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Asymmetric synthesis using hydrolytic enzymes in supercritical carbon dioxide

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Abstract—The use of hydrolytic enzymes in supercritical carbon dioxide ($scCO_2$), an environmentally friendly solvent with many uses, is an attractive approach to asymmetric synthesis: several examples are reviewed here.

Contents

1. Introduction

Reactions using hydrolytic enzymes have been usually conducted in non-aqueous organic solvents for esterifications. In order to make these reactions greener, supercritical carbon dioxide ($scCO_2$) can replace the conventional organic solvent, so that the reaction is essentially solventless.[1] Moreover, $scCO_2$ has been attracting increasing attention as a solvent with high potential. Its properties such as density, dielectric constant, diffusivity, viscosity, solubility etc. can be tuned by adjusting the pressure and temperature,[2] which clearly distinguishes this supercritical fluids from conventional solvents and which enables solvent effects to be examined without changing the kind of solvent. Therefore, considerable research using hydrolytic enzymes in $scCO_2$ has been conducted since 1985.[3] This review shows some

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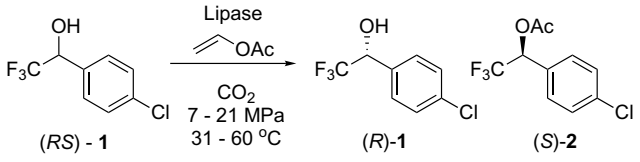
examples of asymmetric synthesis using hydrolytic enzymes in $scCO_2$.[4–6] Enzymatic asymmetric synthesis has been also conducted in other supercritical fluids such as fluoroform, which is described elsewhere.[7]

The aqueous buffer, an environmentally friendly and cheap solvent, for hydrolysis using hydrolytic enzyme is one of the choices for solvents to construct a green process. However, the process often includes the extraction of product using organic solvent unless avoided by selective crystallization for the separation of the product etc., and/or the synthesis of the substrates, racemic esters, using chemical reagents before the enzyme hydrolysis in water. These reactions are reviewed elsewhere, so not described here.

2. Esterification of 1-(*p*-chlorophenyl)-2,2,2-trifluoroethanol by lipase Novozym[4]

The enantioselective acetylation of racemic 1-(*p*-chlorophenyl)-2,2,2-trifluoroethanol (*RS*)-**1** with lipases and vinyl acetate in $scCO_2$ was examined in detail (Scheme 1), and it was found that the enantioselectivity of the reaction catalyzed by lipase Novozym can be controlled by adjusting the pressure and the temperature of $scCO_2$.



Scheme 1.

Table 1. Screening of lipases for enantioselective acetylation of 1-(*p*-chlorophenyl)-2,2,2-trifluoroethanol in $scCO_2$

Lipase	Low pressure conditions (9.1 MPa)		High pressure conditions (14.5 MPa)	
	Yield (%)	E[a]	Yield (%)	E[a]
LPL (*P. aeruginosa*)	52	12	38	16
AY (*C. rugosa*)	8	1[b]	2	2
AH (*P. cepacia*)	3	29	0	—
PS-D (*P. cepacia*)	0	—	0	—
PS-C (*P. cepacia*)	43	8	22	17
Lipozyme (*Rizomucor miehei*)	0	—	0	—
Novozym (*C. antarctica*)	25	38	24	23

Reaction conditions: 40 °C, 4 h.

[a] Enantiomeric ratio, E value, was used to evaluate enantioselectivity. $E = (V_A/K_A)/(V_B/K_B)$ where V_A, K_A and V_B, K_B denote maximal velocities and Michaelis constants of the fast- and slow-reacting enantiomers, respectively. The (*S*)-enantiomer reacted faster than (*R*)-enantiomer.

[b] In this case, the (*R*)-enantiomer reacted slightly faster than the (*S*)-enantiomer.

2.1. Screening of lipases

First, various lipases were screened for the reaction (Table 1). In all cases but one the (*S*)-enantiomer reacted faster than the (*R*)-enantiomer, affording (*S*)-acetate (*S*)-**2** and the remaining (*R*)-alcohol (*R*)-**1**. The highest enantioselectivity ($E = 38$) was obtained using Novozym at 9.1 MPa. Interestingly, the enantioselectivity was significantly affected by pressure.

2.2. Effect of pressure

The effect of pressure on enantioselectivity was investigated in more detail by carrying out the esterification at pressures ranging from 8 and 19 MPa and for different reaction times, while maintaining the temperature at 55 °C. As shown in Figure 2a, the E value decreased from 50 to 10 *continuously* when the pressure was changed from 8 to 19 MPa, regardless of the reaction time.

The effect of pressure on enantioselectivity is indeed noteworthy, although the reason is not clear at present. When the pressure of $scCO_2$ was changed, there was no significant change in the polarity evaluated as dielectric constant[2a] and log P (at 50 °C; 1.4 at 8 MPa and 1.9 at 11 MPa).[2e] It is not clear whether this small change in polarity has a large effect on this reaction. On the other hand, the density of $scCO_2$ does change from 0.20 to 0.42 g/mL when the pressure is changed from 8 to 11 MPa at 55 °C.[2a,b] Ikushima explained the high enantioselectivity of lipase in a very limited pressure range at 304.1 K as resulting from interaction between CO_2 and enzyme molecules.[5a,b,c] We also propose that the large change in density could significantly change the interaction between CO_2 and the enzyme by the formation of carbamates from CO_2 and the free amine groups on the surface of the enzyme.[3a] This can also occur by CO_2 adsorption on the enzyme, as reported for other proteins,[8] and/or by CO_2 incorporation in the substrate-binding pocket of the enzyme, in analogy to the incorporation of organic molecules in enzymes.[9] These interactions may gradually change the conformation of the enzyme in response to pressure, resulting in a continuous change in enantioselectivity.

The effect of pressure on the enantioselective acetylation of (*RS*)-**1** with vinyl acetate in $scCO_2$ by Novozym was also investigated at 31, 40 and 60 °C (Fig. 2b). As in the case at 55 °C, the E value changed continuously according to the pressure. This is most probably due to the change of $scCO_2$ density, as described above. This explanation is in agreement with the observation that at lower temperatures (31 and 40 °C) the decrease of the E value measured at pressure below 10 MPa is steeper whereas at higher temperatures the E values decrease more gradually. These changes correlate well with the change in density as shown in Figure 2c.[2a,b]

2.3. Effect of temperature

However, when E-values obtained at the same density but at different temperatures were compared, a signifi-

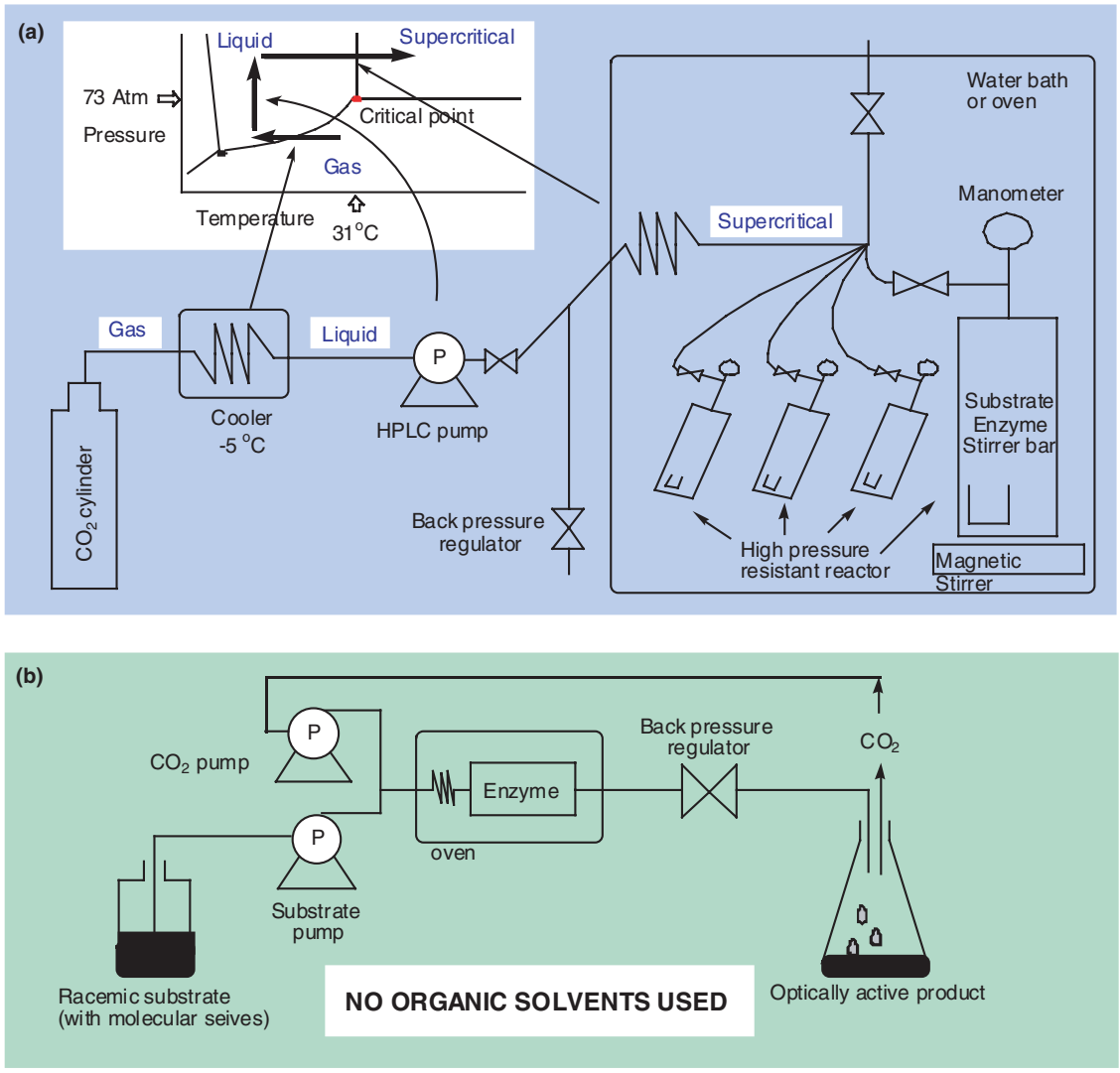


Figure 1. Experimental apparatus for enzymatic reactions in $scCO_2$: (a) batch type reactor; (b) flow type reactor.

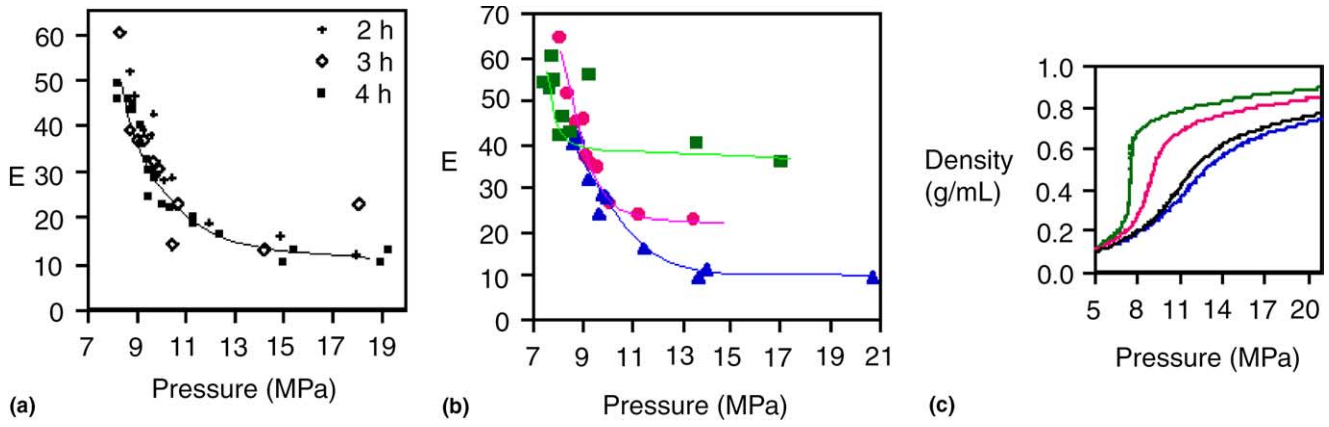


Figure 2. Effect of pressure and temperature on enantioselective acetylation of 1-(*p*-chlorophenyl)-2,2,2-trifluoroethanol by lipase Novozym. (a) Reaction at 55 °C for 2, 3, or 4 h. (b) Reaction at 31 °C (green square), 40 °C (magenta circle) and 60 °C (blue triangle). (c) Density versus pressure[2a,2b] of CO_2 at 31 °C (green), 40 °C (magenta), 55 °C (black) and 60 °C (blue).

cant temperature effect became evident. Therefore, the enantioselectivity is determined not only by density but also by temperature. In a reaction under ambient conditions, the enantioselectivity of a kinetic resolution

is temperature-dependent and obeys a modified Eyring equation:[10]

$$\ln E = -(\Delta\Delta H^{\ddagger}/R)(1/T) + (\Delta\Delta S^{\ddagger}/R) \quad (1)$$

Therefore, according to the Eyring equation, a modulation of stereoselectivity of enzymatic catalysis is possible through temperature variation. Using enzymatic reactions performed at temperatures ranging from 30 to −50 °C, Sakai et al. provided the first experimental evidence supporting the theory of the effect of temperature on stereochemistry.[10b,10c] Here, we examined whether the theory is applicable to the reaction in $scCO_2$. At a density of 0.75 g/mL (31 °C at 9.5 MPa, 35 °C at 11.2 MPa, 40 °C at 13.2 MPa, 45 °C at 15.3 MPa, 50 °C at 17.5 MPa, 55 °C at 19.6 MPa, and 60 °C at 21.8 MPa), ln E was plotted against $1/T$. As shown in Figure 3, the Eyring plot was found to be linear throughout this range and thus indicates the conformational stability of the transition state. The differences in enthalpy and entropy values calculated from the above graph are given in equation Eq. (2). The values are comparable to those for the reaction in organic solvent.[5g]

$$\Delta\Delta H^{\ddagger} = -11 \text{ kcal/mol}$$
$$\Delta\Delta S^{\ddagger} = -28 \text{ cal/K/mol} \quad (2)$$

2.4. Conventional organic solvent versus $scCO_2$

The reaction in $scCO_2$ was compared to the reaction in conventional media in terms of reactivity, enantioselectivity, continuity of the enantioselectivity change, and possibility to examine solvent effect without changing the kind of solvent. The reaction rates largely depended on the kinds of organic solvent or $scCO_2$ conditions and did not differ significantly between conventional and supercritical. Concerning the enantioselectivity, the highest value was obtained using benzene, a hazardous solvent. The large differences between conventional and supercritical solvent appeared when continuity of the enantioselectivity change and the possibility of examining the solvent effect without changing the molecular shape of the solvent were compared. Continuity of the enantioselectivity change was not observed using conventional organic solvents for the same reaction as shown in Figure 4. Moreover, it is unclear whether the E values depended only on the polarity of the organic solvent, because a polarity change is inevitably accompanied by a change in the molecular structure of the solvent (cyclic or acyclic). On the other hand, by using CO_2, the solvent properties can be changed simply by altering the pressure or temperature.

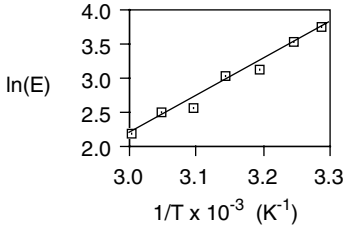


Figure 3. Effect of temperature on enantioselectivity of acetylation of 1-(*p*-chlorophenyl)-2,2,2-trifluoroethanol in $scCO_2$ catalyzed by lipase Novozym at 0.75 g/mL.

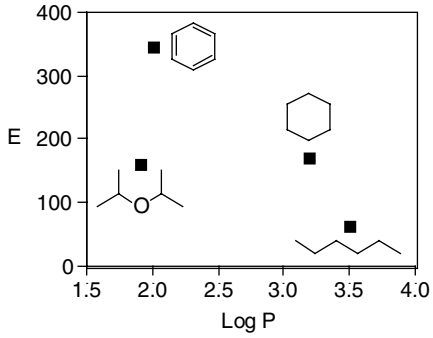


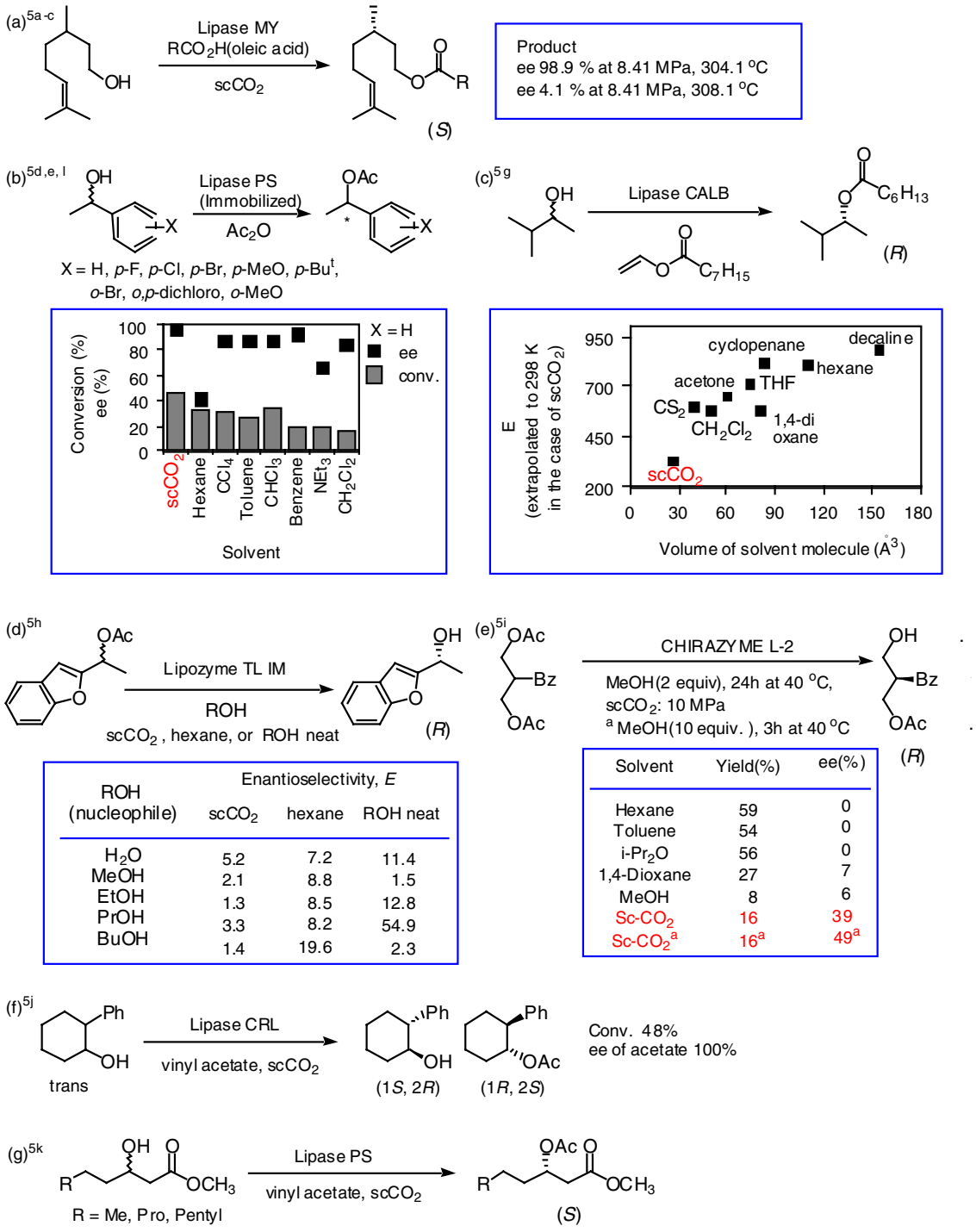
Figure 4. Effect of organic solvent on enantioselectivity of acetylation of 1-(*p*-chlorophenyl)-2,2,2-trifluoroethanol catalyzed by lipase Novozym.

3. Various asymmetric reactions in $scCO_2$[5]

An increasing number of asymmetric reactions in $scCO_2$ with hydrolytic enzymes has been reported.[5] Most of them use batch type reactors while flow type reactions have only been reported recently. Some examples are shown in this section.

3.1. Batch process

Selected examples for asymmetric synthesis using batch type reactors are shown in Figure 5. The ester synthesis from oleic acid and racemic citronellol by lipase was carried out in $scCO_2$ (Fig. 5a).[5a,b,c] The enantioselectivity was largely affected by the $scCO_2$ conditions. For the kinetic resolution of 1-phenylethanol by lipase PS (Fig. 5b), several solvents were examined, and the utilization of $scCO_2$ afforded higher enantioselectivity and conversion values than conventional organic solvents.[5d,e,l] For the resolution of 3-methyl-2-butanol catalyzed by lipase CALB in $scCO_2$ (Fig. 5c), the reaction was also compared with that in organic solvent.[5g] A correlation of the enantioselectivity to the van der Waals volume of the solvent molecules was observed for the organic solvent, but the reaction in $scCO_2$ suggested the mechanism to be of a somewhat different nature in $scCO_2$. Kinetic resolution of 1-(benzofuran-2-yl)ethanols by lipase, Lipozyme TL IM, was conducted in $scCO_2$, hexane and under neat conditions (Fig. 5d) because the benzofuran-based structure are important units for the synthesis of various kinds of biologically active molecules such as antibacterial or antifungal agents, β-blockers and cardiac anti-arrhythmic drugs, etc.[5h] In the hydrolysis reaction in $scCO_2$, water as a nucleophile among several alcohols or water gave the highest selectivity whereas in hexane butanol provided the highest, and under neat conditions the highest selectivity was achieved with propanol. For lipase-catalyzed asymmetrization of 1,3-propanediacetate (Fig. 5e), no enantioselectivity was observed in conven-



ROH (nucleophile)	Enantioselectivity, *E*		
	$scCO_2$	hexane	ROH neat
H_2O	5.2	7.2	11.4
MeOH	2.1	8.8	1.5
EtOH	1.3	8.5	12.8
PrOH	3.3	8.2	54.9
BuOH	1.4	19.6	2.3

Solvent	Yield(%)	ee(%)
Hexane	59	0
Toluene	54	0
i-Pr_2O	56	0
1,4-Dioxane	27	7
MeOH	8	6
Sc-CO_2	16	39
Sc-CO_2[a]	16[a]	49[a]

Figure 5. Examples for enzymatic asymmetric reactions in $scCO_2$.

tional organic solvents, whereas in $scCO_2$, enantioselectivities were improved up to 50% ee, which probably arose from a conformational change of the lipase at the active site due to a transformation of the amino group of lysine into carbamic acid.[5i] A cyclic alcohol was also applied for the kinetic resolution by *Candida rugosa* lipase in $scCO_2$ using vinyl acetate as acyl donor (Fig. 5f).[5j] Attempts to improve reaction yields demonstrated that supercritical CO_2 is the best medium among several organic solvents tested. As a result, *trans*-2-phenyl-1-cyclohexanol was quantitatively resolved. For the kinetic resolution of different 3-hydroxy esters by lipase from *Pseudomonas cepacia*, similar enantioselectivity (ee, up to 99%) was found for transesterifications in organic solvents and in $scCO_2$ (Fig. 5g).[5k] For the reaction in $scCO_2$, the addition of different co-solvents had only a small effect on the reaction. Immobilization of the lipase on VA-epoxy resulted in similar enantiomeric excesses to those found for the crude lipase, but at halved reaction times.

3.2. Flow process

Continuous flow $scCO_2$ phase reactions using heterogeneous, immobilized enzymes were also reported for the synthesis of optically active compounds.[5m] The use of flow reactors enable virtually solventless reactions (Fig. 1b). For example, the use of the continuous flow reaction system for the kinetic resolution of 1-phenylethanol by lipase Novozyme 435 resulted in a completely organic solvent free process and in a significant improvement in the productivity for long periods of reaction times (Fig. 6). The productivity of the optically active compounds, namely space-time yield, was improved by over 400 times compared to the corresponding batch reaction using $scCO_2$.

The reaction of 1-phenylethanol and vinyl acetate, with the molar ratio of 1:0.5 at a flow rate of 0.70 mL/min, over the catalyst under 13 MPa of $scCO_2$ (1.5 mL/min) gave the corresponding acetate with 99.7% ee in 47% yield. The *E* value exceeds 1800 (Fig. 6a). The use of a slight excess of vinyl acetate resulted in an increase in the chemical yield of optically active acetate from 47% to 50%, in which the unreacted alcohol with 98.8% ee was recovered quantitatively. Changing the CO_2 pressure from 8.9 to 20 MPa did not significantly change the outcome of the reaction.

When the substrate specificity of this system was investigated, it was found that aliphatic alcohols, 2-undecanol and 1-tetralol were kinetically resolved with the Novozym catalyst to give a mixture of the corresponding optically active (*R*)-ester and unreacted (*S*)-alcohol (Figs. 6b and c).

This synthetic process is particularly useful for the large-scale production of optically active alcohols. As shown in Figure 6d, the biocatalyst maintained its performance in terms of the reactivity and selectivity during 3-days operation under a supercritical conditions (12.9–13 MPa at 42 °C) and resulted in a quantitative transformation of (*RS*)-1-phenylethanol (221 g) to (*S*)-phenylethanol with 99% ee and the corresponding (*R*)-acetate with 99% ee using 1.73 g of the immobilized enzyme.

4. Ionic liquid/$scCO_2$ systems[6]

Ionic liquids, as well as $scCO_2$, have been attractive solvents for enzyme catalysis to develop green chemistry. The combination of kinetic resolution in ionic liquids and selective extraction with $scCO_2$ provides a new approach to asymmetric synthesis.[6] The system was exemplified by the lipase catalyzed esterification of secondary alcohols as shown in Figure 7.

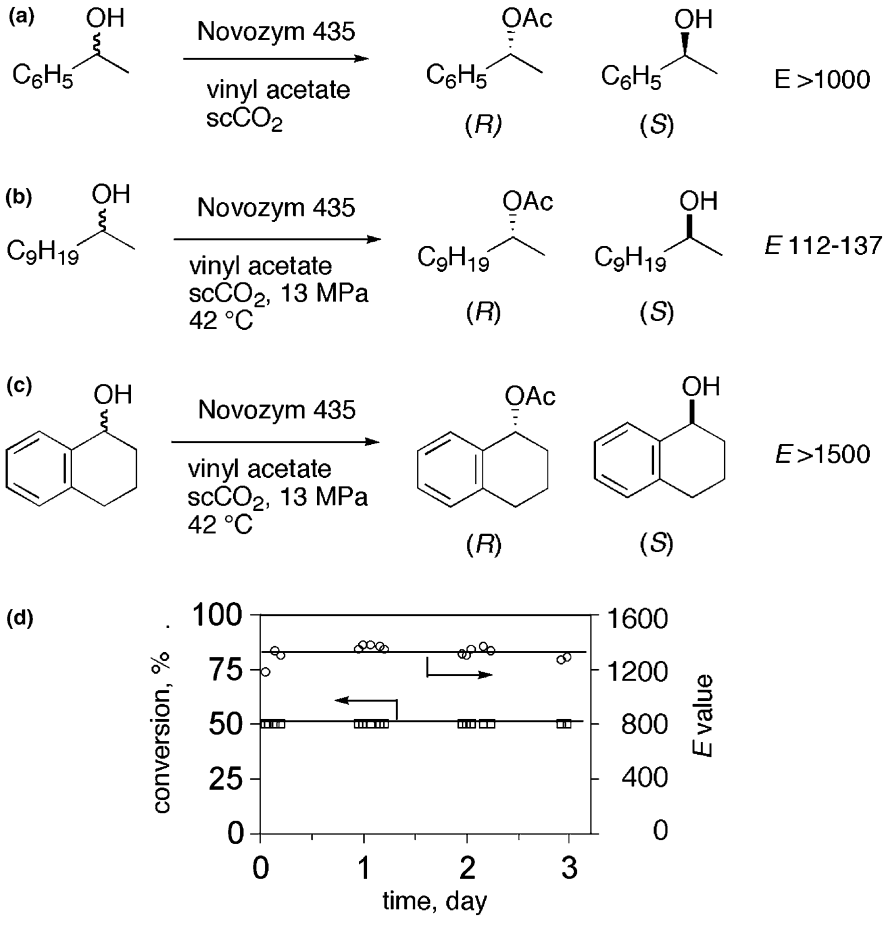


Figure 6. Asymmetric enzymatic reactions using $scCO_2$ flow system.

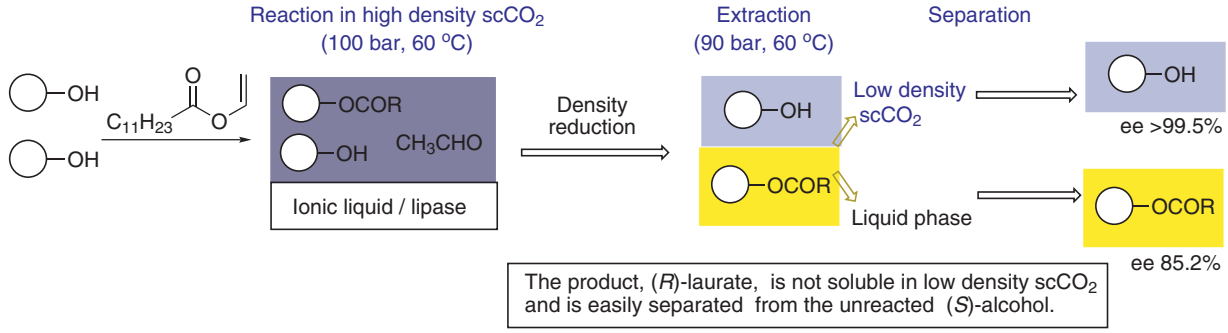


Figure 7. Asymmetric enzymatic reactions using ionic liquid/$scCO_2$ system.

5. Conclusion

Study of biocatalysis in $scCO_2$ has just begun due to the strong concern with the natural environment. Some examples for asymmetric synthesis by hydrolytic enzymes in $scCO_2$ were described in this short review. With more investigations, we believe that it will be developed into industrial applications.

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