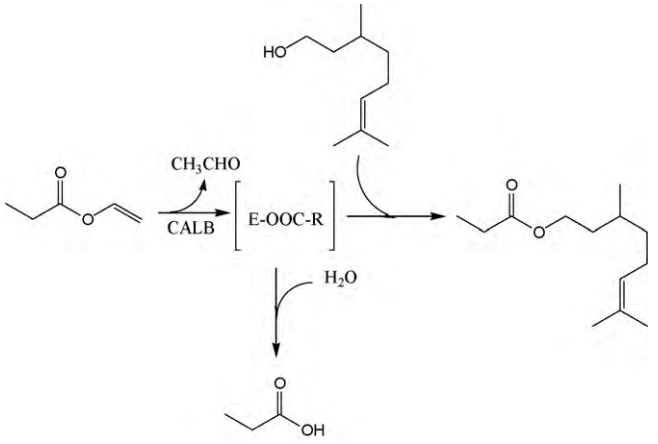
$$V_0 = V_{max}\frac{[PV][Bu]}{[Bu](K_{mPV}([Bu]/K_i + 1)) + (K_{mBu} + [Bu])[PV]} \quad (3)$$

where V_0 and V_{max} are the initial and the maximum reaction rates, respectively, [PV] and [Bu] the initial vinyl propionate and 1-butanol molar concentrations, K_{mPV} and K_{mBU} the Michael's constants of vinyl propionate and 1-butanol, and K_i the inhibition constant of 1-butanol.

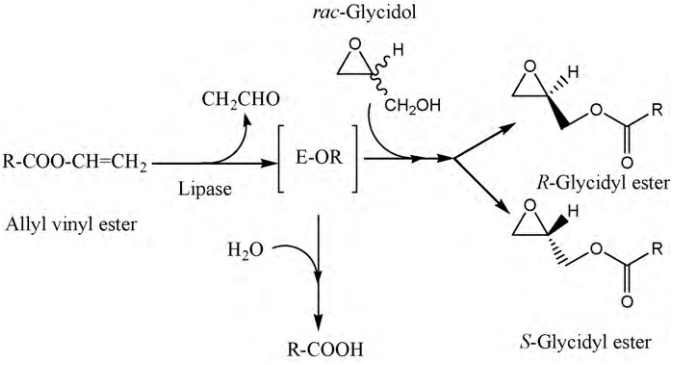
The parameters in the model equation were estimated by minimization of the sum of residual squares using the solver from Microsoft Excel. The results for the kinetic parameters involved: $V_{max} = 0.851\,mmol\,min^{-1}\,cm^{-2}$; $K_{mPV} = 0.9985$ M; $K_{mBU} = 0.102$ M; $K_i = 0.077$ M and the sum of the mean square error was 5.77×10^{-6}. The obtained kinetic parameters may be considered as apparent values because of the possibility of internal and/or external diffusion limitations. The results were demonstrated with more experiments, which showed that the experimental rate data match the values calculated by the model.

5. Applications of lipase catalysis in ionic liquids/SC-CO_2

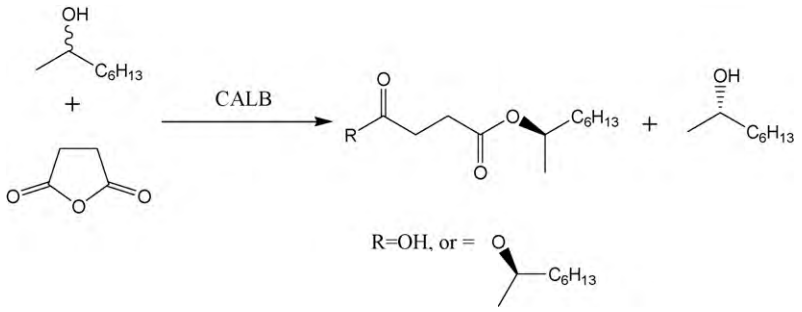
The first application was carried out by Lozano et al. [6,8,38,40,42,44,63], Hernández et al. [40] and de los Rios et al. [49] in lipase-catalyzed synthesis of butyl butyrate or butyl propionate. Lozano et al. employed CALB immobilized on the glass wool in [EMIM][Tf_2N] or [BMIM][Tf_2N]. For the transesterification of vinyl butyrate and 1-butanol to butyl butyrate, a solution of the ester in hexane and a two-fold excess of the alcohol was injected into



Scheme 1. CALB-catalyzed citronellyl propionate synthesis from vinyl propionate and citronellyl by transesterification.



Scheme 2. Lipase-catalyzed kinetic resolution of *rac*-glycidol.



Scheme 3. Lipase-catalyzed reaction for resolution of 2-octanol enantiomers in IL/SC-CO_2.

SC-CO_2, which was then flowed over the immobilized IL/enzyme solution; the butyl butyrate product was recovered by depressurization through a restrictor. For reactions conducted at 40, 50 and 100 °C with 15 MPa pressure, the lowest temperature gave the least enzyme deactivation. For kinetic resolution of *rac*-1-phenylethanol, the aqueous enzyme solution was dissolved in [EMIM][Tf_2N] or [BMIM][Tf_2N] and then immobilized on Celite. A solution of vinyl propionate and a two-fold excess of 1-phenylethanol in hexane was injected into SC-CO_2, which was flowing over the immobilized IL/enzyme layer. At 50 °C (*R*)-phenylethyl propionate was formed in >99.9% enantiomeric excess. The efficiency was the same with both ILs, and the activity was eight times greater than that when the enzyme was immobilized on Celite in the absence of IL. Therefore, "dissolution" of the lipase in the IL was found to provide protection against thermal denaturation as well as through the action of the molecular organic solvent and SC-CO_2. Since the cost of both lipases and ILs is fairly high, their immobilization on a solid support is advantageous. Such immobilized lipase/IL systems would also allow facile screening of various ILs to find the one most suitable for a given lipase-catalyzed reactions.

A suspension of CALB in [BMIM][Tf_2N] was used as lipase-catalyzed reaction to produce octyl acetate in the second application [7,22,64]. The acylation of 1-octanol by vinyl acetate was first investigated in a batch model reaction in which the IL was used as a solvent rather than as a protective layer for the enzyme. In this case, 1-octanol and a two-fold excess of vinyl acetate were directly added to the IL/lipase suspension in the reactor. After reaction for 0.5 h the reactor was connected to a CO_2 compressor and extracted for 1 h at 39 °C and 9.5 MPa. The octyl acetate, acetaldehyde and excess vinyl acetate were collected in a cold trap. A 92% yield of octyl acetate was obtained. The researchers also adopted a continuous-flow system in which a SC-CO_2 stream, into which the reactants were injected, was flowed through the IL-suspended lipase. The output was almost identical to the input, and the system operated for 24 h with a yield of 0.1 kg/L of reactor volume per hour. A batch reaction system was also utilized for the kinetic resolution of *rac*-1-phenylethanol by reaction with vinyl acetate in the IL/lipase suspension. After reaction, extraction with SC-CO_2 efficiently converted the *R*-enantiomer into the corresponding acetate ester. Repeated reactions in the same IL/lipase suspension followed by SC-CO_2 extraction of the product gave no apparent lipase deactivation.

Later, Lozano et al. reported the applications of lipase catalysis in IL/SC-CO_2 in syntheses of citronellyl propionate (Scheme 1) and glycidyl esters (Scheme 2). Terpene esters, such as citronellyl propionate, are very important flavor and fragrance substances, and they may be considered natural when produced enzymatically. In the study, they used monolith-supported-ionic liquid CALB derivative to catalyzed transesterification reactions in SC-CO_2 to synthesize the citronellyl propionate. For all the immobilized derivatives they tested, productivity in the synthetic process increased with temperature, reaching a maximum level at 80 °C, decreasing at 100 °C. This effect was related directly with the decrease in fluid density, which produced a reduction in the internal diffusion limitations within the enzyme particle. At 80 °C and 100 MPa, the highest productivity reached 3.58×10^5 mol product/mol enzyme. Furthermore, all derivatives showed excellent operational behavior, with practically no loss of activity during the assayed time, except in the case of the process carried out at 100 °C. The reaction was also very selective as the enzyme-catalyzed reaction was fully directed towards the synthesis of citronellyl propionate, due to the absence of free water molecules in the reaction system during the operation time.

Chiral glycidol (2,3-epoxy-1-propanol) is one of the most important building blocks for the synthesis of various enantiopure pharmaceuticals, including β-blockers, chiral lactones and antiviral substances. Lozano et al. [38] reported that lipase-catalyzed kinetic resolution of glycidol by transesterification with vinyl alkyl esters in SC-CO_2 with the combination of four different ionic liquids: [EMIM][NTf_2], [BMIM][PF_6], [BMIM][NTf_2] and [TROMA][NTf_2]. The activity and stereoselectivity of three different lipases, including CALA, CALB and MML, free or immobilized were evaluated in the study. The use of enzyme in IL/SC-CO_2 system permitted the proposed kinetic resolution in continuous operation as a clean process.

Butanolysis of triolein by lipase in IL/SC-CO_2 was investigated by Miyawaki and Tatsuno [47]. They used the methyltrioctylammonium trifluoroacetate (MTOATFA) as the ionic liquid. After the lipase-catalyzed reaction in IL, the product was extracted with SC-CO_2.

Bogel-Lukasik et al. [46,50] investigated the resolution of 2-octanol enantiomers with lipase catalysis in IL/SC-CO_2 (Scheme 3). 11 ILs were employed and CALB was used as lipase. In them, [OMIM][PF_6] and [OMIM][$N(CN)_2$] were found as to be the best.

6. Concluding remarks

Recent advances in the research of lipase catalysis in IL/SC-CO_2 suggest a promising future for biphasic IL/SC-CO_2 systems. A primary advantage of such systems using solvents facilitating green processing is the solubility or stability of enzymatic catalysis in ILs and their negligible solubility in SC-CO_2. On the other hand, many organic reactants and products are reasonably soluble in SC-CO_2. The advantages of using CO_2 as an extraction medium include low cost, non-toxic nature, recoverability, and ease of separating from reaction products. The primary disadvantage of these biphasic systems is the cost of the IL and equipment for producing and handling SC-CO_2. Therefore, the development of continuous-flow catalytic systems in which both the IL/lipase and the CO_2 can be recycled would be an important advance en route to large-scale commercial applications. The combination of other technologies, such as membrane technology, immobilized technology, with lipase catalysis in IL/SC-CO_2 can develop the continuous-flow catalytic systems.

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