Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 97 (2006) 324–330
Food Chemistry
www.elsevier.com/locate/foodchem

Comparison of lipase-catalyzed enantioselective esterification of (±)-menthol in ionic liquids and organic solvents

Yi Yuan, Shu Bai, Yan Sun *

Department of Biochemical Engineering, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China

Received 10 January 2005; received in revised form 28 April 2005; accepted 28 April 2005

Abstract

Enantioselective esterification of (±)-menthol was studied using *Candida rugosa* lipase (CRL) in ionic liquids (1-butyl-3-methyl-imidazolium hexafluorophosphate ([BMIM][PF_6]) and 1-butyl-3-methyl-imidazolium tetraflouroborate) and organic solvents of different hydrophobicities. Propionic anhydride was employed as an acylating agent. Because the enzyme showed comparable conversion yield and enantioselectivity in [BMIM][PF_6] and hexane in a 24-h reaction, more work focused on these two reaction media. Comparison of the activity, stability and enantioselectivity of CRL was carried out by examining the effects of the mole ratio of substrates, temperature, incubation time and enzyme recycling. It was found that temperature control was more crucial in the ionic liquid than in hexane to reach high conversion and enantioselectivity. The ionic liquid system showed an advantage of using less acid anhydride to achieve higher (±)-menthol conversion yield and better enantioselectivity. Moreover, during an incubation of 4–60 days in the ionic liquid, CRL activity was 2.5 times higher than its initial value, while that in hexane decreased to less than 60% in 2 days. In addition, the enzyme showed potentiality of recycled use in the ionic liquid. These advantages of the ionic liquid suggest that it would be used as a green alternative to organic solvents for the enantioselective esterification of (±)-menthol.

Keywords: Lipase; Enantioselective esterification; Menthol; Ionic liquid; Propionic anhydride

1. Introduction

Room-temperature ionic liquids are known as green reaction media of the future and have attracted much attention in recent years (Rogers & Seddon, 2002; Sheldon, Lau, Sorgedrager, Rantwijk, & Seddon, 2002). The application of ionic liquids for organic synthesis has been well documented in recent reviews (Sheldon, 2001; Wasserscheid & Keim, 2000; Welton, 1999). Moreover, biocatalysis in ionic liquids has also been investigated increasingly since the research work on thermolysin-catalyzed Z-aspartame synthesis in an ionic liquid was initiated by Erbeldinger, Mesiano, and Russell (2000). Some potential advantages of enzymatic reactions in ionic liquids, such as high activity (Eckstein, Sesing, & Kragl, 2002) thermal and operational stability of biocatalysts (Persson & Bornscheuer, 2003), good enantioselectivity (Kim, Choi, & Lee, 2003) of biotransformation in comparison with conventional reaction media, have been reported. Currently, the design of efficient reaction procedures using the unconventional solvent characteristics of ionic liquids has become the local point in ionic liquids-related biocatalysis research (Itoh, Nishimura, & Ouch, 2003; Nara, Harjani, & Salunkhe, 2002). Various reactions with different ionic liquids and enzymes have been investigated to examine the behavior of biocatalysis in ionic liquids.

(−)-Menthol and its esters are important flavor compounds from an industrial point of view because of their cooling and refreshing effects. Up to now,

* Corresponding author. Tel.: +86 222 740 2048; fax: +86 222 740 6590.
E-mail address: ysun@tju.edu.cn (Y. Sun).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.04.020

enzymatic resolution of (±)-menthol in organic solvent has been investigated by some researchers (Athawale, Manjrekar, & Athawale, 2001). Here, we describe the enzyme-catalyzed esterification of (±)-menthol in ionic liquids with propionic anhydride as an acyl donor. Comparison of the stability, activity and enantioselectivity of lipase in ionic liquids and organic solvents is performed. The effect of various reaction parameters such as solvents, mole ratio of substrates, temperature, incubation time and enzyme recycling on the conversion as well as enantioselectivity was studied.

2. Materials and methods

2.1. Chemicals and enzyme

(±)-Menthol and (−)-menthol were purchased from Aldrich. Propionic anhydride and (−)-menthyl propionate were from Sigma (St. Louis, MO, USA). Methanol, hexane and dichloromethane for gas chromatography were of HPLC grade from Fisher Scientific (Fair Lawn, NJ, USA). All other regents were of analytical grade and obtained from local sources. The organic solvents were anhydrated by molecular sieves of 3 Å (Dalian Institute of Chemical Physics, Dalian, China) before use.

The enzyme *Candida rugosa* lipase (E.C. 3.1.1.3) was also obtained from Sigma. The enzyme activity was determined by the method of olive oil hydrolysis (Cho & Rhee, 1993). The released fatty acids were determined by titration with 5 mM NaOH in ethanol. One unit of enzyme activity was defined as the amount of lipase which liberates 1 μmol fatty acids per minute under the assay condition. So, its concentration was expressed as IU/ml.

2.2. Preparation and characterization of ionic liquids

The ionic liquid 1-butyl-3-methyl imidazolium hexafluorophosphate ([BMIM][PF_6]) was prepared according to the procedure described by Huddleston (Huddleston, Willauer, & Swatloki, 1998). 1-Butyl-3-methyl imidazolium tetraflouroborate [BMIM][BF_4] was prepared by a straightforward adaptation of the procedure described by Cull (Cull, Holbrey, & Seddon, 2000). Both of the ionic liquids were purified according to the procedure described by Park and Kazlauskas (Park & Kazlauskas, 2001) and the purity was determined by elemental analysis.

2.3. Esterification of menthol

In a typical experiment, 1.0 mmol of (±)-menthol and 200 IU of CRL were added to 3 ml of the ionic liquid or solvents (THF, methyl dichloride, phenyl chloride, hexane, heptane and octane) in a 10-ml screw-capped vial. The reaction was started by adding 1.0 mmol of propionic anhydride and run by shaking at 200 rpm at the designated temperature for 48 h. At different time intervals, 300 μl aliquots were taken and suspended in 1 ml of hexane/10%$NaHCO_3$ (1:1, v/v). The multiphasic mixture was rigorously shaken to extract all substrates and product to the hexane phase and remove acid to the aqueous phase. Then, 100 μl hexane extract were diluted with 500 μl hexane, and 2 μl of the diluted solution was analyzed by gas chromatography (GC).

Control experiments were performed in the absence of CRL. As a result, no chemical acyl transfer reaction was detected.

2.4. GC analysis

The GC analysis was performed with an Agilent 6890N GC (Agilent Technologies, DE, USA) equipped with a splitless/split injector, a flame-ionization detector, and a CYCLOSIL-B capillary column (0.25 μm film thickness, 30 m length, 0.25 mm I.D.). The injector and detector were set at 200 and 250 °C, respectively, and nitrogen was used as the carrier gas. The oven temperature was kept at 90 °C for 10 min, programmed to increase from 90 to 150 °C at 2 °C/min, then increased to 165 °C at 5 °C/min, and finally kept at 165 °C for 5 min. Chromatographic data were acquired and analyzed using the Agilent Chemical Station. The retention time were found to be 30.6 and 31.5 min for (−)- and (+)-menthyl propionate, respectively, and 26.5 min for (±)-menthol.

2.5. Calculation of enantioselectivity

The enantiomers of the (±)-menthol and of the product (±)-menthyl propionate were baseline separated in the GC analysis. The conversion in percentage was calculated from the following equation:

$$c = \left(1 - \frac{S_R + S_S}{S_{R0} + S_{S0}}\right) \times 100\%, \quad (1)$$

where S_0 and S stand for the concentrations of (±)-menthol before and after reaction. The enantioselectivity for each reaction was expressed by enantiomeric excess, e.e.(P_-)% and enantiomeric ratio, E-value (Straathof & Jongejan, 1997).

$$\text{e.e.}(P_-)\% = \frac{P_- - P_+}{P_- + P_+} \times 100\%, \quad (2)$$

$$E = \ln(1 - c(1 + \text{e.e.}P_-))/\ln(1 - c(1 - \text{e.e.}P_-)), \quad (3)$$

where P_- and P_+ represent the ratios of (−)- and (+)-menthyl propionate to the total menthyl propionate in the reaction mixture, respectively.

2.6. Enzyme stability during incubation in the absence of substrates

Mixtures of 20 mg CRL and 300 μl hexane (or [BMIM][PF_6]) were added into different screw-capped vials (1.5 ml capacity) at 30 °C in the absence of substrates. The mixtures in different vials were incubated for 0–5 days, and at different time intervals 100 μmol (±)-menthol and 100 μmol propionic anhydride were introduced to different vials to initiate the reaction. The initial esterification rate and enantiomeric excess were determined as described above to follow the changes of the enzyme activity and enantioselectivity during the incubation.

2.7. Enzyme recycling

In this part of experiment, 1.0 mmol (±)-menthol and 200IU CRL were introduced to 3 ml [BMIM][PF_6] or hexane in different 10 ml screw-capped vials. The reaction was started by adding 1.0 mmol propionic anhydride and the mixture was maintained at 30 °C for 24 h by shaking at 200 rpm. Then, 3 ml of anhydrous hexane was added to the vial with ionic liquid and the biphasic mixture was strongly shaken to extract all substrates and products into the hexane phase. The upper organic solvent was removed and the enzyme with ionic liquid was washed two times with fresh hexane. Then, next batch reaction was initiated by the addition of new substrates (1.0 mmol menthol and 1.0 mmol propionic anhydride) to the ionic liquid. For the reaction system with hexane, the reaction mixture was centrifuged at 3000 rpm for 10 min to recover the enzyme from the solvent containing substrates and products. The recovered lipase was washed with 3 ml fresh hexane three times, and used for the next batch reaction by adding 3 ml of fresh hexane to dissolve the substrates. After each cycle, analyses were performed as described in Sections 2.3 and 2.4, and (±)-menthol conversion and enantiomeric excess were determined.

3. Results and discussion

3.1. Effect of solvent

Lipase catalyzed reactions are greatly influenced by the reaction media. Here, the two ionic liquids described above and six organic solvents (tetrahydrofuran, methyl dichloride, phenyl chloride, hexane, heptane and octane) with different log *P* values (the logarithm of the partition coefficient of a given solvent between water and 1-octanol, indicating the nature and polarity of organic solvents) were used for the esterification of (±)-menthol following the typical experimental method at 30 °C (see Section 2.3). Fig. 1 shows the results of esterification for 24 h.

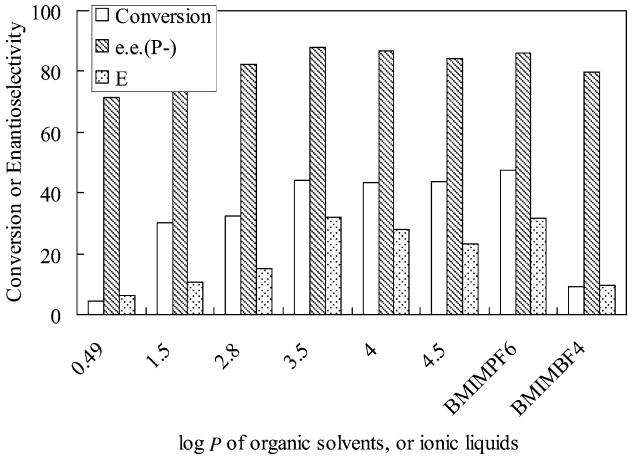

Fig. 1. Comparison of esterification conversion, enantiomeric excess and enantiomeric ratio (*E*) of menthol in different solvents in 24 h. The log *P* values of THF, methyl dichloride, phenyl chloride, hexane, heptane and octane are 0.49, 1.5, 2.8, 3.5, 4.0 and 4.5, respectively. The reaction was performed by the methods described in Section 2.3 with 67 IU/ml CRL.

It is well known that the activity of enzymes is affected by the polarity of organic media. The log *P* values of organic solvents used in our experiment are 0.49, 1.5, 2.8, 3.5, 4.0 and 4.5 for tetrahydrofuran (THF), methyl dichloride, phenyl chloride, hexane, heptane and octane, respectively. It is clear that the conversions increased with the log *P* value. In the organic solvents, the better conversion and enantioselectivity were obtained in hexane, heptane and octane. The result that THF gave low conversion and selectivity fits the rule that organic solvents with log $P < 2.0$ are generally not promising for biocatalysis (Zaks & Klibanov, 1985). From the above results, it can be concluded that the log *P* value was the decisive parameter for the conversion and enantioselectivity in the reaction.

Ionic liquids have been put in the polarity range of lower alcohols (Carmichael & Seddon, 2000; Muldoon et al., 2001) or formamide (Reichardt, 1994). In the two ionic liquids, the enantiomeric excess of menthol esterification was comparable, whereas the conversion (*c*%) in [BMIM][BF_4] (9.21%) was much less than that in [BMIM][PF_6] (47.5%). This is in agreement with that reported by Mohile (Mohile, Potdar, J.R, Nara, & Salunkhe, 2004). Some explanation was that the hydrophilic ionic liquid [BMIM][BF_4] is prone to desorb water from the enzyme surface and decrease the activity of the enzyme (Persson & Bornscheuer, 2003). However, the results by Kaar suggest that enzyme activity in ionic liquids is anion dependent (Kaar, Jesionowski, Berberich, Roger, & Russell, 2003). Anions such as [NO_3], [CH_3CO_2], and [CF_3–CO_2] are more nucleophilic than [PF_6] and may coordinate more strongly to positively

charged sites in the lipase's structure causing conformation changes in the enzyme's structure.

In the study, the enantioselectivity of menthol esterification in [BMIM][PF6] was higher than or similar to those in organic solvents reported previously (Kim, Song, Choi, & Kim, 2001; Krzysztof, Eryka, & Michel, 2003; Lau, Rantwijk, Seddon, & Sheldon, 2000; Park & Kazlauskas, 2001). Highest enantioselectivity was achieved in [BMIM][PF6] ($E = 31.5$) and in hexane ($E = 31.9$) for CRL during the esterification of menthol (Fig. 1). So, in the following work, we further examined [BMIM][PF6] and hexane as the reaction media to find the difference between the ionic liquid and organic solvent.

3.2. Effect of temperature

The enantioselectivity of enzymes is highly temperature sensitive and enzymes are known to show maximum activity in ambient conditions. However, there are some reports about excellent enzymatic activity retention at high temperature such as a reported increase in free lipase (*Candida antarctica*) activity (120%) observed after 100 h of incubation in [BMIM][PF6] at 80 °C (Sheldon et al., 2002). Therefore, we investigated menthol esterification at 20, 25, 30, 35 and 40 °C in hexane and [BMIM][PF6]. The process kinetics showed these esterification reactions at different temperature finished at about 40 h. So the conversion of menthol and the enantiomeric excess of (−)-menthyl propionate in 24 h are summarized in Table 1. The results indicate that the optimum reaction temperature in both of the media as 30 °C. However, the enzyme activity was more affected in [BMIM][PF6] than in hexane. Thus, temperature control is more crucial for the enzymatic reaction in the ionic liquid.

3.3. Effect of mole ratio of substrates

Menthol conversions and e.e.% at mole ratios of propionic anhydride to (±)-menthol of 0.5, 1 and 2 are listed in Table 2. An increase in the acid anhydride concentration to twice that of menthol obviously increased the overall yields in hexane. However, the enantioselectivity of these reactions was lower than the systems with acid anhydride/menthol of 0.5 and 1. The result is similar to that with lipase-AY in organic solvents (Athawale et al., 2001). In contrast to this, however, in [BMIM][PF_6] the yield of menthyl propionate was the highest at the mole ratio of 1 and a better enantiomeric excess was also obtained at this mole ratio. This is an advantage of the ionic liquid, for less acid anhydride is required for the esterification of menthol.

3.4. Enzyme stability during incubation

The stability and the enantioselectivity of CRL was investigated in hexane and [BMIM][PF_6] following the method described in Section 2.6. The stability of CRL

Table 1
Conversion and enantioselectivity of (±)-menthol esterification at various temperatures in hexane and [BMIM][PF_6] by 24-h reaction

Temperature (°C)	Solvent	Menthol conversion (%)	Enantiomeric excess e.e.(P_-) %	Enantiomeric ratio *E*-value
20	Hexane	42.6	82.5	19.4
	[BMIM][PF_6]	39.6	83.7	19.5
25	Hexane	42.8	79.9	16.3
	[BMIM][PF_6]	41.9	84.9	22.8
30	Hexane	43.6	88.0	31.9
	[BMIM][PF_6]	47.5	86.1	31.5
35	Hexane	40.2	74.5	11.2
	[BMIM][PF_6]	42.3	79.2	15.4
40	Hexane	42.1	81.7	18.1
	[BMIM][PF_6]	35.0	78.6	12.6

Reactions were performed for 24 h at a mole ratio of 1 (1.0 mmol menthol and 1.0 mmol propionic anhydride in 3 ml solvent) using 67 IU/ml of CRL.

Table 2
Effect of mole ratio of propionic anhydride to (±)-menthol on the enzymatic reaction in hexane and [BMIM][PF_6] for 24 h

Mole ratio	0. 5		1		2	
	Conversion (%)	e.e.(P_-) %	Conversion (%)	e.e.(P_-) %	Conversion (%)	e.e.(P_-) %
Hexane	43.4	88.5	43.6	88.0	50.2	49.4
[BMIM] [PF_6]	39.3	86.3	47.5	86.1	40.5	75.1

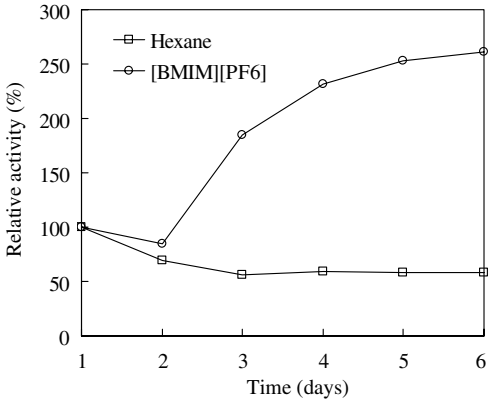

Fig. 2. Effect of incubation time on the relative activity of CRL in hexane and [BMIM][PF_6] at 30 °C. Menthol conversion rate determined in 15 min of the reaction was defined as the esterification activity. The esterification activity achieved after the first-day incubation was set to be 100%, the absolute values of which were 8.67 μmol g^{-1} min^{-1} in [BMIM][PF_6] and 97 μmol g^{-1} min^{-1} in hexane.

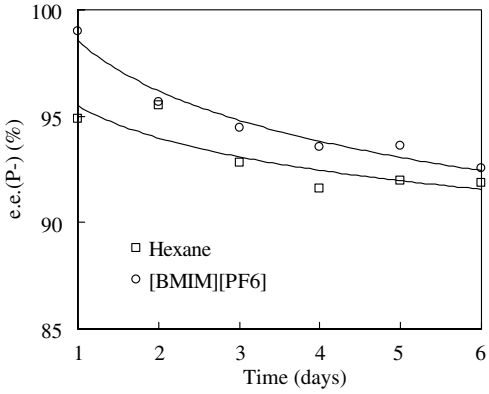

Fig. 3. Effect of incubation time on the enantioselectivity of CRL in hexane and [BMIM][PF_6] at 30 °C. The enantiomeric excess was determined in 15 min of the reaction.

was expressed using the relative activity for (±)-menthol conversion as an index. Here, menthol conversion rate determined in 15 min of the reaction was defined as the esterification activity, and the esterification activity achieved immediately after the incubation was set to be 100%. The results are represented in Fig. 2. Although the absolute value of the activity in hexane (97 μmol g^{-1} min^{-1}) in hexane was much higher than that in [BMIM][PF_6] (8.67 μmol g^{-1} min^{-1}), the relative activity in hexane decreased day by day and dropped to about 60% in the fifth day. In contrast, the enzyme activity showed much different behavior in ionic liquid. It decreased slightly in the first day and then increased thereafter, till 250% of the initial value after the fourth day. CRL incubation in the media was continued for 60 days. As a result, the relative activity was still 250% in the ionic liquid, while no activity was detected in hexane. The increased stability of enzymes in ionic liquids as compared to organic solvents has also been observed for other enzymes, such as α-chymotrypsin (Lozano, 2001) and *C. antarctica* lipase B (Lozano, Diego, Carrie, Vaultier, & Iborra, 2001). It was considered that the solvent polarity influenced the hydration level of an enzyme preparation, and higher water activity of the system resulted in lower stability of enzymes (Wehtje, Costes, & Adlercreutz, 1997; Hansson, Andersson, & Wehtje, 2001). By controlling water activity of enzyme and solvents, other researchers have concluded that the observed increase in enzyme stability in [BMIM][PF_6] was directly related to the solvent effect (Persson & Bornscheuer, 2003). That is, electrostatic interaction was considered to occur between ionic liquids and protein, which could enhance the enzyme stability. In Kaar's opinion, increased enzyme activity must invoke a permanent activating conformational change or an increase in active site concentration to explain this unusual data (Kaar et al., 2003). In this work, water-free media were used, so we prefer the latter explanation.

Fig. 3 depicts the changes of the enantiomeric excess during the incubation. The enantioselectivity decreased in both of the solvents, but the e.e.(*P*-)% in the ionic liquid was always higher than in hexane. In the ionic liquid, the enzyme activity increased (Fig. 2) while the enantioselectivity decreased (Fig. 3) with incubation time. Therefore, although the CRL was activated by electrostatic interaction with the ionic liquid, conformation changes of the active site might occur and resulted in lower enantioselectivity for (±)-menthol esterification.

3.5. Recycled use of enzyme and ionic liquid

The above experiments have demonstrated that CRL displays better stability and enantioselectivity in [BMIM][PF_6] than in hexane, so it is worthwhile checking if the enzyme and ionic liquid can be recycled. Several recycling studies have been reported (Itoh et al., 2003; Nara et al., 2002) in which the time of each recycle was several hours and did not reach the natural ending.

Table 3
Changes of the (±)-menthol conversion and enantioselectivity of (−)-menthol propionate in recycled use of CRL

Cycle	Medium	Conversion (%)	e.e.(P_-) %	*E*-value
1	Hexane	41.0	76.5	12.7
	[BMIM][PF_6]	40.2	84.5	21
2	Hexane	19.9	28.9	1.9
	[BMIM][PF_6]	21.9	73.7	8.1
3	Hexane	17.3	26.3	1.8
	[BMIM][PF_6]	19.1	67.0	5.9

The reaction time for each cycle was 24 h.

Through the process kinetic determination, we kept the reaction for one day until the next recycling operation began. The experimental procedure is described in Section 2.7. The changes of (±)-menthol conversion and enantiomeric excess of (−)-menthyl propionate during recycling are provided in Table 3.

It can be seen that (±)-menthol conversion in both the media decreased drastically during recycling, although the conversion in the ionic liquid was somewhat higher than in hexane. This may be due to the mass loss of the enzyme and/or the activity decrease in the recycling operation. Besides, propionic acid generated in [BMIM][PF_6] could not be removed by extraction with hexane in the recycling operation (see Section 2.7). It may be a main reason for the reduced activity of CRL in the recycled use in the ionic liquid system. If the problem is solved, it is expected that CRL can be recycled with higher activity.

As shown in Table 3, the enantioselectivity in hexane also decreased to a very low value in the second and third cycles; *E*-value decreased to 1.8 in the third-cycle reaction. In the ionic liquid, however, quite high values of enantiomeric excess and enantiomeric ratio of (−)-menthyl propionate were maintained in the second and third cycles. Thus, it is obvious that CRL in [BMIM][PF_6] possesses better enantioselectivity for (±)-menthol esterification than in hexane in the recycling applications.

Commercial CRL consists of different isoenzymes, and the use of a recombinant pure isoenzyme would exhibit significantly higher enantioselectivity than the crude CRL in the resolution of menthol (Vorlová et al., 2002). Thus, the ionic liquid system should be tested with more enzymes for its potential in the enzymatic resolution of menthol.

4. Conclusions

We have here compared the behavior of CRL for the enantioselective esterification of (±)-menthol in ionic liquids and organic solvents. Because the enzyme showed comparable conversion yield and enantioselectivity in [BMIM][PF_6] and hexane, more work focused on these two reaction media. The ionic liquid system showed an advantage of using less acid anhydride to achieve higher (±)-menthol conversion yield and better enantioselectivity. In addition, CRL exhibited higher stability and enantioselectivity during a long-term incubation in the ionic liquid than in hexane. It is more important that the enzyme showed potentiality of recycled use in the ionic liquid. These advantages of the ionic liquid suggest that it would be used as a green alternative of organic solvents for the enantioselective esterification of (±)-menthol.

Acknowledgement

This work was supported by the Natural Science Foundation of China for Outstanding Young Researchers (Grant No. 20025617).

References

Athawale, V., Manjrekar, N., & Athawale, M. (2001). Enzymatic synthesis of chiral menthyl methacrylate monomer by pseudomonas cepacia lipase catalysed resolution of (±)-menthol. *Journal of Molecular Catalysis B-Enzymatic, 16*, 169–173.

Carmichael, A. J., & Seddon, K. R. (2000). Polarity study of some 1-alkyl-3-methylimidazolium ambient-temperature ionic liquids with the solvatochromic dye, Nile Red. *Journal of Physical Organic Chemistry, 13*, 591–595.

Cho, S. W., & Rhee, J. S. (1993). Immobilization of lipase for effective interesterification of fats and oils in organic solvent. *Biotechnology and Bioengineering, 41*, 204–210.

Cull, S. G., Holbrey, J. D., & Seddon, K. R. (2000). Room-temperature ionic liquids as replacements for organic solvents in multiphase bioprocess operations. *Biotechnology Bioengineering, 69*, 227–233.

Eckstein, M., Sesing, M., & Kragl, U. (2002). At low water activity α-chymotrypsin is more active in an ionic liquid than in non-ionic organic solvents. *Biotechnology Letters, 24*, 867–872.

Erbeldinger, M., Mesiano, A. J., & Russell, A. J. (2000). Enzymatic catalysis of formation of Z-aspartame in ionic liquid – an alternative to enzymatic catalysis in organic solvents. *Biotechnology Progress, 16*, 1129–1131.

Hansson, T., Andersson, M., & Wehtje, E. (2001). Influence of water activity on the competition between β-glycosidase-catalysed trans-glycosylation and hydrolysis in aqueous hexanol. *Enzyme and Microbial Technology, 29*, 527–534.

Huddleston, J. G., Willauer, H. D., & Swatloki, R. P. (1998). Room temperature ionic liquids as novel media for 'clean' liquid–liquid extraction. *Journal of the Chemical Society, Chemical Communications*, 1765–1766.

Itoh, T., Nishimura, Y., & Ouch, N. I. (2003). 1-Butyl-2,3-dimethylimidazolium tetrafluoroborate: the most desirable ionic liquid solvent for recycling use of enzyme in lipase-catalyzed transesterification using vinyl acetate as acyl donor. *Journal of Molecular Catalysis B: Enzymatic, 26*, 41–45.

Kaar, J. L., Jesionowski, A. M., Berberich, J. A., Roger, M., & Russell, A. J. (2003). Impact of ionic liquid physical properties on lipase activity and stability. *Journal of the American Chemical Society, 125*, 4125–4131.

Kim, K., Song, B., Choi, M., & Kim, M. J. (2001). Biocatalysis in ionic liquids: markedly enhanced enantioselectivity of lipase. *Organic Letters, 3*, 1507–1509.

Kim, M. J., Choi, M. Y., & Lee, J. K. (2003). Enzymatic selective acylation of glycosides in ionic liquids: significantly enhanced reactivity and regioselectivity. *Journal of Molecular Catalysis B: Enzymatic, 26*, 115–118.

Krzysztof, O., Eryka, G. J., & Michel, T. (2003). Ionic liquids as a new reaction medium for oxidase–peroxidase-catalyzed sulfoxidation. *Tetrahedron: Asymmetry, 14*, 2487–2490.

Lau, R., Rantwijk, V. F., Seddon, K. R., & Sheldon, R. A. (2000). Lipase-catalyzed reactions in ionic liquids. *Organic Letters, 2*, 4189–4191.

Lozano, P. (2001). Stabilisation of *a*-chymotrypsin by ionic liquids in transesterification reactions. *Biotechnology and Bioengineering, 75*, 563–569.

Lozano, P., Diego, D. T., Carrie, D., Vaultier, M., & Iborra, J. L. (2001). Over-stabilization of *Candida antarctica* lipase B by ionic liquids in ester synthesis. *Biotechnology Letters, 23*, 1529–1533.

Mohile, S. S., Potdar, M. K., J.R Nara, S. J., & Salunkhe, M. M. (2004). Ionic liquids: efficient additives for *Candida rugosa* lipase-catalysed enantioselective hydrolysis of butyl 2-(4-chlorophenoxy)propionate. *Journal of Molecular Catalysis B: Enzymatic, 30*, 185–188.

Muldoon, M. J. et al. (2001). Investigations of solvent-solute interactions in room temperature ionic liquids using solvatochromic dyes. *Chemical Society, Perkin Transactions, 2*, 433–435.

Nara, S. J., Harjani, J. R., & Salunkhe, M. M. (2002). Lipase-catalysed transesterification in ionic liquids and organic solvents: a comparative study. *Tetrahedron Letters, 43*, 2979–2982.

Park, S., & Kazlauskas, R. (2001). Improved preparation and use of room-temperature ionic liquids in lipase-catalyzed enantio- and regioselective acylations. *Journal of Organic Chemistry, 66*, 8395–8401.

Persson, M., & Bornscheuer, U. T. (2003). Increased stability of an esterase from Bacillus stearothermophilus in ionic liquids as compared to organic solvents. *Journal of Molecular Catalysis: B Enzymatic, 22*, 21–27.

Reichardt, C. (1994). Solvatochromic dyes as solvent polarity indicators. *Chemical Reviews, 94*, 2319–2358.

Rogers, R. D. & Seddon, K. R. (2002). Ionic liquids. Industrial applications to green chemistry. *ACS Symposium series 818*.

Sheldon, R. A. (2001). Catalytic reactions in ionic liquids. *Chemical Communications (Camb)*, 2399–2407.

Sheldon, R. A., Lau, R. M., Sorgedrager, M. J., Rantwijk, V. F., & Seddon, K. R. (2002). Biocatalysis in ionic liquids. *Green Chemistry, 4*, 147–151.

Straathof, A., & Jongejan, J. (1997). The enantiomeric ratio: origin, determination and prediction. *Enzyme and Microbial Technology, 21*, 559–571.

Vorlová, S., Bornscheuer, U. T., Gatfield, I., Hilmer, J. M., Bertram, H. J., & Schmid, R. D. (2002). Enantioselective hydrolysis of D,L-menthyl benzoate to L-menthol by recombinant *Candida rugosa* lipases. *Advanced Synthesis and Catalysis, 344*, 1152–1155.

Wasserscheid, P., & Keim, W. (2000). Ionic liquids – new 'solutions' for transition metal catalysis. *Angewandte Chemie International Edition England, 39*, 3772–3789.

Wehtje, E., Costes, D., & Adlercreutz, P. (1997). Enantioselectivity of lipases: effects of water activity. *Journal of Molecular Catalysis B: Enzymatic, 3*, 221–230.

Welton, T. (1999). Room-temperature ionic liquids: Solvents for synthesis and catalysis. *Chemical Reviews, 99*, 2071–2083.

Zaks, A., & Klibanov, A. M. (1985). Enzyme-catalyzed processes in organic solvents. *Proceedings of the National Academy of Sciences of the United States of America, 82*, 3192–3196.