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Production of highly enantioselective (−)-menthyl butyrate using *Candida rugosa* lipase immobilized on epoxy-activated supports

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Abstract

Optically active (−)-menthyl butyrate was synthesized by enantioselective esterification of racemic (±)-menthol and butyric anhydride using lipase from *Candida rugosa* immobilized onto epoxy-activated supports of Eupergit C and Eupergit C 250 L through physical adsorption method. The effects of various temperature, storage condition, stability in organic solvent and lipase recyclability were investigated for their influence on the enzymatic enantioselective formation of (−)-menthyl butyrate. The immobilized lipases retained high catalytic activity of up to 31% yield and 100% enantiomeric excess of the desired product, and showed better stability compared to the native lipase. They also exhibited about 50% retained activity even after incubation at higher temperatures, storage at room temperature and after long incubation in hexane. Immobilized lipases also showed considerably efficient reusability.

Keywords: Immobilization; Lipase; Enantioselectivity; Epoxide; Menthyl butyrate

1. Introduction

The synthesis of optically pure products for pharmaceuticals is of great importance due to the fact that the biological activity of enantiomers can differ in kind and intensity because of the chiral nature of life processes (Fessner, 2000). The (−)-menthyl esters, for example are more important from an industrial point of view than the (+)-menthyl esters and the racemic mixture because it has a more distinct cooling and refreshing effect which is lacking in the other enantiomers (Akoh, Phillips, & Wu, 1997). Due to this fact, (−)-menthol and its esters are widely used as components of candy, sweets, chewing gum, beverages, toothpaste, tobacco products, local anesthetics, analgesics, medicines, liniments and cosmetic products (Athawale, Manjrekar, & Athawale, 2001).

For the synthesis of these asymmetric compounds, enzymes have received increasing acceptance as chiral catalysts and reaction conditions optimized for high selectivity and catalytic efficiency (Shaw, Wang, Nag, & Lee, 2002). In contrast to the chemical methods, reactions using enzymes can usually be performed under mild reaction conditions, avoiding tedious and costly methods. Furthermore, enzymatic catalysis is most attractive especially for the synthesis and modification of biologically relevant classes of organic compounds that are typically multifunctional and water-soluble such as amino acids and carbohydrates, which are difficult to prepare by the conventional chemical methods (Sonke, Kaptein, Boesten, & Broxterman, 2000).

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In order to further enhance enzyme activity and enantioselectivity, it is always possible to redesign their physical and functional properties so that they have the desired and beneficial properties such as increased activity, stability as well as to allow enzyme recyclability and ease of enzyme-product separation (Adlercreutz, Persson, Mladenoska, & Wehtje, 2002). In this study, lipase from *Candida rugosa* was immobilized onto polymer beads of Eupergit C and Eupergit C 250 L to further enhance its catalytic activity.

Eupergit is an uncharged, spherical acrylic polymer, with a diameter of 150 μm, containing 0.8 mM epoxy groups per g dry weight as the reactive components. The oxirane group functions as reactive component capable of covalently binding ligands containing mercapto, sulphydryl, amino or hydroxyl compounds, but does not introduce any alteration of electric charge into the matrix or the ligand. This interesting feature is accommodating for protein molecules as it allows immobilization of enzymes with high activity yields (Crout & Hernaiz, 2000). Besides that, this matrix has a large content of oxirane groups that allow a high water uptake of 3.0 mL per g of dry beads (Madoery & Fidelio, 2001). Furthermore, reaction between the nucleophiles in proteins with epoxy groups on the support is hardly affected by stearic hindrances and can be easily stopped by blocking the epoxy groups with very different reagents such as mercaptoethanol, ethanolamine and glycine, among others (Guisan, Mateo, Abian, & Fernandez-Lafuente, 2000).

In this study, we report the highly enantioselective esterification of (±)-menthol with butyric anhydride as acyl donor using Eupergit C and Eupergit C 250 L immobilized lipases. The effects of reaction parameters such as thermostability, storage condition, stability in organic solvent and lipase recyclability were studied for their influence on the enzymatic enantioselective formation of (±)-menthyl butyrate.

2. Materials and methods

2.1. Materials

Lipase from *Candida rugosa* (EC 3.1.1.3.) was purchased from Sigma Chemicals Co., St. Louis, MO. Eupergit C and Eupergit C 250 L were kindly supplied by Röhm, Darmstadt, Germany. Optically pure menthol and its racemic mixture, butyric anhydride and ethyl caproate were obtained from Fluka, Buchs, Switzerland. Other chemicals, reagents and solvents used in this work were purchased from J.T. Baker, Phillipsburg, NJ, USA and Mallinckrodt, Griesheim, Germany and were of analytical grade.

2.2. Preparation of partially purified lipase

Crude lipase from *Candida rugosa* (1.5 g) was extracted with 15.0 mL of distilled water. The mixture was stirred and centrifuged at 10,000 rpm (10,062*g*) for 15 min. The undissolved solid suspension was discarded after centrifugation while the supernatant was used as partially purified lipase or stored at −20 °C prior to use. Some of the supernatant was frozen and lyophilized and used as native lipase (NL).

2.3. Immobilization of lipase

The partially purified lipase (15.0 mL, 0.1 g/mL) was added to 2.0 g of support. The mixture was immersed in a water bath at room temperature by continuous agitation at 100 rpm for 1 h. The enzyme-loaded supports were separated from the supernatant by filtering through Whatman No. 1 filter paper. The immobilized lipases were then lyophilized in a freeze drier (Labconco 195, Kansas City, MO, USA).

2.4. Protein assay

Protein concentration was determined by Bradford (1976) Coomasie blue assay procedure using bovine serum albumin (BSA) as standard. The protein concentration was determined spectrophotometrically at 595 nm using the calibration curve of BSA.

2.5. Enzymatic esterification

The reaction mixture was composed of (±)-menthol (1 mmol) and butyric anhydride (1 mmol) in hexane (2.0 mL) and was carried out in screw-capped vials and incubated for 24 h at 30 °C in 150 rpm shaking water bath. Native lipase (50.0 mg) or immobilized lipases containing equivalent amount of protein were used as catalysts and added immediately before incubation. A control without the presence of lipase was simultaneously incubated.

2.6. Enantioselective analysis of (±)-menthyl esters by chiral column gas chromatography (GC)

Esterified samples were analyzed by GC using ethyl caproate as the internal standard. Quantitative determinations were performed using a Hewlett–Packard 4890 D gas chromatograph (Hewlett–Packard, Avondale, PA) equipped with a flame ionization detector (FID) and a chiral Rt-βDEXsm fused silica capillary column (30 m × 0.25 mm id, 0.25 μm film thickness: Restek, Bellfonte, PA). The injector and detector temperatures were 230 and 250 °C, respectively, and the carrier gas used was helium. The initial temperature was 110 °C, then programmed to rise 4 °C per min to reach 150 °C and further elevated to 200 °C at 2 °C per min. The amount of each enantiomer was estimated by peak area recorded and integrated by a 3395 Hewlett–Packard Integrator.

The activities of the native and immobilized lipases were estimated from the integrated peak areas of each menthyl ester and expressed as a percentage of the activity of that with the highest activity. Percent yield of menthyl butyrate was defined as (mmol ester/initial mmol alcohol present in

the system) × 100%. The enantioselectivity is expressed as percentage of enantiomeric excess (ee), defined as $\{[-]-[+]/[-]+[+]\} \times 100\%$ (Shaw et al., 2002). The activity of the lipases was expressed as a percentage of the activity of that with the highest activity.

2.7. Thermostability

Native and immobilized lipases were incubated at various temperatures (30, 40, 50, 60 and 70 °C) for 1 h in sealed vials. After incubation, the biocatalysts were cooled to room temperature. The esterification activity and enantioselectivity of the lipase preparations were analyzed using GC. Percent relative activity and enantiomeric excess were determined as described earlier.

2.8. Storage stability

The native and immobilized lipases were stored at various temperatures (−20 °C, 0 °C, 4 °C and room temperature) for 60 days in sealed vials. The esterification activity and enantioselectivity of the native and immobilized lipases were analyzed using GC. Percent relative activity and enantiomeric excess were determined as described earlier.

2.9. Stability in organic solvent

Native and immobilized lipases were incubated in hexanes at room temperature starting from day one to day ten. After incubation, ability of native and immobilized lipases in catalyzing the esterification of (±)-menthol and butyric anhydride was analyzed using GC. Percent relative activity and enantiomeric excess was determined as described earlier.

2.10. Leaching study

To investigate the effect of washing on the immobilized lipases, immobilized lipases were carefully washed with hexane at 4.0, 8.0, 12.0, 16.0 and 20.0 mL with 4.0 mL at each washing. Percent relative activity and enantioselectivity of the lipase preparations was analyzed using GC and determined as described earlier.

2.11. Operational stability

To investigate the operational stability of the immobilized lipase preparations, lipases were incubated in the reaction mixture. After each cycle, the reaction mixture was carefully drained out and percent relative activity as well as enantioselectivity of the immobilized lipases after each cycle were analyzed using GC and determined as described earlier.

2.12. Statistical analysis

All experiments were conducted in triplicate and statistical analysis was performed according to the MINITAB User's Guides (Meyer & Krueger, 2005). Analysis of variance was carried out using the ANOVA procedure by the Bonferroni multiple comparison procedure to determine significant differences between the means.

3. Results and discussion

3.1. Immobilization of lipase

Immobilization of lipase from *Candida rugosa* on the epoxy-activated supports was confirmed using a scanning electron microscope (SEM) (XL30 ESEM, Philips, Washington, DC, USA). Properties of supports as well their protein loading after immobilization is shown in Table 1. Eupergit C and Eupergit C 250 L possess almost similar characteristics. The essential difference between the two supports is the content of epoxide groups, which is >600 μmol/g of dried beads for Eupergit C and >250 μmol/g dried beads for Eupergit C 250 L. However, Eupergit C 250 L (4.81 mg protein/g support) was found to absorb more protein than Eupergit C. In the case of Eupergit C, the support contained a large amount of active groups which hardly permitted lipase particles from being adsorbed while for the Eupergit C 250 L (11.16 mg protein/g support), less amount of active group allows enough lipase particles to be adsorbed.

3.2. Thermostability

Fig. 1 shows thermostability of lipases in the esterification of (−)-menthyl butyrate. All lipases including native lipase exhibited optimum thermostability at 30 °C. Stability of the native lipase, however, decreased as temperature increased. Immobilized lipases retained their catalytic activity, even at higher temperatures compared to native lipase. At these temperatures, native lipase had probably been denatured, while immobilized lipases such as the Eupergit C-lipase and Eupergit C 250 L-lipase were more rigid in terms of conformation and therefore were able to remain stable and retain 72–91% of relative activity even at higher temperatures (60–70 °C). Herein, thermostability of the Eupergit C 250 L-lipase was found to be higher than that of the Eupergit C-lipase and native lipase.

As described earlier, the main difference between the two supports used is the content of epoxide groups, which is more for Eupergit C compared to Eupergit C 250 L. Stability of an enzyme is proportional to the number of cross-linkages between enzyme and the immobilization matrix that locked the enzyme into the active conformation and prevents irreversible unfolding of the protein. However, excess of linkages to the enzyme may also disturb the globular structure of the protein and hence its stability (Crout & Hernaiz, 2000). This could be due to the fact that thermostability of Eupergit C-lipase is lower than that of Eupergit C 250 L-lipase.

Enantioselectivity shown by percent yield of (−)-menthyl butyrate and percent enantiomeric excess of lipases

Table 1
Properties of the supports used and their protein loadings

Support	Properties		Protein loading (mg protein/g support)[a]
	Content of active group (μmol/g of dry beads)	Average pore diameter (Å)	
Eupergit C[b]	>600	150	4.81
Eupergit C 250 L[b]	>250	150	11.16

[a] Protein loading was determined by Bradford Coomasie blue assay procedure (Bradford, 1976) spectrophotometrically at a wavelength of 595 nm using the calibration curve of bovine serum albumin.
[b] Eupergit C and C 250 L were gifts from Röhm, Darmstadt, Germany.

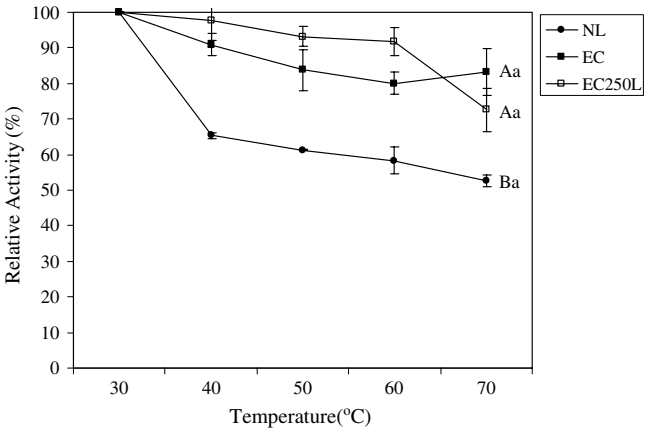

Fig. 1. Percent relative activity of native and immobilized lipases after 1 h of incubation at various temperatures. NL, native lipase; EC, Eupergit C-lipase; EC250L, Eupergit C 250 L-lipase. Error bars indicate standard deviation of triplicates of different enzymes analyzed. Values with same letter (a, b) are not significantly different ($P < 0.05$), between incubation temperatures. Values with same letter (A, B) are not significantly different ($P < 0.05$), between enzymes.

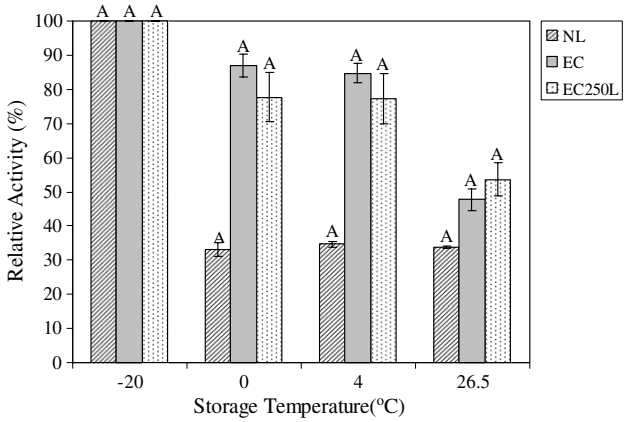

Fig. 2. Percent relative activity of native and immobilized lipases after 60 days of incubation at different storage temperatures. NL, native lipase; EC, Eupergit C-lipase; EC250L, Eupergit C 250 L-lipase. Error bars indicate standard deviation of triplicates of different enzymes analyzed. Values with same letter (A, B) are not significantly different ($P < 0.05$), between enzymes.

in Table 2 were not much affected by heat treatment and enantioselectivity of the immobilized lipases was much higher than that of the native lipase. This could be due to the direct interaction between lipases and the supports used which allows lipase to obtain suitable conformation that facilitates the (−)-enantiomer and treatment of the immobilized lipases with different temperature did not seem to affect this conformation.

3.3. Storage stability

Generally, native and immobilized lipases were most active when stored at −20 °C for 60 days (Fig. 2). When storage temperatures were increased all lipases showed a decrease in storage stability. The immobilized lipases were, however, more stable than the native lipase. This stabilization may be a result of adsorption of the enzyme to the support. Supports used in the immobilization process may protect and prevent lipase from proteolysis and aggregation; thus creating a more rigid enzyme molecule (Dabulis & Klibanov, 1993; Secundo, Carrea, Soregaroli, Varinelli, & Morrone, 2001).

As purity and chirality are concerned, immobilized lipases yielded very high percent enantiomeric excess under all storage conditions and exhibited optical purity of about 10-folds higher than that shown by the native lipase (Table

Table 2
Values of percent yield of (−)-menthyl butyrate and percent enantiomeric excess of (±)-menthyl butyrates synthesized by native and immobilized lipase after 1 h of incubation at various temperatures

Temperature (°C)	Yield of (−)-menthyl butyrate (%)[a]			Enantiomeric excess (%)[b]		
	NL	EC	EC250L	NL	EC	EC250L
30	6.77 ± 0.37	35.00 ± 3.91	43.37 ± 4.05	14.00 ± 0.73	90.38 ± 0.37	93.99 ± 0.55
40	6.41 ± 4.06	31.89 ± 0.44	41.80 ± 1.48	10.76 ± 1.50	94.48 ± 1.05	90.99 ± 0.73
50	7.00 ± 5.25	30.07 ± 0.02	39.94 ± 2.63	14.46 ± 0.41	90.89 ± 1.91	91.37 ± 0.06
60	6.32 ± 0.17	29.28 ± 2.20	39.11 ± 0.35	12.88 ± 0.81	91.67 ± 2.30	91.56 ± 0.45
70	3.29 ± 0.30	27.01 ± 0.02	30.70 ± 0.19	13.98 ± 0.66	93.08 ± 2.84	90.68 ± 0.15

[a] Yield is defined as (mmol ester/initial mmol alcohol present in the system) × 100%.
[b] Enantiomeric excess is defined as {[−] − [+]/[−] + [+]} × 100% (Shaw et al., 2002). NL, native lipase; EC, Eupergit C-lipase; EC250L, Eupergit C 250 L-lipase. The values are reported as mean ± SD.

Table 3
Values of percent yield of (−)-menthyl butyrate and percent enantiomeric excess of (±)-menthyl butyrates synthesized by native and immobilized lipase after 60 days of incubation at different storage temperatures

Storage temperature (°C)	Yield of (−)-menthyl butyrate (%)[a]			Enantiomeric excess (%)[b]		
	NL	EC	EC250L	NL	EC	EC250L
−20	8.51 ± 0.00	33.88 ± 2.26	47.69 ± 4.25	13.54 ± 2.32	100 ± 0.00	94.79 ± 0.05
0	3.27 ± 0.01	27.49 ± 0.74	36.32 ± 0.33	9.87 ± 2.38	91.89 ± 1.26	91.90 ± 0.23
4	3.60 ± 0.40	27.78 ± 0.73	36.02 ± 0.01	9.70 ± 1.82	93.75 ± 0.82	90.95 ± 0.93
26.5	3.17 ± 0.04	15.96 ± 0.04	25.51 ± 0.04	9.41 ± 0.50	90.98 ± 0.72	92.91 ± 0.03

[a] Yield is defined as (mmol ester/initial mmol alcohol present in the system) × 100%.
[b] Enantiomeric excess is defined as {[−] − [+]/[−] + [+]} × 100% (Shaw et al., 2002). NL, native lipase; EC, Eupergit C-lipase; EC250L, Eupergit C 250 L-lipase. The values are reported as mean ± SD.

3). This high yield and optical purity could be attributed to the better distribution of lipase on the larger surface area of the supports. Restriction of lipase onto larger support surface area was found to increase lipase catalytic activity via interfacial activation.

3.4. Stability in organic solvent

The stability pattern of lipase preparations in hexane at room temperature over a period of 10 days is illustrated in Fig. 3. Interestingly, the activities of immobilized lipases remained above 50% even after 10 days of incubation in hexane. When enzymes are used in organic solvents, they are not only surrounded by the solvent itself, but also by water. The hydration on enzyme which is a molecular monolayer of water is required in order for an enzyme to be catalytically active (Basri et al., 1997).

Lipase activity and enantioselectivity of (−)-menthyl butyrate were very much influenced by hydrophobicity of solvent as they play an important role for the retention of enzymatic activity. Researchers have shown that activities of lipases are often very good in hydrophobic solvents with log *P* value of between 2 and 4 (e.g. hexane). However, these solvents may cause inhibitory effects due to the withdrawal of water from the enzyme surfaces necessary for the enzymatic activity, but are still low compared to hydrophilic solvents (Laane, Boeren, Vos, & Veeger, 1987).

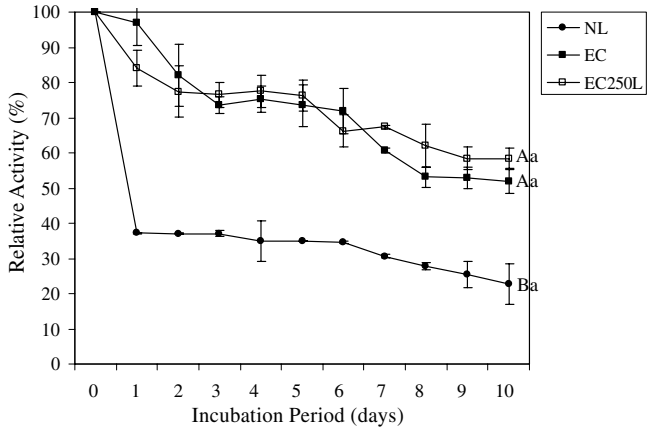

Fig. 3. Percent relative activity of native and immobilized lipases as affected by incubation in hexane from 1 to 10 days. NL, native lipase; EC, Eupergit C-lipase; EC250L, Eupergit C 250 L-lipase. Error bars indicate standard deviation of triplicates of different enzymes analyzed. Values with same letter (a, b) are not significantly different ($P < 0.05$), between incubation periods. Values with same letter (A, B) are not significantly different ($P < 0.05$), between enzymes.

In this study, lipase immobilized onto both Eupergit C and C 250 L were not much affected by the presence of hexane over a period of 10 days and retained high percent yield of (−)-menthyl butyrate and percent enantiomeric excess (Table 4). This can be explained by the immobilization of lipase on porous matrix of the epoxy-activated supports, which prevents lipase from interaction with external interfaces such as air, oxygen and organic solvents, thus not inferring their catalytic activities (Bes, Gomez-Moreno, Guisan, & Fernandez-Lafuente, 1995; Fernandez-Lafuente, Rodriguez, & Guisan, 1998; Gupta, 1991).

3.5. Leaching study

The effect of washing lipase preparations using hexane towards stability and enantioselectivity of the esterification was investigated. Fig. 4 shows that lipase immobilized onto Eupergit C and Eupergit C 250 L retained high activity even after washing with copious amount of hexane.

Besides working as an immobilization matrix for the lipase, supports used in this study were also found to protect lipases from being leached during washing process. This can be observed from the high percent yield of (−)-menthyl butyrate and optical purity achieved by immobilized lipases (Table 5).

To clarify these, the use of these enzymes with 12 cycles of repetition was then done and discussed in the following section (Section 3.6).

3.6. Operational stability

Reusability studies of the lipase were carried out by using the recovered lipases for subsequent cycles as shown in Fig. 5. Overall, relative activity for both lipases decreased gradually with remaining activity for Eupergit C 250 L-lipase and Eupergit C-lipase after 12 cycles being approximately about 46% and 38%, respectively. Percent yield of (−)-menthyl butyrate formed and percent enantiomeric excess of (±)-menthyl butyrate are listed in Table 6.

Table 4
Values of percent yield of (−)-menthyl butyrate and percent enantiomeric excess of (±)-menthyl butyrates synthesized by native and immobilized lipase as affected by incubation in hexane from 0 to 10 days

Incubation period (days)	Yield of (−)-menthyl butyrate (%)[a]			Enantiomeric excess (%)[b]		
	NL	EC	EC250L	NL	EC	EC250L
0	8.51 ± 0.00	33.88 ± 2.26	47.69 ± 4.25	13.54 ± 2.32	100 ± 0.00	94.79 ± 0.05
2	7.43 ± 0.00	27.69 ± 1.22	36.35 ± 4.01	14.64 ± 0.00	94.98 ± 0.14	95.52 ± 0.20
4	6.63 ± 1.90	25.94 ± 5.32	37.61 ± 1.46	14.76 ± 1.57	100 ± 0.00	95.68 ± 0.10
6	6.76 ± 0.15	24.24 ± 0.67	32.92 ± 1.87	12.48 ± 0.14	95.17 ± 0.07	94.87 ± 0.66
8	5.49 ± 0.53	15.66 ± 0.83	32.89 ± 5.71	9.21 ± 0.00	100 ± 0.00	95.68 ± 0.29
10	4.41 ± 1.39	12.45 ± 0.07	28.50 ± 0.61	12.91 ± 0.77	94.77 ± 0.03	95.40 ± 0.06

[a] Yield is defined as (mmol ester/initial mmol alcohol present in the system) × 100%.
[b] Enantiomeric excess is defined as {[−] − [+]/[−] + [+]} × 100% (Shaw et al., 2002). NL, native lipase; EC, Eupergit C-lipase; EC250L, Eupergit C 250 L-lipase. The values are reported as mean ± SD.

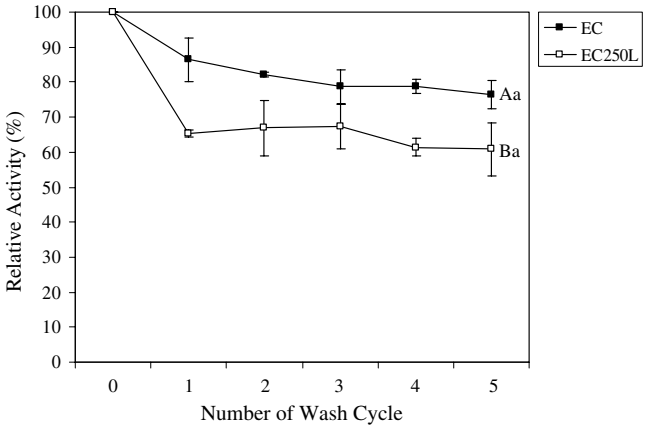

Fig. 4. Percent relative activity of native and immobilized lipases as affected by washing using hexane. Immobilized lipases were carefully washed with 4 mL of hexane at each washing. EC, Eupergit C-lipase; EC250L, Eupergit C 250 L-lipase. Error bars indicate standard deviation of triplicates of different enzymes analyzed. Values with same letter (a, b) are not significantly different ($P < 0.05$), between number of wash cycles. Values with same letter (A, B) are not significantly different ($P < 0.05$), between enzymes.

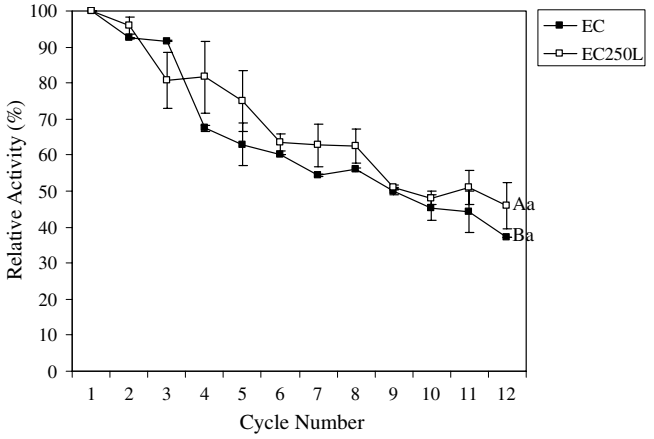

Fig. 5. Percent relative activity of immobilized lipases showing operational stability of the immobilized lipases. EC, Eupergit C-lipase; EC250L, Eupergit C 250 L-lipase. Error bars indicate standard deviation of triplicates of different enzymes analyzed. Values with same letter (a, b) are not significantly different ($P < 0.05$), between number of operation cycles. Values with same letter (A, B) are not significantly different ($P < 0.05$), between enzymes.

Table 5
Values of percent yield of (−)-menthyl butyrate and percent enantiomeric excess of (±)-menthyl butyrates synthesized by immobilized lipases as affected by washing with different volume of hexane

Wash cycle	Yield of (−)-menthyl butyrate (%)[a]		Enantiomeric excess (%)[b]	
	EC	EC250L	EC	EC250L
0	39.11 ± 0.08	47.69 ± 4.25	100 ± 0.00	94.79 ± 0.05
1	33.88 ± 2.26	31.96 ± 2.29	95.24 ± 0.07	95.52 ± 0.34
2	35.28 ± 0.16	33.04 ± 1.70	95.44 ± 0.35	95.44 ± 0.34
3	30.81 ± 2.08	35.23 ± 4.46	94.63 ± 0.05	96.04 ± 0.26
4	33.40 ± 1.68	29.81 ± 1.43	95.24 ± 0.38	95.45 ± 0.01
5	29.78 ± 1.47	29.62 ± 1.18	94.69 ± 0.00	94.45 ± 0.29

[a] Yield is defined as (mmol ester/initial mmol alcohol present in the system) × 100%.
[b] Enantiomeric excess is defined as {[−] − [+]/[−] + [+]} × 100% (Shaw et al., 2002). EC, Eupergit C-lipase; EC250L, Eupergit C 250 L-lipase. The values are reported as mean ± SD.

The initial percent yield of (−)-menthyl butyrate formed was about 34% and 48% for Eupergit C 250 L-lipase and Eupergit C-lipase, respectively. The value decreased gradually after the first cycle for both lipases and was about 22% and 12% for Eupergit C 250 L-lipase and Eupergit C-lipase after 12 cycles, respectively. This decrease may not necessarily be due to inactivation of the biocatalysts since enzyme activity could still be measured in the matrix but may indeed be due to increase of diffusion limitation of the substrate into the pores of the support (Chikere, Galun-

Table 6
Values of percent yield of (−)-menthyl butyrate and percent enantiomeric excess of (±)-menthyl butyrates synthesized by immobilized lipases showing operational stability

Cycle number	Yield of (−)-menthyl butyrate (%)[a]		Enantiomeric excess (%)[b]	
	EC	EC250L	EC	EC250L
2	30.52 ± 1.98	47.59 ± 1.43	100 ± 0.00	100 ± 0.00
4	21.62 ± 0.33	40.80 ± 0.00	95.06 ± 0.10	80.01 ± 0.28
6	19.25 ± 0.90	29.06 ± 4.44	91.20 ± 3.64	96.11 ± 1.52
8	18.39 ± 0.41	28.13 ± 0.00	86.30 ± 3.34	89.55 ± 4.51
10	13.69 ± 0.05	21.96 ± 0.44	85.65 ± 3.79	87.42 ± 0.00
12	11.85 ± 0.10	22.07 ± 2.17	83.28 ± 1.50	85.22 ± 0.00

[a] Yield is defined as (mmol ester/initial mmol alcohol present in the system) × 100%.
[b] Enantiomeric excess is defined as {[−] − [+]/[−] + [+]} × 100% (Shaw et al., 2002). EC, Eupergit C-lipase; EC250L, Eupergit C 250 L-lipase. The values are reported as mean ± SD.

sky, Schünemann, & Kasche, 2001). However, percent enantiomeric excess remained high for both Eupergit C 250 L-lipase and Eupergit C-lipase and showed less than 20% decrease in activity. Furthermore, immobilization was also found to help increase dispersion of lipase in the reaction media by promoting better substrate binding in the reaction at each cycle.

4. Conclusions

The presence of polymer matrix with a lower distribution of active groups in Eupergit C 250 L was found beneficial for enzymatic stability and activity. Immobilization of lipase onto porous supports of Eupergit C and Eupergit C 250 L by adsorption proved to be a useful technique for improving enzymatic activity and enantioselectivity. Furthermore, the possibility of an efficient reuse of the immobilized lipases shown in this study makes this system attractive for practical applications. This process is therefore applicable to various reactions involving water-insoluble reactants and products.

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