ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 15 (2001) 71–80

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

Resolution of racemic *trans*-2-methyl-1-cyclohexanol by lipase-catalysed transesterification in a membrane reactor

Józef Ceynowa*, Marta Rauchfleisz

Faculty of Chemistry, Nicholas Copernicus University, 7 Gagarin St., 87-100 Toruñ, Poland

Received 25 July 2000; accepted 31 January 2001

Abstract

A kinetic resolution of *trans*-2-methyl-1-cyclohexanol with transesterification was studied using a membrane reactor. The highest enantioselectivity was observed for the reaction with vinyl acetate in an *n*-hexane solution, which was catalysed by lipase from *Pseudomonas* sp. It was also found that the use of both native lipase and that immobilised in a polyamide hollow-fibre membrane leads to conversion of the (1*R*;2*R*)-enantiomer. However, the catalytic activity of the immobilised lipase was equal to 27% of that of the native one. Operating conditions for the membrane reactor, such as the kind of solvent, amount of water, temperature, and concentrations of substrates, were examined and optimised. Under the optimum conditions, the kinetics of the process are characterised by the constants of the Michaelis–Menten equation, K_M and V_{max}, being as high as 1.702×10^{-2} M and 3.903×10^{-4} mol/h mg, respectively. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Enzyme membrane reactor; Enantioselective transesterification; Kinetic resolution; Immobilised lipase; *trans*-2-Methyl-1-cyclohexanol

1. Introduction

Enantiomers of a compound can display various biological activities, ranging from distinguishable smell and flavour to an opposite influence on living organisms. Thus, an increasing trend towards production of the materials such as pharmaceuticals, agricultural chemicals, flavours and fragrances from optically pure components can be observed [1–3].

One of the most useful and practical ways to prepare compounds of high optical purity is the enzyme catalysis [4,5]. Enzymes exhibit a high catalytic action in aqueous solutions as well as in many organic solvents under mild conditions [6] and can be applied using rather simple technical equipment. They can easily be immobilised on supports, which enhances their stability and simplifies both structure and performance of a reactor. The immobilised enzymes can also easily be recovered from the reaction medium and reused many times in order to attain low operational costs [7–9]. Moreover, processes with enzymes immobilised in membranes can conveniently be performed in a continuous, steady state mode of operation [10–14]. Hydrolytic enzymes such as lipase are often applied to kinetic resolution of racemic acids, alcohols and esters by hydrolysis, esterification and transesterification in single-phase (organic) or two-phase (aqueous-organic) media [15–17].

The aim of this work was to separate (±)-*trans*-2-methyl-1-cyclohexanol by means of the kinetic resolution in the lipase-catalysed transesterification being performed in an enzyme membrane reactor. Vinyl

* Corresponding author. Tel.: +48-56-6114-517; fax: +48-56-6542-477.
E-mail address: ceynowa@chem.uni.torun.pl (J. Ceynowa).

1381-1177/01/$ – see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S1381-1177(01)00010-8

acetate was applied as an acyl donor because unstable vinyl alcohol released in the reaction immediately tautomerises to acetaldehyde, which shifts the reaction equilibrium to the product side [18]. The lipase molecules were chemically immobilised directly in the polyamide membrane. Parameters of the immobilisation were estimated using the simplex method for experimental optimisation. Influence of the factors such as the type of solvent, amount of added aqueous phase, temperature, concentrations of substrates and the alcohol/ester molar ratio on the rate of transesterification and on enantioselectivity of the kinetic resolution of (±)-*trans*-2-methyl-1-cyclohexanol was examined as well.

2. Experimental

2.1. Materials

Lipases from *Pseudomonas* sp., Type XIII (EC 3.1.1.3), *Candida cylindracea*, Type VII and *Porcine pancreas*, Type VI-S with specific activities of 1500, 2160 and 205 U/mg solid, respectively, were purchased from Sigma (USA).

Racemic *trans*-2-methyl-1-cyclohexanol (99%) was purchased from Aldrich-Chemie (Germany). Vinyl acetate (>99%), isopropenyl acetate (99%) and 1,4-diaminobutane (pure) were obtained from Fluka Chemie AG (Switzerland). *n*-Hexane (HPLC grade) was purchased from Mallinckrodt Baker B.V. (The Netherlands) and *tert*-butyl methyl ether (HPLC grade) from Labscan Ltd. (Ireland). The solvents: *n*-heptane (Mallinckrodt Baker B.V.), isopropyl ether (Fluka), 2-metoxyethyl ether (Janssen Chimica), diethyl ether, benzene, toluene, chloroform and tetrahydrofuran (all from PPH POCh, Poland) were of analytical grade. Glutaric dialdehyde (25% aqueous solution) was purchased from Sigma. All the reagents were used without additional purification.

2.2. Membrane reactor

An enzyme membrane module was the main part of the membrane reactor. It consisted of five hollow-fibre membranes (200 mm long) placed in a glass tube. The enzyme membranes were prepared by means of immobilisation of lipase with a nominal molecular weight cut-off of 50 kDa (a kind gift from Berghof, Germany) within the aromatic polyamide hollow-fibre membranes (i.d. = 0.6 mm, o.d. = 1.2 mm). The reactor was connected to a peristaltic pump that recirculated the liquid mixture of the reactants.

2.3. Instruments

Progress of the reaction and enantiomeric purity of the reactants were controlled by means of an HPLC chromatograph equipped with a Chiralcel OD-H chiral column (i.d. = 0.46 cm, i.l. = 25 cm) and a Chiralcel OD precolumn (i.d. = 0.46 cm, i.l. = 5 cm), both from Daicel Chemical Industries, Ltd. A Shodex SE-61 refractive index detector, a Spectra-Physics SP 8810 isocratic pump and a PC computer with the PL LogiCal program completed the instrument, 80 vol.% of *n*-hexane and 20 vol.% of *tert*-butyl methyl ether was the optimal composition of the mobile phase. The flow rate of the mobile phase was set at a level of 1.0 ml/min under the pressure of 50–60 bar. The measurements of optical rotation were carried out on a Polamat A polarimeter from Carl Zeiss, Jena (Germany). The NMR spectra were obtained with a Varian Gemini 2000 instrument operating at 200 MHz, using the $CDCl_3$ solution of TMS as an internal standard. The detection of enantiomers was performed using tris[3-(heptafluoropropylhydroxymethylene)-d-camphorato]europium(III) (Fluka Chemie AG, Switzerland) as an NMR chemical shift reagent.

2.4. Methods

2.4.1. Immobilisation

The immobilisation of the lipase molecules within the hollow-fibre membranes was accomplished through chemical bonding that occurred in four steps: (i) acidolysis of a part of amide groups with HCl (1.8 M, 3 h); (ii) modification with 1,4-butadiene (0.1 M, 3 h); (iii) activation with glutaraldehyde (5% solution in the 0.1 M phosphate buffer, pH = 7.2, 3 h, 275 K); and (iv) bonding of lipase from its solution (3 mg/100 ml) in the same phosphate buffer at 310 K [19]. Each of these steps was attained by means of forced permeation of the specified solution through the membrane and recirculation of the solution. Values of the above mentioned parameters as well as temperature of the process have been established with

the simplex planning procedure presented in Section 3.1. The load density of the immobilised lipase was determined according to the Sigma diagnostics procedure based on the Tietz–Fiereck method [20].

2.4.2. Transesterification

The reaction mixture was prepared by mixing 2 mmol of racemic *trans*-2-methyl-1-cyclohexanol dissolved in 48 g of *n*-hexane with 20 mmol of vinyl acetate in a 50 ml flask. Transesterification was performed in a one-phase reactor by circulating the reaction mixture at both sides of the membrane while applying a forced, partial permeation through the membrane. The reactor was placed in a thermostatic box at 310 K. Samples (25 μl) were periodically taken from the reactor and analysed with HPLC.

Enantiomeric ratio (E), representing enantioselectivity of the process, is defined by the relation:

$$E = \frac{(k_{\text{cat}}/K_{\text{M}})_R}{(k_{\text{cat}}/K_{\text{M}})_S} \tag{1}$$

where k_{cat} denotes the catalytic rate constant, K_{M} is the Michaelis constant, and the subscripts R and S correspond to the relevant isomers. As suggested by Chen et al. [21,22], the enantiomeric ratio for *trans*-2-methyl-1-cyclohexanol was calculated from the extent of conversion (c) and the enantiomeric excess of the alcohol (e.e.):

$$E = \frac{\ln[(1-c)(1-\text{e.e.})]}{\ln[(1-c)(1+\text{e.e.})]} \tag{2}$$

where

$$\text{e.e.} = \frac{|C_{1S;2S} - C_{1R;2R}|}{C_{1R;2R} + C_{1R;2R}} \tag{3}$$

and $C_{1S;2S}$ and $C_{1R;2R}$ denote the concentrations of the 1*S*;2*S* and 1*R*;2*R* isomers, respectively. The absolute configurations of (1*S*;2*S*)-(+)-*trans*-2-methyl-1-cyclohexanol, (1*R*;2*R*)-(−)-*trans*-2-methyl-1-cyclohexanol, (1*S*;2*S*)-(+)-*trans*-2-methyl-1-cyclohexylacetate and (1*R*;2*R*)-(−)-*trans*-2-methyl-1-cyclohexylacetate were established by comparing the measured values of optical rotation with the literature data [23]. The spectroscopic data for the substrate and the product were as follows. For *trans*-2-methyl-1-cyclohexanol, ^{1}H NMR (in $CDCl_3$): δ 1.00 (d, 3H, –C*H*$_3$), δ 1.21–1.32 (m, 4H, –C*H*$_2$–), δ 1.50–1.78 (m, 4H, –C*H*$_2$–), δ 3.11 (d-t, 1H, –C*H*–COH); for *trans*-2-methyl-1-cyclohexylacetate, ^{1}H NMR (in $CDCl_3$): δ 0.86 (d, 3H, trans –C*H*$_3$), δ 1.01–1.76 (m, 9H, (C*H*$_2$)$_4$–C*H*–CH$_3$), δ 2.03 (s, 3H, –O–C*H*$_3$), δ 4.39 (d-t, 1H, –C*H*OAc).

3. Results and discussion

3.1. Establishing of conditions of immobilisation

The simplex designing method of multiparameter experiments [24] has been applied to optimise the immobilisation procedure. The method enables to estimate the optimum parameters at the minimum number of experiments: the parameters of the next experiment are calculated from the results of the preceding experiment.

The parameters of the simplexes were calculated according to the following matrix [25]:

$$A = \begin{vmatrix} k_1 & k_2 & k_3 & \cdots & k_{n-1} & k_n \\ -R_1 & k_2 & k_3 & \cdots & k_{n-1} & k_n \\ 0 & -R_2 & k_3 & \cdots & k_{n-1} & k_n \\ 0 & 0 & -R_3 & \cdots & k_{n-1} & k_n \\ 0 & 0 & 0 & \cdots & -R_{n-1} & k_n \\ 0 & 0 & 0 & \cdots & 0 & -R_n \end{vmatrix},$$

$$k_i = \left[\frac{1}{2i(i+1)}\right]^{1/2}, \quad R_i = \left[\frac{i}{2(i+1)}\right]^{1/2},$$

$$x_{n+2,i} = \frac{2}{n}\sum_{m-1}^{n+1} x_{mi} - x_{ji}, x_{mi} = x_{0i} + z_i k_i,$$

$$x_{mi} = x_{0i} - z_i r_i \tag{4}$$

where A represents the simplex matrix in the unspecified co-ordinate system, n the number of parameters, $i = 1, 2, 3, \ldots, n$, $m = 1, 2, 3, \ldots, n+1$, R_i and k_i the dimensionless values of the '*i*th' parameter, x_{0i} the value of the '*i*th' parameter in a co-ordinate system relating to the experiments forming the starting simplex, x_{mi} the value of the '*i*th' parameter in the '*m*th' experiment, and z_i the scaling factor of the '*i*th' parameter.

The analysis of the results of a particular experiment was based on the load density of the catalytically active lipase bonded within the membrane. The load density was assumed as a criterion for quality of the procedure.

Table 1
Parameters and results of initial experiments

Parameter	Experiment I	Experiment II
Concentration of HCl (M)	1.8	3.6
Time of acidolysis with HCl (h)	3	6
Concentration of 1,4-diaminobutadiene (M)	0.01	0.02
Time of modification by 1,4-diaminobutadiene (h)	3	6
Concentration of glutaraldehyde (%)	5	10
Time of activation by glutaraldehyde (h)	3	6
Temperature of activation (K)	298	298
Concentration of lipase from *Pseudomonas* sp. (mg/100 ml)	2	2
Temperature of immobilisation (K)	278	278
Load density of lipase in the membrane (10^{-2} mg/cm^2)	2.87	2.93

The immobilisation parameters that had to be considered were: concentration of HCl, time of acidolysis due to HCl, concentration of 1,4-diaminobutadiene, time of modification with 1,4-diaminobutadiene, concentration of glutaraldehyde, time of activation by glutaraldehyde, temperature of activation, concentration of lipase, and temperature of the process. Two experiments (see Table 1) were performed in order to find the parameters that might not influence the immobilisation. As seen, the values of the quality criterion for both experiments are close to each other. This allows one to eliminate from the optimisation procedure the concentration of HCl, the time of the acidolysis, the concentration of 1,4-diaminobutadiene, the time of the modification, the concentration of glutaraldehyde, and the time of the activation. Thus, the temperature of activation by glutaraldehyde, the concentration of lipase and the temperature of the immobilisation were finally used as the parameters subjected to the optimisation. The values of these parameters, which were taken for calculating the starting simplex, are presented in Table 2.

The values of the parameters for the successive experiments and of the quality criterion are presented in Table 3. The parameters were calculated while analysing the quality criteria of the experiments belonging to the consecutive simplexes, the experiment with the lowest criterion being eliminated. As Table 3 shows, the load density of the catalytically active enzyme increases with the decreasing temperature of the immobilisation of lipase. Thus, the optimum temperature of the immobilisation was found to be between 274 and 275 K (at 274 K, the whole reaction mixture freezes), the optimum temperature of activation by glutaraldehyde is 310 K, and the optimum concentration of the lipase solution −3 mg/100 ml. Consequently, the process of the immobilisation of lipase, described in Section 2.4.1, was carried out using these values.

It is noteworthy that the catalytic activity of the lipase immobilised in a polyamide hollow-fibre equals to 27% of the activity of the native lipase. This indicates that the immobilisation of the lipase originating from *Pseudomonas* sp. does not affect the catalytic activity of this enzyme and that essentially no changes in the conformation of the active site occur during the immobilisation.

Table 2
Parameters for the experiments forming the starting simplex

Parameter	Value	Accuracy	Axis unit
Temperature of immobilisation (K)	278	1	2
Concentration of lipase (mg/100 ml)	2	0.05	1
Temperature of activation (K)	298	1	10

3.2. Selection of lipase, ester and solvent

The catalytic activities and the enantioselectivities of lipases from *Pseudomonas* sp., *Candida cylindracea* and *Porcine pancreas* have been tested in the reaction of transesterification of *trans*-2-metyl-1-cyclohexanol with vinyl acetate. It was found that only lipases from *Pseudomonas* sp. and *Candida cylindracea* revealed a stereo-preference to (1*R*;2*R*)-(−)-*trans*-2-methyl-1-cyclohexanol (Fig. 1). The results of the enantiomeric excesses of the

Table 3
Parameters and results of the optimisation process

Experiment	Parameter			Criterion of quality (Y) (10^{-2} mg/cm^2)
	Temperature of immobilisation (x_1) (K)	Concentration of lipase (x_2) (mg/100 ml)	Temperature of activation (x_3) (K)	
X_1	278	2.0	298	2.80
X_2	279	2.3	300	2.63
X_3	277	2.3	300	2.40
X_4	278	1.5	300	2.16
X_5	278	2.0	292	1.80
X_6	278	2.1	308	3.19
X_7	278	3.0	305	3.17
X_8	280	2.6	309	2.77
X_9	278	2.9	315	3.00
X_{10}	276	2.7	310	3.83
X_{11}	276	2.3	301	3.88
X_{12}	275	3.3	303	3.38
X_{13}	274	2.6	304	3.65
X_{14}	274	3.5	310	4.05

alcohol, obtained at the 45% conversion, are presented in Table 4. As seen, the lipase from *Pseudomonas* sp. exhibits the highest enantioselectivity. Therefore, this lipase has been chosen as the most effective catalyst for further experiments.

A series of three experiments was carried out to establish the best acyl donor for the studied transesterification. Ethyl acetate, isopropenyl acetate and vinyl acetate were used in these experiments and the results are presented in Fig. 2. According to these data, vinyl acetate was accepted as the best reagent for the studied process.

Table 4
Enantioselectivities of different lipases in transesterification of *trans*-2-methyl-1-cyclohexanol with vinyl acetate

Source of lipase	e.e. (%)
Pseudomonas sp.	81
Candida cylindracea	59
Porcine pancreas	0

A solvent for the reactants can influence the studied process as it determines not only the restriction due to diffusion but also the catalytic activity of lipase. Therefore, the effect of a number of solvents of

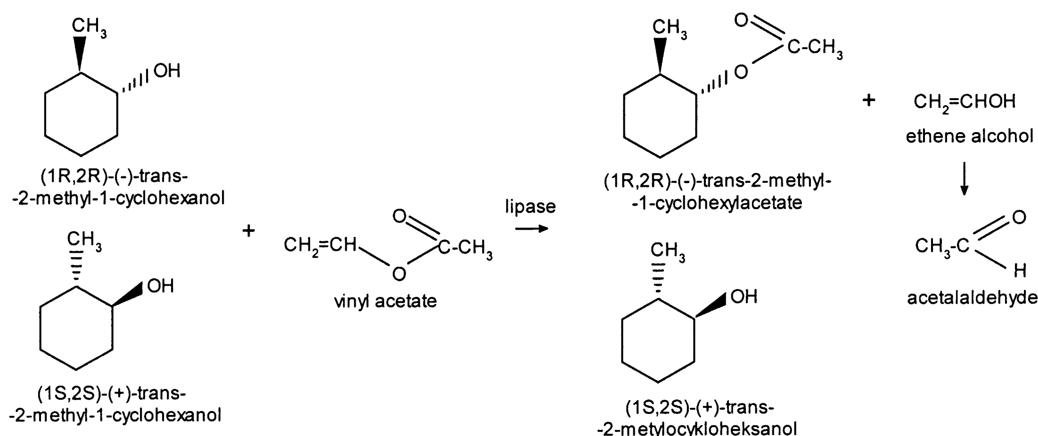

Fig. 1. Stereoselective transesterification of *trans*-2-methyl-1-cyclohexanol with vinyl acetate, catalysed by lipase from *Pseudomonas* sp.

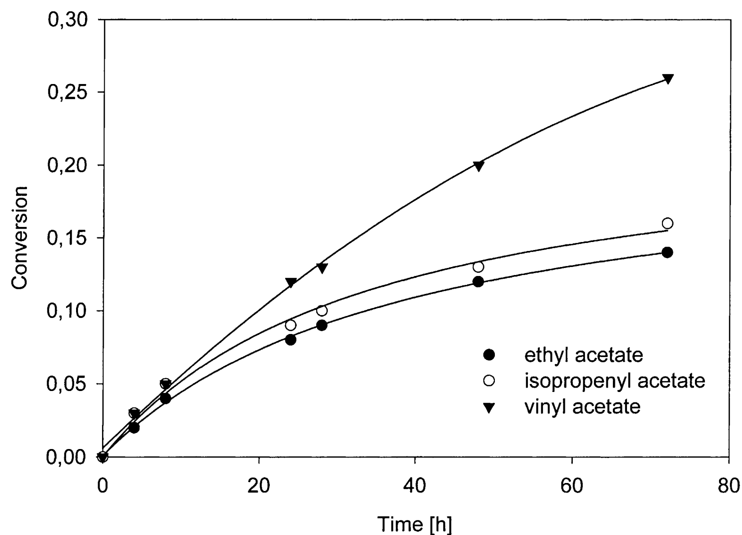

Fig. 2. Extent of conversion vs. reaction time in transesterification of *trans*-2-methyl-1-cyclohexanol with various esters, catalysed by lipase from *Pseudomonas* sp.

different hydrophobicities on the course of the process has been investigated. The result is represented by the logarithm of the partition coefficient ($\log P$) for the solvent in the octanol–water system. The highest catalytic activity of the lipase from *Pseudomonas* sp. as characterised by the enantiomeric excess at the 45% conversion is observed for the most hydrophobic solvents ($\log P > 3$), *n*-hexane and *n*-heptane (Table 5). Hydrophilic solvents ($\log P < 2$) reduce the catalytic activity of lipase because of their ability to penetrate the protein molecules and to strip the water molecules necessary to maintain the catalytic activity of the lipase. Thus, *n*-hexane was accepted as the most adequate solvent. This compound was also selected as a component of the mobile phase in the HPLC analysis.

Table 5
Enantioselectivities (e.e.) of lipase from *Pseudomonas* sp. in transesterification of *trans*-2-methyl-1-cyclohexanol with vinyl acetate in organic solvents of different hydrophobicities ($\log P$)

Solvent	Log *P*	e.e. (%)
Acetone	−0.2	5
2-Metoxyethyl ether	0.3	34
Tetrahydrofuran	0.5	10
Diethyl ether	0.8	18
Diisopropyl ether	1.1	27
Benzene	2.0	48
Chloroform	2.0	53
Toluene	2.5	59
n-Hexane	3.5	79
n-Heptane	4.0	79

3.3. Effect of water content, temperature and molar ratio of reagents

It is important to control the water content in the system subjected to the reaction of transesterification with lipase in organic media because a definite amount of water is necessary to maintain the lipase in a catalytically active conformation [26]. The influence of water on the reaction is presented in Fig. 3. The figure shows that the reaction carried out without the presence of water is very slow and the enantiomeric excess is as low as 45%. Addition of a small amount of aqueous solution of phosphate buffer (pH = 7.2) leads to an essential increase in the reaction yield. At the optimum water concentration of ca. 1% (v/v), the e.e. value reaches 83%. The further decrease in e.e. with the rising concentration of water is caused probably by an unwanted process of hydrolysis [27].

The influence of temperature on transesterification of *trans*-2-methyl-1-cyclohexanol with vinyl acetate was examined in the range of 293–318 K (Fig. 4). Nearly opposite influence of temperature on the

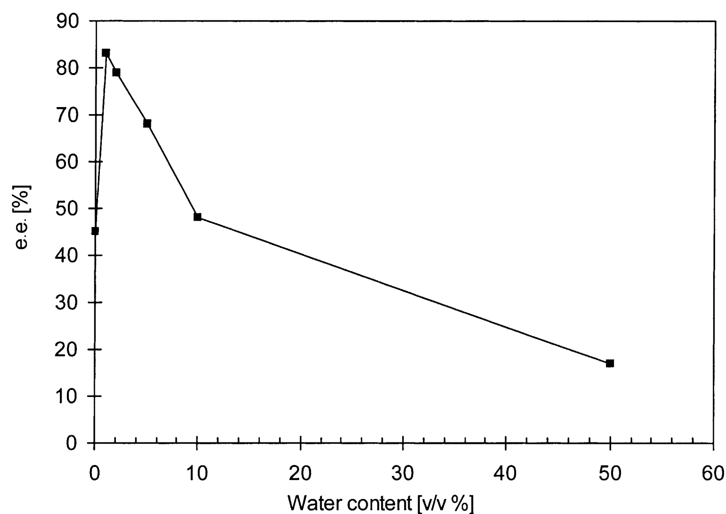

Fig. 3. Effect of water added to the reaction medium on enantioselectivity in transesterification of *trans*-2-methyl-1-cyclohexanol with vinyl acetate, catalysed by lipase from *Pseudomonas* sp.

reaction rate as compared to the effect on enantioselectivity is observed. The reaction rate increases with the temperature rising up to only ca. 310 K while the enantioselectivity of lipase decreases steadily, which agrees with the theoretical considerations [28].

In order to find an optimum concentration of vinyl acetate, transesterifications of *trans*-2-methyl-1-cyclohexanol were carried out at different concentrations of vinyl acetate and at a constant concentration of *trans*-2-methyl-1-cyclohexanol. Enantioselectivities

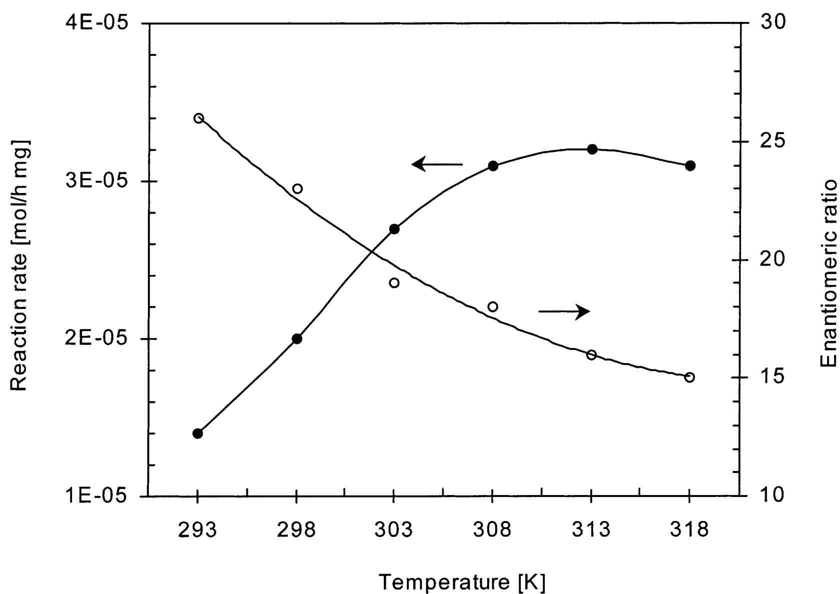

Fig. 4. Influence of temperature on the rate of transesterification of *trans*-2-methyl-1-cyclohexanol with vinyl acetate catalysed by lipase from *Pseudomonas* sp.

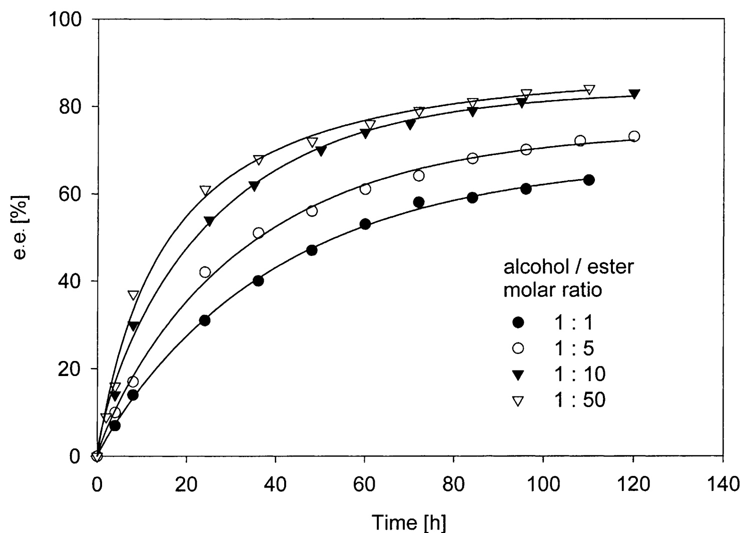

Fig. 5. Influence of the molar ratio of alcohol to ester on enantioselectivity in transesterification of *trans*-2-methyl-1-cyclohexanol with vinyl acetate, catalysed by lipase from *Pseudomonas* sp.

of these processes expressed as enantiomeric excesses of the alcohol are presented in Fig. 5. The course of the curves indicates that the highest values of the enantiomeric excesses were obtained for a significant (at least 10-fold) molar excess of vinyl acetate. Thus, the next (kinetic) experiments were performed using that excess.

3.4. Kinetic parameters of enzymatic transesterification

In order to determine the kinetic parameters of the studied reaction, the processes were carried out with different initial concentrations of both *trans*-2-methyl-1-cyclohexanol and vinyl acetate, using the constant molar ratio of the substrates equal to 1:10. The results (Fig. 6) show that the initial concentrations of both substrates influence the reaction rate but the final values of the kinetic resolution are close to one another: e.e. ≅ 80%.

The obtained results were used to estimate the kinetic parameters of the studied process while applying the Michaelis–Menten equation:

$$V = -\frac{d[S]}{dt} = \frac{V_{max}[S]}{K_M + [S]} \quad (5)$$

where V is the reaction rate, [S] the substrate concentration, t time, V_{max} the maximum reaction rate, and K_M the Michaelis constant [29]. Here, the Michaelis constant and the maximum reaction rate were estimated with a classical method using both linear forms of the Michaelis–Menten equation and the original Eq. (5).

In the first approach, the linear transformations of the Michaelis–Menten equation were used [30]. They were obtained according to:

1. Lineveaver–Burk:

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}[S]} \quad (6)$$

2. Hofstee–Eadie:

$$V = V_{max} - \frac{K_M}{[S]} \quad (7)$$

3. Hanes:

$$\frac{[S]}{V} = \frac{K_M}{V_{max}} + \left(\frac{1}{V_{max}}\right)[S] \quad (8)$$

The results of the calculation are listed in Table 6. As seen, the values of K_M vary from 0.0162 to 0.0169 M and those of V_{max} from 3.82×10^{-4} to 3.89×10^{-4} mol/h mg.

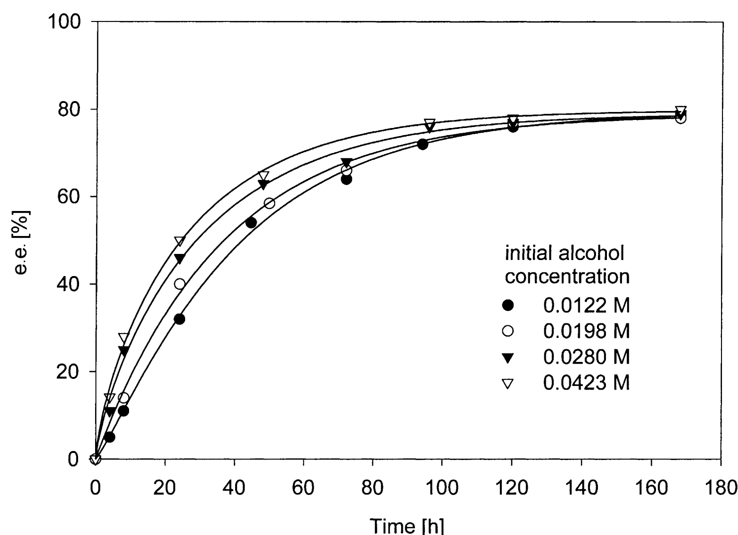

Fig. 6. Influence of the initial concentration of *trans*-2-methyl-1-cyclohexanol on enantioselectivity in transesterification with vinyl acetate catalysed by lipase from *Pseudomonas* sp., the molar ratio of alcohol to ester was equal to 10.

Table 6
Maximum reaction rates (V_{max}) and the Michaelis constants (K_M), derived from linear transformations of the Michaelis–Menten equation and from the non-linear regression, for transesterification of *trans*-2-methyl-1-cyclohexanol with vinyl acetate, catalysed by lipase from *Pseudomonas* sp.

Method	*y*-intercept	Slope	V_{max} (10^{-4} mol/h mg)	K_M (10^{-2} M)
Lineveaver–Burk	$1/V_{max}$	K_M/V_{max}	3.82	1.62
Hofstee–Eadie	V_{max}	$-K_M$	3.85	1.65
Hanes	K_M/V_{max}	$1/V_{max}$	3.89	1.69
Non-linear regression			3.90	1.70

K_M and V_{max} were estimated also directly, i.e. simply by fitting the hyperbolic Michaelis–Menten Eq. (5) to the experimental data with use of the SigmaPlot® 5.0 Regression Wizard program. The obtained relation is of the form:

$$V = \left(\frac{3.903 \times 10^{-4}[\mathrm{S}]}{1.702 \times 10^{-2} + [\mathrm{S}]} \right) \quad (9)$$

which gives $K_M = 1.702 \times 10^{-2} \pm 7.8 \times 10^{-4}$ M and $V_{max} = 3.903 \times 10^{-4} \pm 7.2 \times 10^{-6}$ mol/h mg. These values are inserted in Table 6, too.

Comparison of the latter values with those obtained from the linear transformations indicates that, from among Eqs. (6)–(8), Eq. (8) gives the best results: the K_M and V_{max} values calculated with the Hanes equation agree, within the experimental error, with those derived directly from the experimental data.

4. Conclusions

Lipase from *Pseudomonas* sp. immobilised within the polyamide hollow-fibre membrane can be used to resolve a racemic mixture of (±)-*trans*-2-methyl-1-cyclohexanol by stereoselective transesterification. This lipase exhibits high enantioselectivity towards (1*R*;2*R*)-(−)-*trans*-2-methyl-1-cyclohexanol. The immobilisation procedure of the lipase in the polyamide membrane does not affect dramatically activity nor enantioselectivity of the enzyme. Both the rate of transesterification and optical purity of a

product depend on factors such as the type of solvent, the amount of added aqueous phase, temperature, and the molar ratio of alcohol to ester. The optical purity of the products is also related to the extent of conversion and not to the initial concentrations of substrates. The concentrations of substrates influence only the reaction rate.

To exploit the advantages of the studied process, it is necessary to elaborate an efficient method for separation of the produced chiral esters from unreacted alcohol. The membrane reactor, built for the studied process, exhibits a satisfactory performance during 120–170 h operation and, after careful washing, can be used within 1 month.

References

[1] E. Schoffers, A. Golebinowski, C.R. Johnson, Tetrahedron 11 (1996) 3769–3826.
[2] A.L. Paiva, F.X. Malcata, J. Mol. Catal. B: Enzym. 3 (1997) 99–109.
[3] E. Sataniello, P. Ferraboschhi, P. Grisenti, A. Manzocchi, Chem. Rev. 92 (1992) 1071–1087.
[4] A. Rahman, Stereoselective Synthesis in Organic Chemistry, Springer, Berlin, 1993.
[5] V.S. Parmar, K.S. Bisht, A. Singh, A. Iha, Proc. Indian Acad. Sci. 18 (1996) 575–583.
[6] A.M. Klibanov, Acct. Chem. Res. 23 (1990) 114–119.
[7] J. Ceynowa, I. Sionkowska, Acta Biotechnol. 13 (1993) 177–183.
[8] L. Giorno, E. Drioli, J. Chem. Technol. Biotechnol. 69 (1997) 11–14.
[9] J. Ceynowa, Chem. Anal. 43 (1998) 929–932.
[10] J.L. Lopez, S.L. Matson, J. Membr. Sci. 125 (1997) 189–211.
[11] F.X. Malcata, C.G. Hill, C.H. Amundson, Biotechnol. Bioeng. 39 (1992) 984–1001.
[12] D.R. Wu, S.M. Cramer, G. Belfort, Biotechnol. Bioeng. 41 (1993) 979–990.
[13] W. Pronk, P.J.A.M. Kerkhof, C. van Helden, K. van't Riet, Biotechnol. Bioeng. 32 (1988) 512–518.
[14] J. Ceynowa, P. Adamczak, M. Staniszewski, Acta Biotechnol. 17 (1997) 161–176.
[15] R. Azerad, Bull. Soc. Chim. Fr. 132 (1995) 17–51.
[16] S.M. Roberts, J. Chem. Soc., Perkin Trans. 1 (1999) 1–21.
[17] B.A. Persson, A.L.E. Larson, M.L. Ray, J.E. Backvall, J. Am. Chem. Soc. 121 (1999) 1645–1650.
[18] H. Stecher, K. Faber, Synthesis (1997) 1–16.
[19] J. Ceynowa, P. Adamczak, J. Appl. Polym. Sci. 46 (1992) 749–755.
[20] N.W. Tietz, E.A. Fiereck, Clin. Chim. Acta 13 (1966) 352.
[21] C.S. Chen, C.J. Shin, Angew. Chem. Int. Ed. Engl. 28 (1989) 695–707.
[22] C.S. Chen, Y. Fujimoto, G. Girdaukas, C.J. Shin, J. Am. Chem. Soc. 104 (1982) 7294–7299.
[23] W. Klyne, J. Buckingham, Atlas of Stereochemistry, Absolute Configurations of Organic Molecules, Chapman & Hall, London, 1974, pp. 37–39.
[24] W. Spandley, G.R. Hext, F.R. Hinsworth, Technometrics 4 (1962) 441–443.
[25] W.G. Gorskij, W.Z. Brodskij, Zawod. Lab. 31 (1965) 831.
[26] M.G. Kim, S.B. Lee, J. Ferment. Bioeng. 81 (1996) 269–271.
[27] S. Parida, J.S. Dordick, J. Am. Chem. Soc. 113 (1991) 2253–2259.
[28] E. Holmberg, K. Hult, Biotechnol. Lett. 13 (1991) 323–326.
[29] C.H. Wong, G.M. Whitesides, Enzymes in Synthetic Organic Chemistry, Pergamon Press, Oxford, 1995, pp. 4–8.
[30] I.H. Segel, Enzyme Kinetics, Wiley, New York, 1975, pp. 59–62.