Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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Online publication date: 15 September 1999

To cite this Article Ceynowa, J. and Koter, I.(1999) 'Selection of Pure Enantiomers of 1-Phenyl Alcohols by Sequenced Processes of Ester Hydrolysis and Transesterification in Enzyme Membrane Reactors', Separation Science and Technology, 34: 13, 2663 — 2678
To link to this Article: DOI: 10.1081/SS-100100797
URL: http://dx.doi.org/10.1081/SS-100100797

SEPARATION SCIENCE AND TECHNOLOGY, **34**(13), pp. 2663–2678, 1999

Selection of Pure Enantiomers of 1-Phenyl Alcohols by Sequenced Processes of Ester Hydrolysis and Transesterification in Enzyme Membrane Reactors

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ABSTRACT

Research aimed on selecting the particular enantiomers in sequenced processes is described. The work presents experimental results on performing hydrolysis of 1-phenyl alcohol ester and transesterifications in the system vinyl acetate and 1-phenyl alcohols in batch and in membrane reactors. Lipase from *Pseudomonas* sp. has been used as the biocatalyst for both processes. This catalyst exhibits high catalytic activity and the same enantioselectivity when used in solution (batch reactor) and when immobilized within polyamide membranes (membrane reactor). Using the mixture of products from the hydrolysis of 1-phenylethyl propionate, which can exhibit a moderate or low enantiomeric purity, to the next process of transesterification with vinyl acetate leads to products of high enantiomeric purity (enantiomeric excess $> 97\%$). This procedure could be a good way to produce substances with the pure enantiomeric block needed for further syntheses of enantiomerically pure compounds. It is convenient to perform the processes in reactors with lipase chemically immobilized within the polyamide membranes.

INTRODUCTION

Enzymes, especially hydrolases, are known to be very useful for the production of enantiomerically pure compounds by the kinetic resolution method. Such compounds have been used as chiral blocks for pharmaceuticals, pesticides, pheromones, and so on. In the enzymatic kinetic resolution of a racemic

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mixture, the optical purity of the product depends on an enzyme enantioselectivity in the system. It was shown recently that kinetic resolution can be improved by carrying out two enzymatic reactions simultaneously or sequentially (1–4), and the enantiomeric excess of the product in double kinetic resolution exceeds that gained in one step. The benefits of that strategy in overall yield and enantiomeric purity of the enantiomers produced are especially clear in processes of low or moderate enantioselectivity. It is convenient to perform such processes in continuous reactors with the catalyst immobilized within a membrane. As a stable carrier of the enzyme catalyst, the membrane separates the reactants, which results in shifting the equilibrium reaction toward the products. Membranes with an immobilized enzyme were used earlier in processes carried out in biphasic systems, such as the hydrolysis of achiral oils (5–7), as well as in the enantioselective hydrolysis of chiral acids esters (8), alcohols (9), and glycidol derivatives (10).

It should be noted that the opical purity of the products of kinetic resolution, expressed conveniently by enantiomeric excess (*ee*), decreases dramatically at conversions higher than 50% (11).

This work is aimed at kinetic resolution to obtain the particular enantiomers of 1-phenyl alcohols, and the final goal of the research is to estimate the conditions for performing this process in two sequenced steps in a continuous membrane reactor. Three different lipases were investigated to choose the best catalyst for the group of chosen substrates, both in hydrolysis and transesterification. The influence of some factors, like alcohol structure, the acyl donor excess, and the amount of water, on the final optical purity of the product and on the lipase activity have been tested in the batch system. Based on the results, single transesterification of racemic alcohols as well as sequenced hydrolysis and transesterification processes have also been performed in an enzyme membrane reactor.

THEORETICAL OUTLINE OF THE KINETICS OF CHIRAL RESOLUTION PROCESSES

Most chiral resolution reactions are actually reactions with more than one substrate and/or product, often with two substrates, called bi-bi reactions. The second substrate may influence the enantiomeric excess of the product and the remaining substrate. This ought to be observed in the system used in this work, i.e., during the transesterification of chiral alcohol by vinyl acetate. A general scheme of the reaction (Eqs. 1a and 1b) and its mechanism (Eq. 2) regarding the participation of the enzyme catalyst, Enz, can be represented by the following scheme (11, 12):

$$A + B^R \underset{}{\overset{\text{Enz}}{\rightleftharpoons}} P + Q^R \qquad (1a)$$

$$A + B^S \underset{}{\overset{Enz}{\rightleftharpoons}} P + Q^S \quad (1b)$$

$$Enz'^R + B^R \underset{k_3^R}{\overset{k_3^R c_B^R}{\rightleftharpoons}} EnzQ^R \underset{k_4^R c_Q^S}{\overset{k_4^R}{\rightleftharpoons}} Enz + Q^R \quad (2a)$$

$$Enz + A \underset{k_1}{\overset{k_1 c_A}{\rightleftharpoons}} EnzA \underset{k_{2cP}}{\overset{k_2}{\rightleftharpoons}} P$$

$$Enz'^S + B^S \underset{k_3^S}{\overset{k_3^S c_B^S}{\rightleftharpoons}} Enz'Q^S \underset{k_4^S c_Q^S}{\overset{k_4^S}{\rightleftharpoons}} Enz + Q^S \quad (2b)$$

where A is the nonchiral substrate (vinyl acetate); B^R and B^S are the chiral (R and S) substrates (alcohol); P is the nonchiral product; Q^R and Q^S are chiral products (acetate enantiomers); EnzA is the transition complex of the enzyme substrate A; Enz'^R, Enz'^S, $EnzQ^R$, and $EnzQ^S$ are transition complexes of enzyme products; Enz is the free enzyme; k_i represents the rate constant of the particular step of the process; and c_P, c_A, c_B^R, c_B^S, c_Q^R, and c_Q^S are the respective concentrations of the particular compounds.

The ratio of the overall reaction rates of Reaction (2a) and (2b), v^R/v^S, can be represented (12) by

$$\frac{v_{B^R}}{v_{B^S}} = \frac{c_{B^R}(E \cdot c_A + c_{Q^R}/\alpha^R) - c_{Q^R}(E \cdot c_P/K_{eq} + c_{B^S}/\alpha^R)}{c_{B^S}(c_A + c_{Q^R}/\alpha^R) - c_{Q^S}(c_P/K_{eq} + c_{B^R}/\alpha^R)} \quad (3)$$

where K_{eq} is the equilibrium constant; kinetic parameter α^R is the ratio of V_1/K_{mA} and V_{-1}/K_{mQR}; V_1 and V_{-1} are the maximum reaction rates for the overall forward and reverse reaction, respectively; K_{mA} and K_{mQ^R} are the Michaelis constants for substrate A and product Q^R; and E (enantiomeric ratio) represents enantioselectivity defined as the ratio $(k_{cat}/K_m)^R/(k_{cat}/K_m)^S$ (11).

For $c_P = 0$ (as is the case when using vinyl alcohol because it tautomerizes to acetic aldehyde), Eq. (3) reduces to

$$\frac{v_{B^R}}{v_{B^S}} = \frac{c_{B^R}(E \cdot \alpha^R \cdot c_A + c_{Q^S}) - c_{Q^R} \cdot c_{B^S}}{c_{B^S}(\alpha^R c_A + c_{Q^R}) - c_{Q^S} \cdot c_{B^R}} \quad (4)$$

Numerical integration of Eq. 4 leads to a relationship between enantiomeric excess and the degree of conversion. Enantiomeric excess is defined as $ee_i = (c_i^R - c_i^S)/(c_i^R + c_i^S)$, where the subscript i refers to chiral substrate (ee_S) or chiral product (ee_P).

MATERIALS AND METHODS

Chemicals

Commercial lipases from *Candida rugosa* (Sigma, 26,000 u/mg), *Porcine pancreas* (Sigma, 160 u/mg), and from *Pseudomonas* sp. (Type XIII, Sigma, 2160 u/mg) were used without any additional purification.

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The *n*-heptane was of HPLC grade (Aldrich). Racemic alcohols and (*R*,*S*)-1-phenylethyl propionate were purchased from Fluka and Aldrich. The following alcohols were used: 1-phenyl-2-propanol **(1),** 1-phenyl-1-propanol **(2),** 1-phenylethanol **(3),** and 2-phenylethanol **(4).** Racemic 1-phenylethyl acetate and 1-phenyl-1-propyl acetate were synthesized by reaction with acetic anhydride and sodium acetate. The optical purity was checked by optical rotation measurements. The commercial reagents were used without additional purification except for vinyl acetate which was distilled before use. The reagents for transesterification and ester hydrolysis were dried over the molecular sieves 4Å.

Analytical

The enantiomeric excess of the esters and the reaction conversion were determined from estimations by HPLC. The system was composed of a Spectra Physics SP 8810 isocratic pump, a Rheodyne injector equipped with a 10-μL loop, the chiral analytical column NUCLEOSIL CHIRAL-2 ET 250/8/4 (4.6 × 250 mm), and a Shodex RI SE 61 refractive index detector. The optimal composition of the mobile phase system consisted of *n*-heptane with 0.1% (v/v) isopropanol and 0.05% (v/v) trifluoroacetic acid. The flow rate of the mobile phase was 1.0 mL/min. Chromatograms were managed by a PL LogiCal program. Chromatographic data are given in Table 1. Additionally, optical rotations were measured by means of a Polamat A polarimeter (Carl Zeiss Jena, Germany).

The water content in the organic phase was determined colorimetrically by titration using ReAquant reagents (J. T. Baker, Holland).

TABLE 1
Characteristics of HPLC Chromatograms: Peak Capacity Factors k'_1 and k'_2 for *R*- and *S*-Enantiomers[a]

Compound	k'_1	k'_2
(*R*,*S*)-1-Phenyl-2-propyl acetate	4.36	5.11
(*R*,*S*)-1-Phenylethyl propionate	4.87	5.55
(*R*,*S*)-1-Phenylethyl acetate	9.78	10.11
(*R*,*S*)-1-Phenyl-1-propyl acetate	12.34	12.86
(*R*,*S*)-1-Phenyl-2-propanol	15.23	15.23
(*R*,*S*)-1-Phenyl-1-propanol	17.85	17.85
(*R*,*S*)-1-Phenylethanol	21.10	21.10

[a] Peak capacity factors k'_1 and k'_2 are equal to the ratio of masses of the compound in the stationary and mobile phases and are calculated by $k' = (T_r - T_0)/T_0$, where T_r and T_0 represent the retention due to the compound and the dead time of the system, respectively.

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Enzyme Membranes

Aromatic polyamide hollow-fiber membranes with a molecular weight cut-off of 50,000 Da, ID 0.6 mm, and OD 1.2 mm were obtained from Berghof (Germany). The hydrodynamic permeability of these membranes was estimated to be 42 $g \cdot m^{-2} \cdot s^{-1} \cdot MPa^{-1}$. The membranes (5 fibers of 20 cm length) were used in a laboratory module with a total (geometrical) area of the membranes equal to 30 cm^2.

Immobilization of the Lipase

The membranes were treated with 3.6 M HCl (for 2 hours) to hydrolyze a part of the amide groups and then placed into a 0.01 M solution of 1,4-diaminobutane (4 hours, 293 K). This was followed by activation of the membrane with a 5% (v/v) solution of glutaraldehyde in phosphate buffer, 0.02 M, pH 7.2. The membranes were then washed several times with phosphate buffer. In the next step the lipase solution in 0.02 M phosphate buffer, pH 7.2 was forced to circulate slowly on the support (shell) side of the membrane (24 hours at 280 K). The solution that permeated through the membrane was directed back to the circulating solution. The amount of lipase immobilized in the membrane was estimated from the mass balance based on enzyme activity determinations in the hydrolysis of olive oil (according to the SIGMA procedure) by lipase solution, before and after the immobilization. Enzyme loading was equal to 0.05 and 0.03 mg/cm^2 of the membrane (geometrical) surface in the two hollow-fiber modules, respectively.

Membrane Reactors

Experiments of enzymatic hydrolysis were carried out in a biphasic system reactor with both (100 cm^3) the ester solution in *n*-heptane and the phosphate buffer 0.5 M, pH 7.20, circulating countercurrently on both sides of the enzyme membrane. Small samples (50 μL) of the organic phase were taken out periodically for HPLC analysis to determine the degree of conversion and the enantiomeric excess of the remaining unreacted ester.

Transesterifications were performed in a monophasic reactor in a crossflow system. The feed solution recirculated on one side of the enzyme membrane and partially permeated through the membrane. Schemes of the reactors are presented as Fig. 1.

Transesterification with Native Lipase

In a typical experiment, 2 mL of an alcohol solution in *n*-heptane (0.1 M, if not stated otherwise) was placed in a capped vial and fresh distilled vinyl acetate was added. The reaction was initiated by the addition of lipase powder,

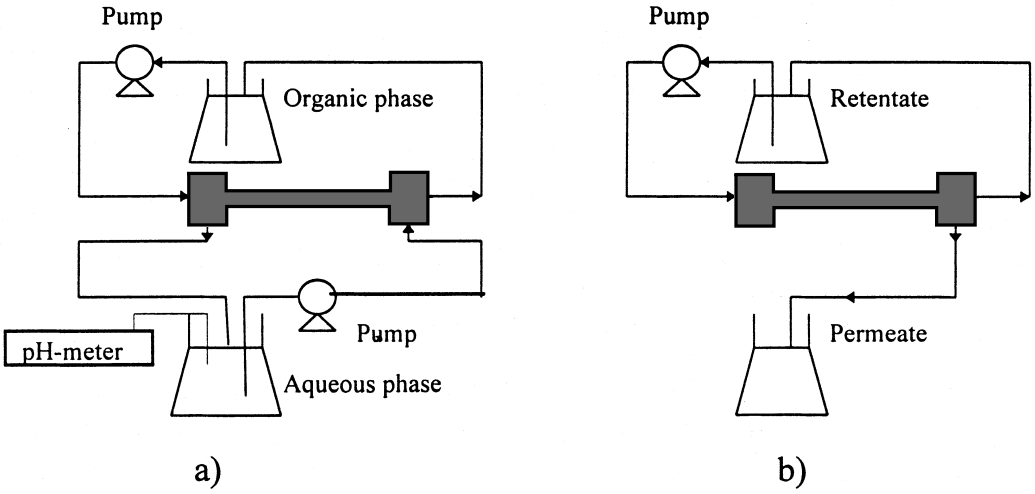

FIG. 1 Scheme of the enzyme membrane reactors. a) Biphasic hollow-fiber enzyme membrane reactor. b) Enzyme membrane reactor for transesterification processes.

and the vial was put in a thermostated box with a magnetic agitator. Small volumes (25 μL) of the reaction mixture were removed periodically for analyses.

Transesterification in an Enzyme Membrane Reactor

In the experiments, 100 mL of the reaction mixture containing racemic alcohol (0.01 mol) and a 25-fold excess of vinyl acetate in *n*-heptane was placed in a flask and recirculated on the inner side of the enzyme membranes. Small volumes (50 μL) of permeate were removed for HPLC analyses.

Sequenced Hydrolysis and Transesterification with Native Lipase

In the first stage, 2.5 mL of the racemic 1-phenylethyl propionate solution in heptane (0.2 M) was placed in a vial with a magnetic stirring bar and a Teflon cap, and 2.5 mL of lipase solution in phosphate buffer (0.5 M, pH 7.2) was added. The total amount of lipase was equal to 0.1 mg. The mixture was stirred vigorously at 303 K. After 24 hours the concentration of the product and the enantiomeric excess of the remained propionate were determined. The phases were separated, 2.5 mL of the heptane solution containing 0.2 mM of 1-phenylethanol and 4 mM of vinyl acetate was placed into an another vial, and transesterification was carried out for 48 hours.

Sequenced Hydrolysis and Transesterification in Enzyme Membrane Reactors

In the first stage, hydrolysis of (*R,S*)-1-phenylethyl proprionate was performed. Ester solution in *n*-heptane (100 mL, 0.1 M) was recirculated on the

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inner (lumen) side of the membrane, and 0.5 M phosphate buffer solution (100 ml, pH 7.2) was recirculated countercurrently on the outer (shell) side. The process was maintained for 4 days and then the conversion and enantiomeric excess of the remained ester were determined by HPLC. The organic phase, after the addition of 4 g of dried vinyl acetate, was used for the second process. The mixture containing 1-phenylethanol (with *R*-enantiomer in excess), unreacted 1-phenylethyl propionate, and vinyl acetate in heptane was forced to permeate through the membrane in a crossflow system. Small aliquots of the reaction mixture (the retentate and permeate) were taken out periodically to determine the conversion of transesterification and optical purity of the 1-phenylethyl acetate produced.

Choice of the Biocatalyst

Three raw commercial lipases were tested in the hydrolysis of the racemic 1-phenylethyl propionate, as well as in the transesterification of 1-phenylethanol, in a batch reactor in order to determine the best biocatalyst for both processes. The results (estimation of initial reaction rates and enantioselectivities) are presented in Table 2.

Lipase from *Pseudomonas* sp. exhibits the best enantioselectivity, both in the ester hydrolysis and in transesterification with vinyl acetate, although the enantioselectivity in the latter process is rather low.

RESULTS

Enantioselectivity and Catalytic Activity of Lipase toward Different Alcohols

The chiral alcohols used as substrates in transesterification processes, **1, 2,** and **3,** differ in lengths of their alkyl chains. The similar first-order alcohol, 2-

TABLE 2
Results of the Hydrolysis of (*R*,*S*)-1-Phenylethyl Propionate and Transesterification of (*R*,*S*)-1-Phenylethanol with Different Lipases. Batch Reactor[a]

	Hydrolysis		Transesterification	
Lipase from	Initial reaction rate (mol/h·mg)	Enantiomeric ratio, *E*	Initial reaction rate (mol/h·mg)	Enantiomeric ratio, *E*
Candida rugosa	2.4×10^{-7}	<2	1.0×10^{-8}	<2
Porcine pancreas	1.7×10^{-7}	<2	1.1×10^{-6}	5
Pseudomonas sp.	1.4×10^{-4}	56	2.3×10^{-6}	11

[a] The initial substrate concentration (both ester and alcohol) was 0.1 M, the excess of vinyl acetate was 25, and the amount of lipase was 0.1 mg.

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phenylethanol **(4)** was also investigated for comparison of the lipase reactivity toward first- and second-order alcohols. All of the processes were carried out in a batch reactor at identical conditions: at 303 K, alcohol concentration equal to 0.1 M, and 25-fold excess of vinyl acetate. The amount of suspended lipase was equal to 0.1 mg, and the water concentration in all the processes was no higher than 0.05% (w/v). The total volume of the reaction mixture was 5 mL.

It was found in the case of transesterification of chiral alcohols that lipase from *Pseudomonas* sp. preferentially catalyzes reactions of the corresponding *R*-enantiomers. The dependence of the degree of conversion and of the enantiomeric excess of the product on the time of reaction for the particular alcohols are given in Figs. 2 and 3.

The best enantioselectivity and the highest initial reaction rate were observed in the reaction of racemic 1-phenyl-2-propanol **(1).** 1-Phenylethanol **(3)** reacts much slower and with the lowest enantioselectivity ($E = 29$, 21, and 11 for **1, 2,** and **3,** respectively). The catalytic activity of lipase from *Pseudomonas* sp. toward 2-phenylethanol is much lower than that exhibited in reactions with second-order alcohols with a similar formula. This could indicate that alcohols with a longer alkyl chain fit better to the active site of the enzyme. This can be seen in Fig. 4 which shows schemes of the active site of lipase (13) from *Pseudomonas* sp. placed in alcohols.

Influence of Acyl Donor Excess on Enantioselectivity in Transesterification

According to the mechanism of bi-bi reactions, vinyl acetate is the first substrate that enters the active site of the enzyme (see Eq. 2). Its influence

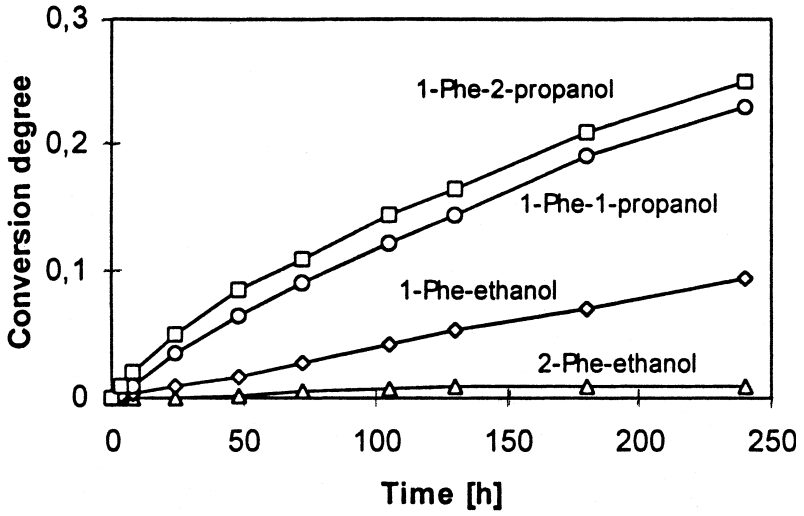

FIG. 2 Degree of conversion vs reaction time in the transesterification of various alcohols catalyzed by the native lipase from *Pseudomonas* sp.

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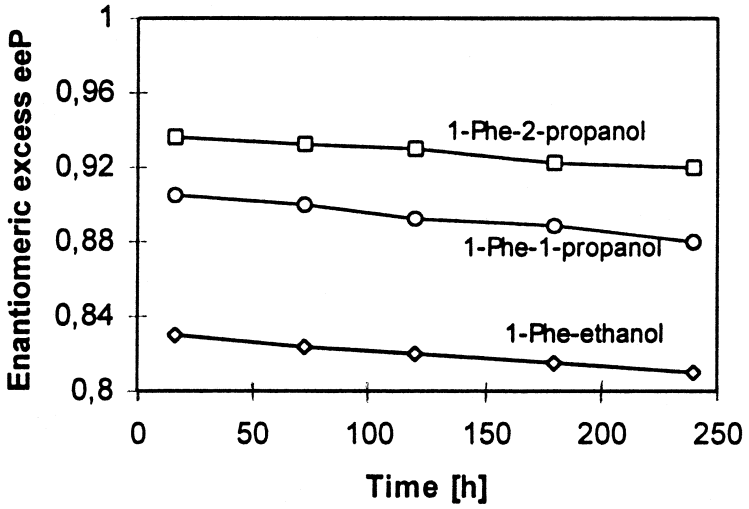
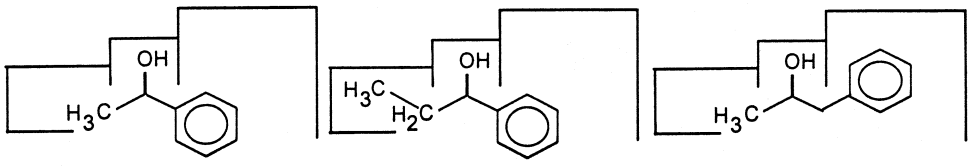


FIG. 3 Enantiomeric excesses of the products as a function of the reaction time in the enzymatic transesterification of racemic alcohols.

on reaction progress may usually be omitted because it is used in large excess in relation to the second substrate. It was of interest, however, to examine the dependence of the enantiomeric excess of the product and the remained substrate on the concentration ratio $c_{(\text{vinyl acetate})}/c_{(\text{alcohol})}$. The progress of transesterification of racemic **2** at different vinyl acetate excesses is shown in Fig. 5. The dependence of an enantiomeric excess of the product (ee_P) on the degree of conversion in these reactions is given in Fig. 6.

The results indicate a strong influence of the acyl donor concentration on reaction performance. Although in all the cases the reaction goes to completion, a decrease of the enantiomeric excess of the product with reaction time is essential at smaller values of c_{A0}/c_{B0}. Evidently, the lower the ratio c_{A0}/c_{B0}, the larger are the deviations from the irreversible uni-uni mechanism, which is reflected in the lower values of enantioselectivity. The dependence of sub-



R-1-Phenylethanol $E = 11$ | R-1-Phenyl-1-propanol $E = 21$ | R-1-Phenyl-2-propanol $E = 29$

FIG. 4 Schemes of the active site of lipase from *Pseudomonas* sp. placed in alcohols.

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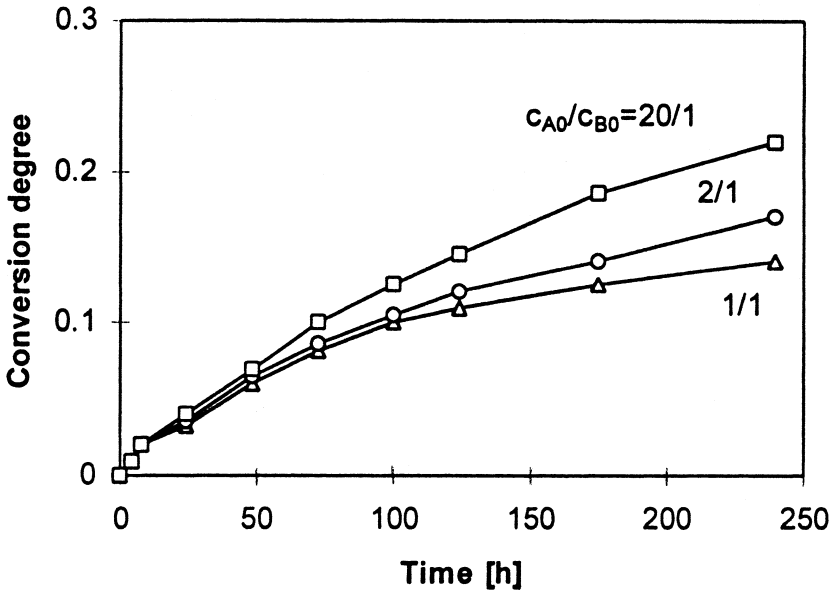

FIG. 5 Degree of conversion vs reaction time in the transesterification of racemic 1-phenyl-1-ropanol at various ratios of the initial vinyl acetate (c_{A0}) and alcohol (c_{B0}) concentrations.

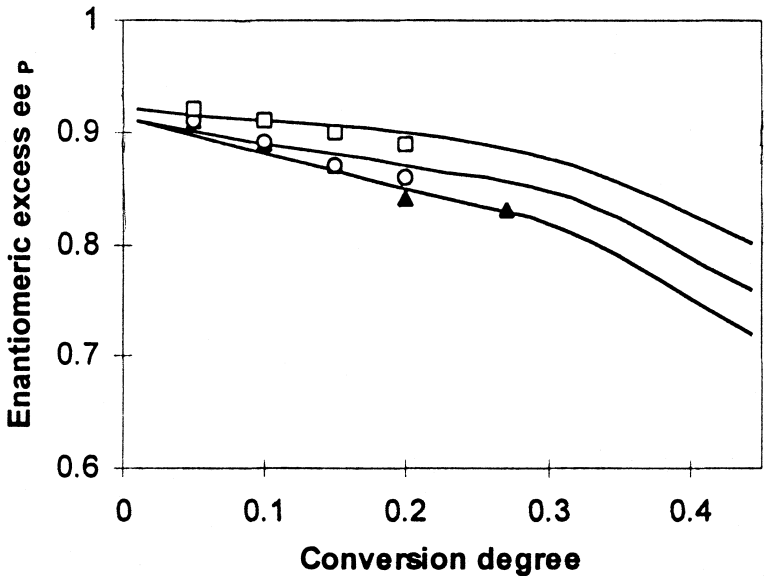

FIG. 6 Enantiomeric excess of the product (1-phenyl-1-propyl acetate) as a function of the degree of conversion at different acyl donor excesses: (▲) $c_{A0}/c_{B0} = 1$, (○) $c_{A0}/c_{B0} = 2$, (□) $c_{A0}/c_{B0} = 20$. Curves were calculated for $E = 21$ and various c_{A0}/c_{B0} ratios according to Eq. (4)

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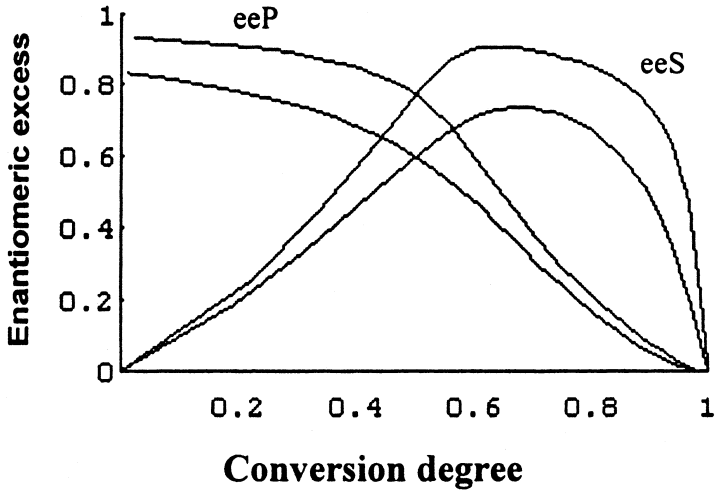

FIG. 7 Enantiomeric excess of a substrate (ee_S) and a product (ee_P) at $c_{A0}/c_{B0} = 2$ (calculated according to Eq. 4). The lower curves are for $E = 11$ and the upper curves are for $E = 29$.

strate and product enantiomeric excesses, ee_P and ee_S, on the degree of conversion for two different values of E is shown in Fig. 7. The two curves were calculated according to Eq. (4) for two arbitrarily taken values of E and the same relative excess of the second substrate. As can be seen, in transesterification at a small excess of the acyl donor it is impossible to obtain either the product or the remaining substrate with good optical purity.

Dependence of the Reaction Rate on the Water Content

It is well known that hydrolytic enzymes like lipases need a definite amount of water in order to exhibit catalytic activity. However, in processes performed in organic media, where water is an undesired reagent (like in esterification and transesterification), its concentration should be minimized. The optimum amount of water ought to be estimated experimentally. This has been done for the transesterification of racemic 1-phenyl-1-propanol with vinyl acetate. The results are shown in Fig. 8.

When all the reagents had been dried over molecular sieves (4Å) and the lipase powder had been dried under vacuum, the reaction was slow and reached ca. 50% of the maximum initial reaction rate. The best catalytic activity was obtained at a water concentration of 0.03–0.05% (w/v) and decreased at higher water concentrations. When the amount of water exceeded the solubility in the system alcohol–heptane–vinyl acetate (in a biphasic system), the reaction stopped at a conversion of ca. 20%. Evidently water competes with alcohol in the reaction of the [acyl–enzyme] complex, thus causing an undesired hydrolysis process.

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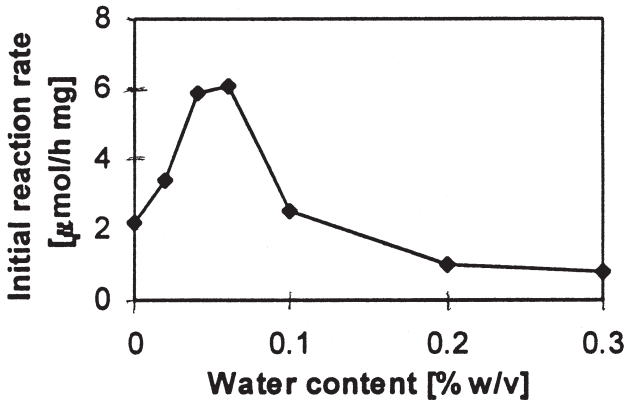

FIG. 8 Dependence of the initial reaction rate in the transesterification of racemic 1-phenyl-1-propanol on the water content in the reaction mixture.

Transesterification of (*R,S*)-1-Phenyl-1-propanol and (*R,S*)-1-Phenylethanol in Enzyme Membrane Reactors

The membrane reactor was used in the transesterification of racemic 1-phenyl-1-propanol **(2)** and 1-phenylethanol **(3)** as described in the Materials and Methods section. (The enzyme loading in the membrane is 0.05 mg/cm^2.) The process was carried out in a crossflow system. The feed solution (the reagents in heptane) was recirculated and partially permeated through the membrane. The results of the degree of conversion and the enantiomeric excess of the product as a function of time of reactor performance are shown in Fig. 9.

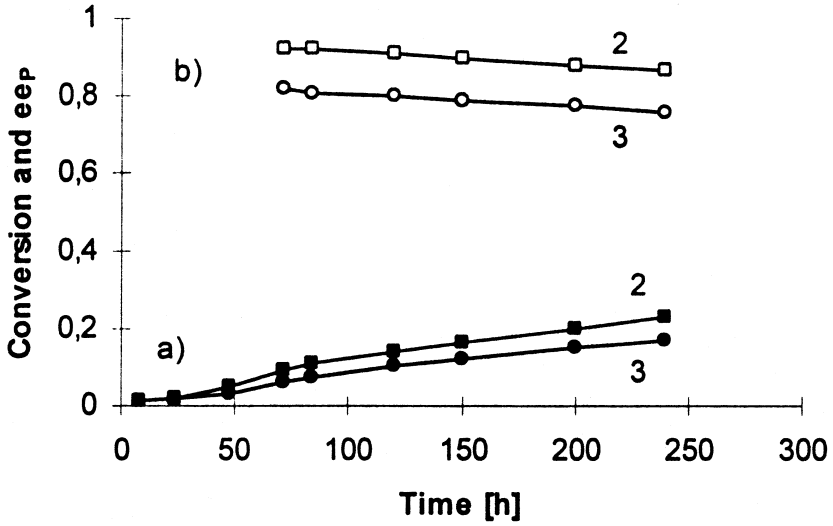

FIG. 9 Degree of conversion (a) and enantiomeric excess of the product, ee_P (b), in the transesterification of racemic 1-phenyl-1-propanol **(2)** and 1-phenylethanol **(3)** in the enzyme membrane reactor. Lipase loading = 0.05 mg/cm^2.

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As could be predicted, transesterification of racemic **3** was slower and less enantioselective than the reaction of **2.** The second process was also performed in a hollow fiber module with lower loading (0.03 mg/cm^2) of the immobilized lipase, in which case the reaction ran with the same enantioselectivity, but slower.

Sequenced Hydrolysis/Transesterification with Native Lipase

As already mentioned, tandem reactions can be used for the preparation of substances with enhanced enantiomeric purity (1–4). This possibility has been proved in the following tandem reactions: hydrolysis of 1-phenylethyl propionate in a biphasic system and transesterification of vinyl acetate with the 1-phenylethanol formed. According to the scheme of both reactions:

$$(R,S)\text{-Phe-CH(OCOCH}_2\text{CH}_3\text{)CH}_3 + \text{H}_2\text{O} \rightarrow (R)\text{-Phe-CH(OH)CH}_3 + (S)\text{-Phe-CH(OCOCH}_2\text{CH}_3\text{)CH}_3 + \text{CH}_3\text{CH}_2\text{COOH} \quad \text{(I)}$$

$$(R)\text{-Phe-CH(OH)CH}_3 + \text{CH}_2{=}{=}\text{CHOCOCH}_3 \rightarrow (R)\text{-Phe-CH(OCOCH}_3\text{)CH}_3 + \text{CH}_3\text{CHO} \quad \text{(II)}$$

the alcohol produced during hydrolysis is enriched in *R*-enantiomer. Such a mixture, taken to the second reaction, ought to be transformed to products with a much higher enantiomeric excess of 1-phenylethyl acetate than in the transesterification of racemic alcohol alone.

Hydrolysis of racemic 1-phenylethyl propionate occurs fast and irreversibly in a biphasic water–organic system while transesterification ought to be performed in a nonaqueous organic phase. That is why the above processes ought to be performed in the following subsequent stages: 1) hydrolysis of 1-phenylethyl propionate in a biphasic system in order to obtain the alcohol with some enantiomeric excess, 2) separation of the phases, and 3) transesterification performed by the addition of vinyl acetate to the organic phase. The degrees of conversion and enantiomeric excesses results, as well as the enantioselectivities, are presented in Table 3.

TABLE 3
Results of 1-Phenylethyl Propionate Hydrolysis Followed by Transesterification of the 1-Phenylethanol Produced with Vinyl Acetate

Reaction	Time of reaction (h)	Degree of conversion	ee_S	ee_P	E
Hydrolysis	24	0.53	0.96	0.85[a]	56
Transesterification	48	0.30	—	0.98	12

[a] Calculated from the equation $\xi = ee_S/(ee_S + ee_P)$ (12).

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As can be seen in the sequenced processes presented, it was possible to obtain the final product with a large enantiomeric excess (*ee* equal to 98%) despite the low enantioselectivity of transesterification ($E = 12$). It was of interest to analyze a run of the second reaction, i.e., transesterification on the basis of dependence of the enantiomeric excess ee_P on the degree of conversion (calculated according to Eq. 4) for the reaction of the racemic substrate and for the non-racemic, enantiomerically enriched substrate. The results are given in Fig. 10.

In a single transesterification with $E = 12$ at 30% conversion, the enantiomeric excess of the product would be $<80\%$. However, using a substrate with a higher initial enantiomeric excess makes it possible to obtain a product of high optical purity, $ee > 98\%$. The results encouraged us to perform such a sequenced process in the enzyme membrane reactors.

Sequenced Ester Hydrolysis/Transesterification in the Enzyme Membrane Reactor

After 100 hours of hydrolysis in the membrane reactor, conversion reached 55%, the enantiomeric excess of the remaining propionate was 99%, and that of the alcohol produced increased ca. 78%. Lipase activity in the membrane reactor was lower than that of native lipase and was equal to 3.7×10^{-5} mol·h^{-1}·mg^{-1} of the enzyme. It was, however, quite stable during the reaction. The transesterification of racemic and nonracemic 1-phenylethanol is presented for the dependence of enantiomeric excess, ee_P, on the degree of conversion (Fig. 11).

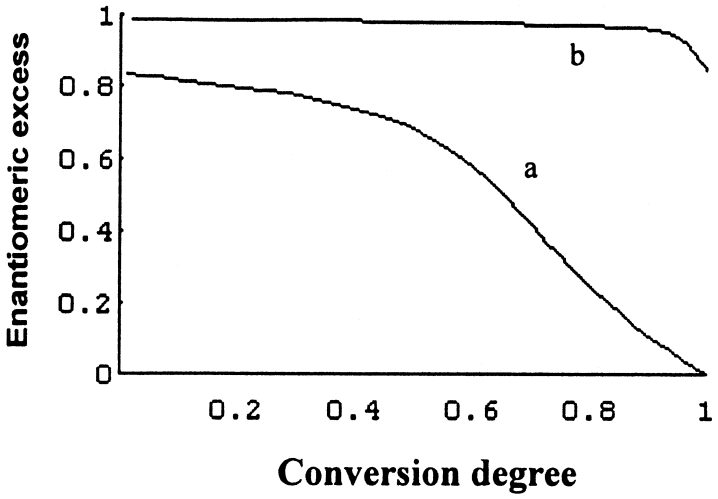

FIG. 10 Enantiomeric excess of the product in the reaction with $E = 12$ of (a) the racemic substrate, $ee_S = 0$, and (b) the nonracemic substrate, $ee_S = 0.85$.

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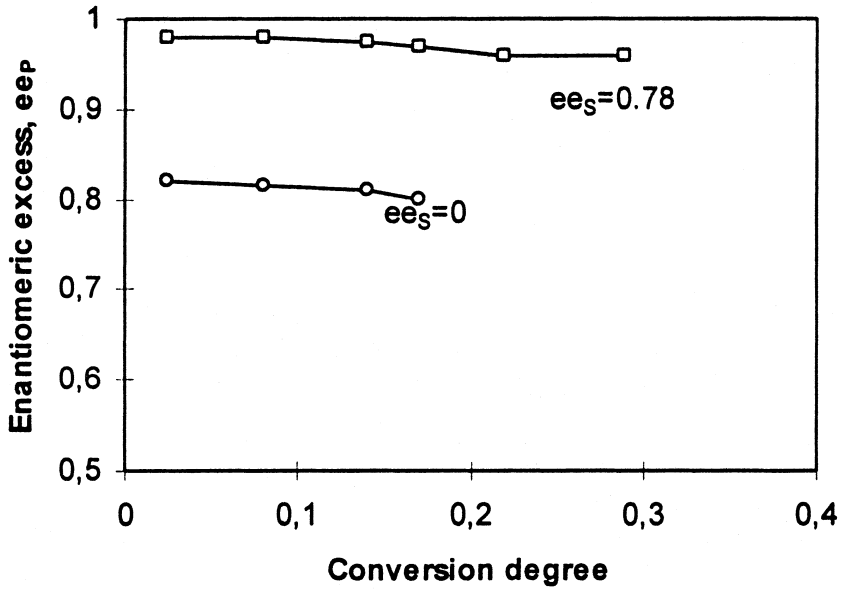

FIG. 11 Enantiomeric excess of the product (1-phenylethyl acetate) in the transesterification of the racemic ($ee_S = 0$) alcohol and the mixture obtained with enantioselective hydrolysis with $ee_S = 0.78$ in the enzyme membrane reactor.

Transesterification of a nonracemic mixture of 1-phenylethanol evidently gives better results than does that of the racemic substrate. The enantiomeric excess of the ester formed during the process is >97%, while in the reaction of racemic alcohol it does not exceed 82%.

CONCLUSIONS

The best biocatalyst for both transesterification of 1-phenylalcohols and hydrolysis of their esters was lipase from *Pseudomonas* sp.

This lipase, native as well as immobilized in a polymer membrane, exhibits good catalytic activity and high enantioselectivity for *R*-enantiomers, both in hydrolysis and transesterification. Enantiomerically enriched products of the hydrolysis of chiral esters, created in a biphasic system, can be used directly to transesterification which will result in obtaining products of very high enantiomeric purity.

Both processes can be easily performed in enzyme membrane reactors.

ACKNOWLEDGMENTS

The authors thank Berghof (Germany) for donating samples of polyamide membranes.

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Received by editor June 16, 1998
Revision received December 1998

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