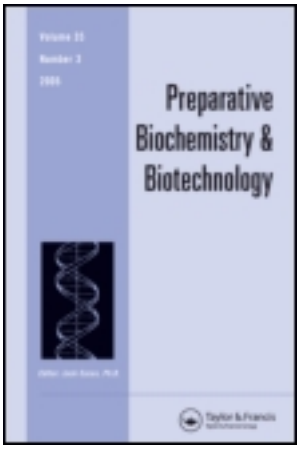

Preparative Biochemistry and Biotechnology

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lpbb20

OPTIMIZATION OF LIPASE-CATALYZED ENANTIOSELECTIVE PRODUCTION OF 1-PHENYL 1-PROPANOL USING RESPONSE SURFACE METHODOLOGY

Aslı Soyer [a], Emine Bayraktar [a] & Ülkü Mehmetoglu [a]

[a] Faculty of Engineering, Department of Chemical Engineering, Ankara University, Ankara, Turkey

Version of record first published: 24 Nov 2010.

To cite this article: Aslı Soyer, Emine Bayraktar & Ülkü Mehmetoglu (2010): OPTIMIZATION OF LIPASE-CATALYZED ENANTIOSELECTIVE PRODUCTION OF 1-PHENYL 1-PROPANOL USING RESPONSE SURFACE METHODOLOGY, Preparative Biochemistry and Biotechnology, 40:4, 389-404

To link to this article: http://dx.doi.org/10.1080/10826068.2010.525433

Preparative Biochemistry & Biotechnology, 40:389–404, 2010

ISSN: 1082-6068 print/1532-2297 online
DOI: 10.1080/10826068.2010.525433

Taylor & Francis
Taylor & Francis Group

OPTIMIZATION OF LIPASE-CATALYZED ENANTIOSELECTIVE PRODUCTION OF 1-PHENYL 1-PROPANOL USING RESPONSE SURFACE METHODOLOGY

Aslı Soyer, Emine Bayraktar, and Ülkü Mehmetoglu

Faculty of Engineering, Department of Chemical Engineering, Ankara University, Ankara, Turkey

□ *Optically active 1-phenyl 1-propanol is used as a chiral building block and synthetic intermediate in the pharmaceutical industries. In this study, the enantioselective production of 1-phenyl 1-propanol was investigated systematically using response surface methodology (RSM). Before RSM was applied, the effects of the enzyme source, the type of acyl donor, and the type of solvent on the kinetic resolution of 1-phenyl 1-propanol were studied. The best results were obtained with* Candida antartica *lipase (commercially available as Novozym 435), vinyl laurate as the acyl donor, and isooctane as the solvent. In the RSM, substrate concentration, molar ratio of acyl donor to the substrate, amount of enzyme, temperature, and stirring rate were chosen as independent variables. The predicted optimum conditions for a higher enantiomeric excess (ee) were as follows: substrate concentration, 233 m*M*; molar ratio of acyl donor to substrate, 1.5; enzyme amount, 116 mg; temperature, 47° C; and stirring rate, 161 rpm. A verification experiment conducted at these optimized conditions for maximum ee yielded 91% for 3 hr, which is higher than the predicted value of 83%. The effect of microwave on the ee was also investigated and ee reached 87% at only 5 min.*

Keywords 1-phenyl 1-propanol, enantiomeric ratio, enantioselectivity, kinetic resolution, lipase, response surface methodology

INTRODUCTION

The different forms of enantiomeric products can cause quite different biological effects. Chiral drugs, agrochemicals, food additives, and fragrances are classes of compounds with high economic and scientific potential. Therefore, during the last decade there has been increased interest in using enzymes for asymmetric synthesis and kinetic resolution to obtain pure enantiomers. Lipases are frequently used both in kinetic resolutions of racemates and in asymmetrizations of prochiral and meso

Address correspondence to Emine Bayraktar, Faculty of Engineering, Department of Chemical Engineering, Ankara University, 06100 Tandogan, Ankara, Turkey. E-mail: bayrakta@eng.ankara.edu.tr

compounds.[1] Lipase activity and selectivity are strongly influenced by the medium used for the desired reaction.[2] Lipases successfully resolve chiral secondary alcohols.

Chiral secondary alcohols are biologically active naturally occurring compounds, and are important intermediates for halides, amines, esters, and ethers. They are also chiral auxiliaries in the syntheses of some drugs and insecticides.[3–11]

The kinetic resolution is dependent on the different rates of two enantiomers. The theoretical yield of kinetic resolution of racemate is 50%. It was reported that secondary alcohol is a sluggish reactant; therefore, to obtain an efficient kinetic resolution, the irreversible condition must be provided.[5]

In order to improve the enantioselectivity of the kinetic resolution of alcohols, the combinations of the lipase, solvent, and acyl donor are important. Lipases from *Pseudomonas cepacia*, *Candida rugosa*, and *Candida antarctica* have been used in secondary alcohol resolution.[12–15] Vinyl esters are commonly used as acyl donors for irreversible acylation. Hirose et al.[12] demonstrated the effective kinetic resolution of 2-phenyl-1-propanol by using an acyl donor, vinyl 3-phenylbutanoate. Isopropyl ether was used as a solvent and *Pseudomonas cepacia* lipase as an enzyme. They obtained a high ee of 85% and enantiomeric ratio (E) of 98. It was also reported that vinyl 3-phenylpropanoate was effective for the resolution of 2-phenyl-1-propanol.

Suan and Sarmidi[13] investigated the enzyme activity and enantioselectivity by acyl length of fatty acid from C12 to C18 in the enantioselective esterification of (*R,S*)-1-phenylethanol. They reported that the carbon number of fatty acids did not influence the enantioselectivities of the enzymes.

The solvent effect is important for the application of lipase in the racemate resolution. The solvent hydrophobicity, characterized by log P value, had a certain effect on the activity of the catalyst. Log P above 2 has been suggested as the best value. However, it was reported that there was no satisfactory correlation between log P of a solvent and enantioselectivity.[5] Frings et al.[16] reported that no dependency of enantioselectivity on log P could be detected in the kinetic resolution of 1-phenylethanol.

Temperature control is regarded as a simple and applicable method for the lipase catalyzed kinetic resolution of alcohols. Sakai[17] demonstrated that the low-temperature method was easily applied for the kinetic resolution of primary and secondary alcohols. For example, in the resolution of solketal, while a low enantiomeric ratio ($E = 16$) was obtained at 23°C, a high E ($E = 55$) was obtained at −40°C.

Immobilization of the enzyme was also affected enantioselectivity of secondary alcohol. Ghanem and Schuring[7] investigated the effect of immobilized lipase in sol-gel on the kinetic resolution secondary alcohol. They found that immobilization increased the enantioselectivity.

Recently, microwave irradiation on the organic synthesis has been applied. It has provided a powerful method to enhance the reaction rate and to improve yields.[18,19]

As can be seen, many parameters affect the enantioselectivity of enzymes. In the literature, most studies on secondary alcohols are based on 1-phenyl ethanol. The aim of this work is to investigate the enantioselective kinetic resolution of a racemic secondary alcohol, 1-phenyl 1-propanol, systemically. This study includes statistical optimization of some process parameters that have been reported to play a very significant role in changing the enantioselective resolution of racemates, namely, the substrate concentration, the molar ratio of acyl donor to the substrate, the amount of enzyme, and the stirring rate. The effect of microwave irritation on enantioselectivity was also investigated under optimum conditions.

EXPERIMENTAL

Enzyme and Chemicals

Candida antartica (Novozym 435) and *Rhizomucor meihei* (Lipozyme RMIM) were obtained from Novozymes. Lipases from *P. cepacia*, *P. stutzeri*, *P. fluorescens*, and Amona lipase PS-CI were purchased from Aldrich (Germany) and *Mucor mihei* lipase from Sigma (Germany). (*R,S*)-1–Phenyl-1-propanol, isopropenyl acetate, and vinyl acetate were purchased from Aldrich (Germany); vinyl laurate and vinyl butyrate from Fluka (Japan); and heptane, toluene, isooctane, tetrahydrofuran (THF), hexane, and thin-layer chromatography (TLC) sheets from Merck (Germany). The molecular sieve (4 Å) was purchased from Sigma (Germany). All chemicals were analytical grade.

Resolution of (*R,S*)-1-Phenyl 1-Propanol

The desired amount of (*R,S*)-1-phenyl-1-propanol was dissolved in 3 mL of solvent. The desired amount of acyl donors, molecular sieves (4 Å), and desired amount of enzyme were added to the reaction medium. The transesterification reaction was performed in a 10-mL bottle with a screw cap on an orbital shaker at the desired stirring rate and temperature. The progress of the reaction was monitored by thin-layer chromatography (TLC), and it was finished when the conversion reached about 50%. TLC was carried out on precoated aluminum sheets 60 F254 (Merck) using ethyl acetate:hexane (1:3) solution. The spots were visualized with ultraviolet (UV) light ($\lambda = 254$ nm).

Microwave Irradiation Experiments

The microwave enzyme reactor was configured with a glass reactor; 233 m*M* (*R,S*)-1-phenyl-1-propanol, 200 m*M* vinyl laurate, 116 mg Novozym 435, and 3 mL solvent were used in microwave irradiation experiments. The reaction mixture was placed into a 5-mL glass reactor with an air cooling system. The transesterification reaction was performed in a microwave synthesis system (Biotage initiator, 400 W magnetron).

HPLC Analysis

The reaction progress, monitored by TLC, was stopped when the conversion reached about 50%. After the solvent was evaporated using a rotary evaporator, the substrates were analyzed with high-performance liquid chromatography (HPLC) using a Chiralcel-OB column. The mobile phase was hexane/2-propanol (97/3) with 0.8 mL/min flow rate at column temperature 30°C, and the compounds were detected at 254 nm with a diode array detector. The conversion and the enantiomeric excess (ee) of the substrate were calculated using the equations $x = [1 - (C_S + C_R)/(C_{S0} + C_{R0})]100$ and $ee = [(C_S - C_R)/(C_S + C_R)]100$ (for $C_S > C_R$), respectively. Here, C_{S0} and C_{R0} are the initial substrate concentrations and C_S and C_R the substrate concentrations at certain times of *S* and *R* form, respectively.

The "Design Expert" (version 6.01, Stat. Ease, Inc., Minneapolis, MN) was used for regression and graphical analyses of the data obtained.

RESULTS AND DISCUSSION

Lipase-catalyzed enantioselective transesterification of 1-phenyl-1-propanol was selected as a model system for the enzymatic resolution of secondary alcohols. The enantioselectivity of kinetic resolution of alcohol is considerably influenced by the source of enzyme, and type of acyl donor and solvent. In the preliminary experiments, the effects of enzyme source, and type of acyl donor and solvent on the kinetic resolution of (*R,S*)-1-phenyl-1-propanol were investigated. Six commercial lipases were screened with vinyl laurate at 40°C in isooctane.

The enzymes used were *Mucor miehei* lipase, *Pseudomonas cepacia* lipase, *Pseudomonas stutzeri* lipase, *Pseudomonas fluorescens* lipase, Lipozyme RMIM, and Novozym 435. All of these enzymes were capable of catalyzing the reaction. (*R*)-1-phenyl-1-propanol was the fastest reacting enantiomer, yielding the (*R*)-acetate and leaving (*S*)-1-phenyl-1-propanol as enantiomerically pure unreacted enantiomer (Figure 1).

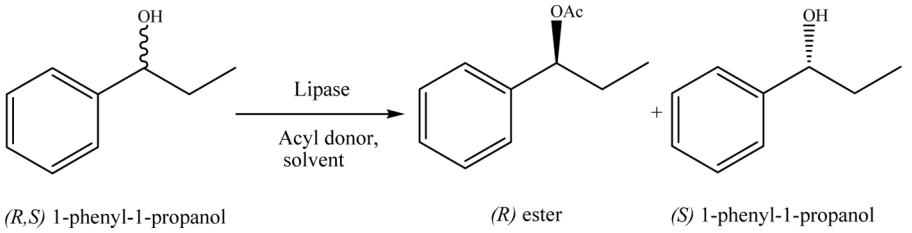

FIGURE 1 Transesterification reaction of (*R,S*)-1-phenyl-1-propanol.

As can be seen from Table 1, the highest ee for substrate was obtained with Novozym 435. All the enzymes except for *Mucor miehei* lipase are immobilized enzymes. While the minimum ee value was obtained with *Mucor miehei* lipase, ee increased with the use of Lipozyme TL IM, which is immobilized lipase produced from *Mucor miehei*. Immobilization of an enzyme is known to affect the enzyme conformation, rigidity, and reactivity. In an organic solvent, lipase molecules usually aggregate, which reduces the activity, but when the lipases are immobilized, they can be highly dispersed.[16,20]

The enantioselectivity of the reaction using Novozym 435 was much better than that of the reaction with other enzymes. This commercial lipase is in immobilized form on acrylic resin. Novozym 435 has been proven to be an efficient biocatalyst for esterification and transesterification of secondary alcohol.[11,13] After lipases were screened, the effect of solvent was investigated for the reaction with vinyl laurate as an acyl donor and Novozym 435. In all cases, the (*R*)-alcohol was more rapidly reacting enantiomer, yielding the (*R*)-ester in high enanatiomeric ester. Thus (*S*)-alcohol was obtained in enantiomerically pure form.

The influence of organic solvents on the enzymatic reaction is different. The hydrophobicity of the solvents can be expressed in terms of log P. The enzymes can show higher activity with the nonpolar (log $P > 4$) and

TABLE 1 The Effect of Enzyme Type on ee

Type of Enzyme	ee (%)	Time (hr)
Mucor meihei	2	0.5
Pseudomanas cepacia	25	0.5
Pseudomanas stutzeri	38	0.5
Pseudomanas floresans	9	0.5
Rhizomucor meihei (Lipozyme RM IM)	10	0.5
Candida antartica (Novozym 435)	71	0.5

Note. Resolution was carried with alcohol (0.5 mmol), vinyl laurate (1 mmol), enzyme (0.1 g), molecular sieve (0.1 g), isooctane (3 mL), temperature (40°C), stirring rate (150 rpm).

TABLE 2 The Effect of Solvent Type on ee

Solvent	ee (%)	Time (hr)
THF	15	4
Toluene	70	4
Hexane	68	3.5
Heptane	63	1
Isooctane	71	0.5

Note. Resolution was carried with alcohol (0.5 mmol), vinyl laurate (1 mmol), Novozym 435 (0.1 g), molecular sieve (0.1 g), solvent (3 mL), temperature (40°C), stirring rate (150 rpm).

midpolar solvents ($2 < \log P < 4$), whereas they have the lowest activity with the polar solvents ($\log P < 2$).[21] Therefore, in order to investigate the solvent effect on the enantioselectivity of enzyme, solvents having different log P were used. For this aim, THF (log P 0.5), toluene (log P 2.5), hexane (log P 3.5), heptanes (log P 4), and isooctane (log P 4.5) were used. The results are summarized in Table 2. While the enantioselectivity was the lowest with THF, ee values showed almost no change when used with other solvents having up to log P 2.5. At the same time, hydrophobic solvents give a lower reaction time than hydrophilic ones. This can be due to the hydrophobic properties of solvents. Hydrophobicity increases the transesterification reaction rate but it does not affect the enantioselectivity.

Afterward, the effect of acyl donor on enantioselectivity for transesterification was investigated by using ethyl acetate, vinyl acetate, isopropenyl acetate, vinyl butyrate, and vinyl laurate, which have different carbon numbers. As shown in Table 3, a vinyl group seems to affect the ee value. Isopropenyl acetate and ethyl acetate do not show any enantioselectivities. Similarly, to solve the reversibility problem of esterification and transesterification, Reetz[22] used vinyl acetate as an acylating agent, with the reactions being irreversible because of the formation of acetaldehyde.

In our study, an acyl donor having a vinyl group gave a high ee. The maximum ee obtained was 71%, with vinyl laurate. If we look at the acyl

TABLE 3 The Effect of Acyl Donor Type on ee

Acyl Donor	ee (%)	Time (hr)
Ethyl acetate (C4)	3	0.5
Vinyl acetate (C4)	19	0.5
Izoprophenyl acetate (C5)	0	0.5
Vinyl butyrate (C6)	26	0.5
Vinyl laurate (C14)	71	0.5

Note. Resolution was carried with alcohol (0.5 mmol), vinyl laurate (1 mmol), Novozym 435 (0.1 g), molecular sieve (0.1 g), iso-octane (3 mL), temperature (40°C), stirring rate (150 rpm).

donors from the point of view of carbon number, ee increased with increasing chain length of the acyl donor having a vinyl group. In the literature, Suan and Sarmidi[13] investigated the effect of chain length of fatty acids on the esterification reaction of (*R,S*)-1-phenyl ethanol. Lauric, myristic, palmitic, and stearic acids were used as acylating agents. The authors reported that although the carbon number of fatty acids affected the reaction rate, it did not influence the enantioselectivities of enzymes. However, our results are similar to those reported by Ottoson and Hult,[23] that the enantioselectivity of *C. antartica* lipase B was strongly influenced by the achiral chain length of vinyl ester in the transesterification of 3-methyl-2-butanol.

After determining the source of enzyme, solvent, and acyl donor, we investigated the other reaction conditions using RSM. The classical method of parameter optimization involves changing one variable at a time, keeping the others at fixed levels. Being single-dimensional, this laborious and time-consuming method often does not guarantee determination of the optimal conditions. Previous experiments helped us to identify the five experimental variables markedly influencing the enantioselective production of 1-phenyl-1-propanol: concentration of alcohol, molar ratio of acyl donor to the alcohol, amount of enzyme, temperature, and stirring rate.

The range and levels of the variables investigated in this study, which were chosen based on previous studies, are given in Table 4. In the regression equation, the test variables were coded according to the equation:[24,25]

$$x_i = \frac{(X_i - X_i^*)}{\Delta X_i} \tag{1}$$

where x_i is the coded value of the ith independent variable, X_i is the uncoded value of the ith independent variable, X_i^* is the uncoded value

TABLE 4 Experimental Range and Levels of the Independent Variable

	Range and Levels				
Variables	−2.38	−1	0	1	2.38
Alcohol concentration (m*M*), X_1	8, 34	100	167	233	325
Acyl donor/alcohol (mol/mol), X_2	0, 81	1, 5	2	2, 5	3, 19
Enzyme (mg), X_3	40, 54	75	100	125	159, 46
Temperature (°C), X_4	16, 22	30	40	50	63, 78
Stirring rate (rpm), X_5	31, 08	100	150	200	268, 92

TABLE 5 Full Factorial Central Composite Design Matrix of Five Variables and Natural Units Along with Observed Responses (ee %)

Number	X_1 (Alcohol Concentration, m*M*)	X_2 (Acyl Donor/ Alcohol, mol/mol)	X_3 (Enzyme, mg)	X_4 (Temperature, °C)	X_5 (Stirring Rate, RPM)	Experimental Response (ee %)
1	100.00	1.50	75.00	30.00	100.00	31
2	233.00	1.50	75.00	30.00	100.00	55
3	100.00	2.50	75.00	30.00	100.00	42
4	233.00	2.50	75.00	30.00	100.00	32
5	100.00	1.50	125.00	30.00	100.00	41
6	233.00	1.50	125.00	30.00	100.00	59
7	100.00	2.50	125.00	30.00	100.00	63
8	233.00	2.50	125.00	30.00	100.00	31
9	100.00	1.50	75.00	50.00	100.00	36
10	233.00	1.50	75.00	50.00	100.00	74
11	100.00	2.50	75.00	50.00	100.00	75
12	233.00	2.50	75.00	50.00	100.00	58
13	100.00	1.50	125.00	50.00	100.00	31
14	233.00	1.50	125.00	50.00	100.00	89
15	100.00	2.50	125.00	50.00	100.00	33
16	233.00	2.50	125.00	50.00	100.00	53
17	100.00	1.50	75.00	30.00	200.00	57
18	233.00	1.50	75.00	30.00	200.00	58
19	100.00	2.50	75.00	30.00	200.00	51
20	233.00	2.50	75.00	30.00	200.00	52
21	100.00	1.50	125.00	30.00	200.00	24
22	233.00	1.50	125.00	30.00	200.00	68
23	100.00	2.50	125.00	30.00	200.00	27
24	233.00	2.50	125.00	30.00	200.00	72
25	100.00	1.50	75.00	50.00	200.00	52
26	233.00	1.50	75.00	50.00	200.00	70
27	100.00	2.50	75.00	50.00	200.00	60
28	233.00	2.50	75.00	50.00	200.00	54
29	100.00	1.50	125.00	50.00	200.00	33
30	233.00	1.50	125.00	50.00	200.00	79
31	100.00	2.50	125.00	50.00	200.00	26
32	233.00	2.50	125.00	50.00	200.00	74
33	8.34	2.00	100.00	40.00	150.00	77
34	324.66	2.00	100.00	40.00	150.00	34
35	166.50	0.81	100.00	40.00	150.00	62
36	166.50	3.19	100.00	40.00	150.00	72
37	166.50	2.00	40.54	40.00	150.00	14
38	166.50	2.00	159.46	40.00	150.00	43
39	166.50	2.00	100.00	16.22	150.00	38
40	166.50	2.00	100.00	63.78	150.00	30
41	166.50	2.00	100.00	40.00	31.08	38
42	166.50	2.00	100.00	40.00	268.92	42
43	166.50	2.00	100.00	40.00	150.00	79
44	166.50	2.00	100.00	40.00	150.00	71
45	166.50	2.00	100.00	40.00	150.00	77
46	166.50	2.00	100.00	40.00	150.00	65

(*Continued*)

TABLE 5 Continued

Number	X_1 (Alcohol Concentration, m*M*)	X_2 (Acyl Donor/ Alcohol, mol/mol)	X_3 (Enzyme, mg)	X_4 (Temperature, °C)	X_5 (Stirring Rate, RPM)	Experimental Response (ee %)
47	166.50	2.00	100.00	40.00	150.00	68
48	166.50	2.00	100.00	40.00	150.00	70
49	166.50	2.00	100.00	40.00	150.00	70
50	166.50	2.00	100.00	40.00	150.00	65

of the *i*th independent variable at the center point, and ΔX_i is the step change value. A 2^5 full factorial composite for five independent variables was used in this study. The full factorial composite design consists of a complete $2k$ factorial design, where k is the number of test variables, n_0 center points ($n_0 \geq 1$), and two axial points on the axis of each design variable at a distance of α ($=2^{k/4}$, ($=2.378$ for $k=5$) from the design center. The total number of design points is $N=2^k+2k+n_0$ (50 experiments for 5 independent variables).

A second-order polynomial model was fitted to obtain the ee value of the transesterification reaction of 1-phenyl-1-propanol. The experiments performed and the results obtained under the operational conditions employed are listed in Table 5.

The application of RSM yielded the following regression equation, which is an empirical relationship between the ee value and the experimental parameters in coded units:

$$\begin{aligned} y = {} & 71.41 + 7.93x_1 - 1.37x_2 - 2.21x_3 + 3.22x_4 + 0.80x_5 - 8.69x_1^2 \\ & + 0.065x_2^2 - 3.03x_3^2 - 5.77x_4^2 - 4.71x_5^2 - 7.19x_1x_2 + 5.91x_1x_3 \\ & + 4.55x_1x_4 + 2.09x_1x_5 - 1.47x_2x_3 + 0.66x_2x_4 - 0.78x_2x_5 \\ & - 1.78x_3x_4 - 1.84x_3x_5 - 0.84x_4x_5 \end{aligned} \quad (2)$$

The results of the second-order response surface model fitting in the form of analysis of variance (ANOVA) are given in Table 6. The Fisher *F*-test with a very low probability value ($P_{model} > F < 0.0001$) demonstrates a very high significance for the regression model and confirms the adequacy of the quadratic model. This result indicates that it is statistically significant at 99.999% confidence level. The significance of each coefficient was determined by *F*-value and Prop > *F*-value (Table 6). Values of "Prop > *F*" less than 0.0500 indicate that the model terms are significant. In this case, the coefficients of x_1, x_1^2, x_1x_2, and x_1x_3 are significant model terms. The first order and the quadratic main effects of alcohol concentration, $x_1x_1^2$ (Prop > F $\ll$ 0.0001), are the most significant. The interaction terms

TABLE 6 Analysis of Variance (ANOVA) for the Quadratic Model

Source	Coefficient Estimate as Coded Value	Sum of Squares	df	Mean Square	*F*-Value	Prob > *F*
Intercept	71.41					
x_1	7.93	2723.94	1	2723.94	23.19	<0.0001
x_2	−1.37	80.96	1	80.96	0.69	0.4132
x_3	−2.21	212.42	1	212.42	1.81	0.1891
x_4	3.32	478.56	1	478.56	4.07	0.0529
x_5	0.80	27.50	1	27.50	0.23	0.6321
x_1^2	−8.69	4192.41	1	4192.41	35.69	<0.0001
x_2^2	0.065	0.23	1	0.23	1.969E-003	0.9649
x_3^2	−3.03	509.86	1	509.86	4.34	0.0461
x_4^2	−5.77	1849.48	1	1849.48	15. 74	0.0004
x_5^2	−4.71	1231.93	1	1231.93	10.49	0.0030
x_1x_2	−7.16	1638.78	1	1638.78	13.95	0.0008
x_1x_3	5.91	1116.28	1	1116.28	9.50	0.0045
x_1x_4	4.53	657.03	1	657.03	5.59	0.0249
x_1x_5	2.09	140.28	1	140.28	1.19	0.2835
x_2x_3	−1.47	69.03	1	69.03	0.59	0.4495
x_2x_4	0.66	13.78	1	13.78	0.12	0.7344
x_2x_5	−0.78	19.53	1	19.53	0.17	0.6864
x_3x_4	−1.78	101.53	1	101.53	0.86	0.3602
x_3x_5	−1.84	108.78	1	108.78	0.93	0.3439
x_4x_5	−0.84	22.78	1	22.78	0.19	0.6629
Model		13,672.47	20	683.62	5.82	<0.0001
Residual		3406.65	29	117.47		
Lack of fit		3224.77	22	146.58	5.64	0.0126
Pure error		181.88	7	25.98		
Cor total		17,079.12	49			

Note. *R*-squared = 0.8005, adeq. precision = 10.519.

between alcohol concentration and acyl donor/alcohol concentration (Prop > F = 0.0008) and alcohol concentration and amount of enzyme (Prop > F = 0.0045) are also more significant than the others.

The response surfaces obtained according to the RSM analysis for each ee value are shown in Figures 2–6. Response surface plots provide a method to predict the ee value for different values of the test variables. When the response surface is examined, it is clear that the interaction between alcohol concentration and acyl donor/alcohol is more effective (Figure 2). ee increased with increasing 1-phenyl 1-propanol concentration from 10 to 250 m*M*, and above this alcohol concentration ee decreased. The effect of molar ratio of acyl donor/alcohol is dominant especially at high alcohol concentration. The ee value reached 91% at 250 m*M* alcohol concentration. This result can be explained as follows. In the transesterification reaction, firstly, the enzyme is acylated and then transesterification occurs.[13] Therefore, the amount of acyl donor is important. The depletion of acyl donor on the active site of the

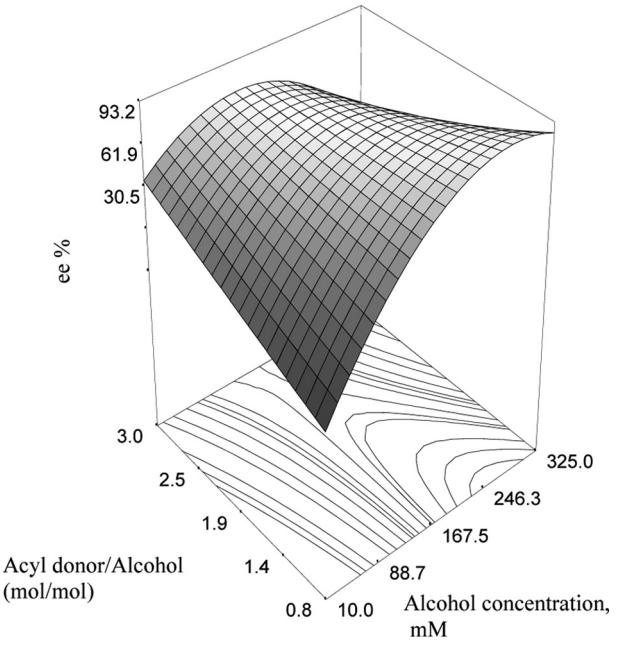

FIGURE 2 Response surface plot showing the mutual effect of acyl donor/alcohol (mol/mol) and alcohol concentration when the other variables are held at zero level.

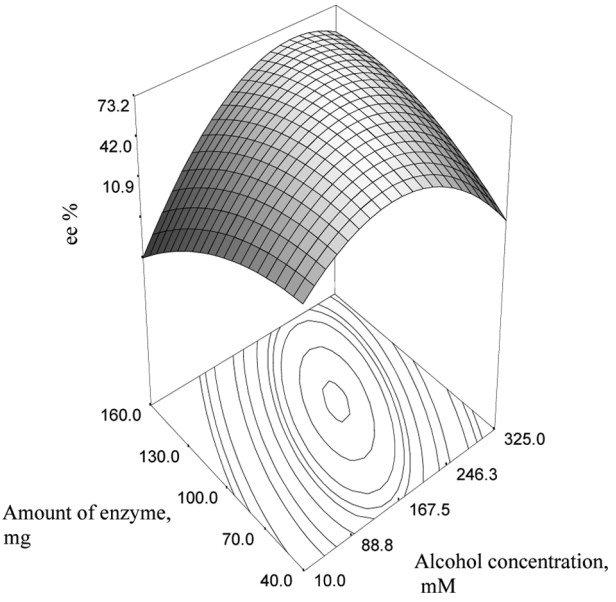

FIGURE 3 Response surface plot showing the mutual effect of amount of enzyme and alcohol concentration when the other variables are held at zero level.

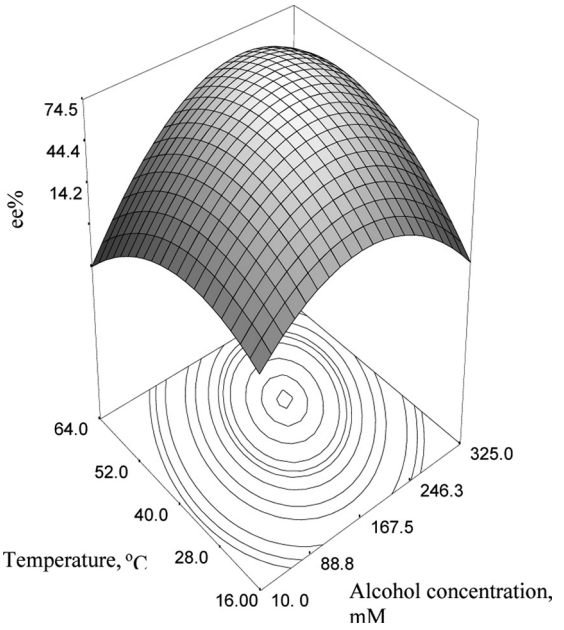

FIGURE 4 Response surface plot showing the mutual effect of temperature and alcohol concentration when the other variables are held at zero level.

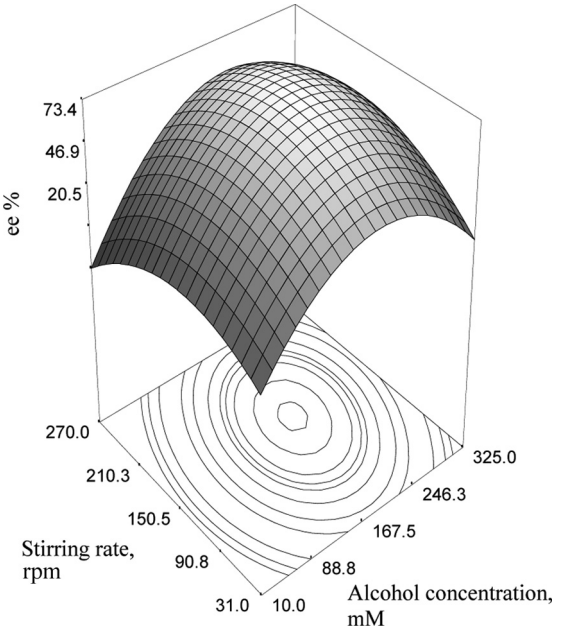

FIGURE 5 Response surface plot showing the mutual effect of stirring rate and alcohol concentration when the other variables are held at zero level.

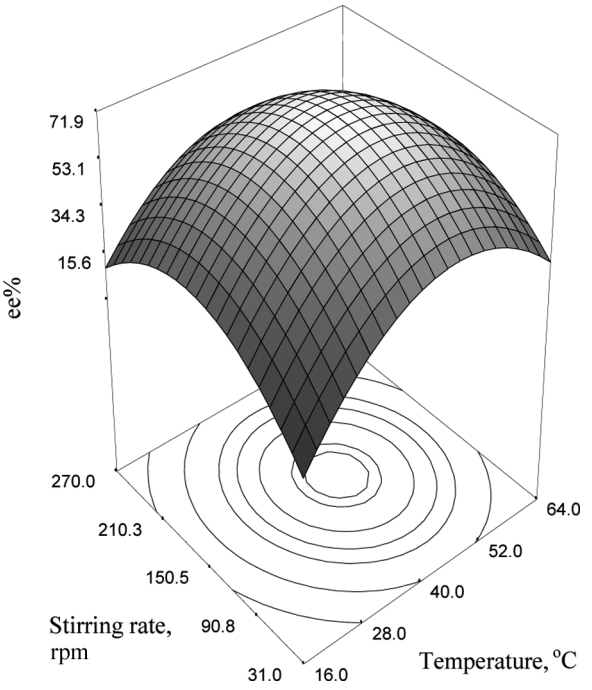

FIGURE 6 Response surface plot showing the mutual effect of stirring rate and temperature when the other variables are held at zero level.

enzyme may be prevented by increasing the molar ratio of the acyl donor/alcohol. Moreover, an increase in alcohol concentration may prevent the acyl donor reaching the active site. The molar ratio of acyl donor/alcohol and alcohol concentration interact with each other. The contour plots help us to identify the types of interactions between test variables. An elliptical or saddle nature of the contour plots indicates the significance of the interactions between the corresponding variables. A similar interaction between alcohol concentration and amount of enzyme is shown in Figure 3. The ee value increases with amount of enzyme up to 120 mg; above this value it remains almost unchanged (Figure 3). Because additional mass transfer limitation can occur with increasing catalyst loading in liquid–solid (catalyst) reaction or the amount of enzyme can be more than the substrate concentration, no free substrate was available to bind the excess enzyme.

The variations in temperature and alcohol concentration with ee can be seen in Figure 4. The ee value increased with increasing temperature up to 47°C. The effect of temperature on the enantioselectivity can differ with the enzyme used and type of reaction. The effect of stirring rate on the ee is shown in Figures 5 and 6. Because of the mass transfer effects, the

enantioselectivity of the enzyme was affected by the stirring rate. It increased with stirring rate between 30 and 175 rpm; above this stirring rate it decreased. This decrease may be due to conformational changes or attrition of the immobilized structure of the enzyme.

The maximum predicted ee value is indicated by the surface confined in the smallest ellipse in the contour diagrams temperature versus the alcohol concentration (Figure 4) and in the contour diagrams stirring rate versus to alcohol concentration (Figure 5). The temperature has no interaction with stirring rate as proved by the relatively circular nature of the contour curves (Figure 6) and shows the optimal stirring rate around 150–200 rpm and optimal temperature around 45°C.

Microsoft Excel software was used to solve the regression equation [Eq. (2)]. The optimum values for the test variables for the maximum ee value (83%) were alcohol concentration 233 mM, acyl donor/alcohol concentration 1.5, amount of enzyme 116 mg, temperature 47°C, and stirring rate 161 rpm. In order to verify these results, enantiomerically pure production of 1-phenyl-1-propanol was carried out at the optimum condition and ee was obtained as 91% for 3 hr. The maximum ee value predicted by the equation (83%) agrees with this experimental value.

Finally, the effect of microwave irradiation on the transesterification of 1-phenyl 1-propanol was investigated using conditions that were optimal except for temperature. The temperature was kept at 60°C under microwave with an air compressor system, and 87% ee was obtained at a considerably short reaction time (5 min). Microwave irradiation increased the rate of the lipase-catalyzed reaction. In the literature, there are limited studies on microwave assisted enantioselective synthesis.[11,18,19,26] There are contradictory statements about the effect of microwave irradiation in the literature. Unlike our study, Leadbeater et al.[26] reported no differences between the effect of microwave heating and conventional heating on the conversion and reaction rate of lipase-catalyzed transesterification reaction. Souze et al.[19] studied on the kinetic resolution of *rac*-1-phenylethanol with microwave irradiation. They reported that microwave irradiation displayed no changes in both activity and selectivity of the immobilized lipases according to the conventional heating at the same temperature. On the other hand, Lin and Lin[18] demonstrated the reaction rate and enantioselectivities of lipase catalyzed reaction significantly enhanced under microwave irradiation. Similarly, Bachu et al.[11] also obtained high conversion under the microwave irradiation. Therefore, the effect of microwave irradiation on the enzyme-catalyzed reaction in organic solvents must be investigated particularly. Our studies have continued in this direction.

CONCLUSION

The kinetic resolution of *rac*-1-phenyl-1-propanol was carried out successfully with a transesterification reaction. Enantiomerically pure secondary alcohols are important synthetic intermediates and chiral auxiliaries. For this resolution, the best results were obtained using Novozym 435, vinyl laurate, and isooctane. The methodology of response surface optimization was shown to be very useful for the determination of relevant variables for process optimization. This made it possible to consider a large number of variables and avoid the loss of information that might be essential in the optimization of the process. It was possible to determine optimal operating conditions for high ee value by using the method of experimental factorial design and response surface analysis. In order to verify the results of RSM, experiments were carried out and ee was found to be 91% at the optimum conditions for three hours. Moreover, at these optimum conditions (except temperature), microwave irritation was applied and 87% ee was obtained at only 5 min.

ACKNOWLEDGMENT

The authors gratefully acknowledge the financial support given to this work by Ankara University, Institute of Biotechnology (project 2001K120140-162).

REFERENCES

1. Berglund, P. Controlling Lipase Enantioselectivity for Organic Synthesis. *Biomol. Eng.* **2001**, 18, 13–22.
2. Theil, F. Enhancement of Selectivity of Reactivity of Lipases by Additives. *Tetrahedron* **2000**, 56, 2905–2919.
3. Soai, K.; Niwa, S. Enantioselective Addition of Organazinc Reagents to Aldehydes. *Chem. Rev.* **1992**, 92(5), 833–856.
4. Bakker, M.; Spruijt, A.S.; Van Rantwijk, F.; Sheldon, R.A. Highly Enantioselective Aminoacylase-Catalyzed Transesterification of Secondary Alcohols. *Tetrahedron Asymmetr.* **2000**, 11, 1801–1808.
5. Parada, J.; Herrera, J.; Pedraza, A. Enantioselective Addition of Diethylzinc to Benzaldehyde Catalyzed by an Organometallic Ti(IV) Compound and a Xylose Derivative. *J. Braz. Chem. Soc.* **2009**, 1, 74–79.
6. Kamal, A., Sandbhor, M.; Raman, K.V. One-Pot Lipase Catalyzed Synthesis of Enantiopure Secondary Alcohols From Carbonyl Compounds: A New Protocol for Lipase-Mediated Resolution. *Tetrahedron Asymmetr.* **2002**, 13, 815–820.
7. Ghanem, A.; Schuring, V. Entrapment of *Pseudomonas cepacia* Lipase With Peracetylated *β*-Cyclodextrin in Sol-Gel: Application to the Kinetic Resolution of Secondary Alcohols. *Tetrahedron Asymmetr.* **2003**, 14, 2547–2555.
8. Martin-Matude, B.; Edin, M.; Bogar, K.; Kaynak, F.B.; Backvall, J.E. Combined Ruthenium(II) and Lipase Catalysis for Efficient Dynamic Kinetic Resolution of Secondary Alcohols. Insight Into the Racemization Mechanism. *J. Am. Chem. Soc.* **2005**, 127, 8817–8825.

9. Nyhlén, J.; Matin-Mattude, B.; Sandström, A.G.; Bocola, M.; Backvall, J.E. Influence of δ-Functional Groups on the Enantiorecognition of Secondary Alcohols by *Cancida antarctica* Lipase B. *Chem BioChem* **2008**, 9, 1968–1974.
10. Kiss, V.; Egri, G.; Balint, J.; Fogassy, E. Enantioseperation of Secondary Alcohols by Diasterepisomeric Salt Formation. *Chirality* **2006**, 18, 116–120.
11. Bachu, P.; Gibson, J.S.; Sperry, J.; Brimble, M.A. The Influence of Microwave Irradiation on Lipase-Catalyzed Kinetic Resolution of Racemic Secondary Alcohols. *Tetrahedron Asymmetr.* **2007**, 18, 1618–1624.
12. Hirose, K.; Naka, H.; Yano, M.; Ohashi, S.; Naemura, K.; Tobe, Y. Improvement of Enantioselectivity in Kinetics Resolution of a Primary Alcohol Through Lipase-Catalysed Transesterification by Using a Chiral Acyl Donor. *Tetrahedron Asymmetr.* **2000**, 11, 1199–1210.
13. Suan, C.; Sarmidi, M.R. Immobilized Lipase-Catalysed Resolution of (*R,S*)-1-Phenyl Ethanol in Recirculated Packed Bed Reactor. *J. Mol. Catal. B Enzymol.* **2004**, 28, 111–119.
14. Kiss, V.; Egri, G.; Bailent, J.; Ling, I.; Barkoczi, J.; Fogassy, E. Kinetic and Chemical Resolution of Different 1-Phenyl-2-Propanol Derivatives. *Tetrahedron Asymmetr.* **2006**, 17, 2220–2234.
15. Kawasaki, M.; Goto, M.M.; Kawabata, S.; Kometani, T. The Effect of Vinyl Esters on the Enantioselectivity of the Lipase-Catalysed Transesterification of Alcohols. *Tetrahedron Asymmetr.* **2001**, 12, 585–596.
16. Frings, K.; Koch, M.; Hatmeier, W. Kinetic Resolution of 1-Phenyl Ethanol With High Enantioselectivitiy With Native and Immobilized Lipase in Organic Solvents. *Enzyme Microb. Technol.* **1999**, 25, 303–309.
17. Sakai, T. Low-Temperature Method for a Dramatic Improvement in Enantioselectivity in Lipase-Catalyzed Reactions. *Tetrahedron Asymmetr.* **2004**, 15, 2749–2756.
18. Lin, G.; Lin, W.Y. Microwave-Promoted Lipase-Catalyzed Reactions. *Tetrahedron Lett.* **1998**, 39, 4333–4336.
19. Souza, R.O.M.A.; Antunes, O.A.C.; Kroutil, W.; Kappe, C.O. Kinetic Resolution of rac-1–Phenylethanol With Immobilized Lipases: A Critical Comparison of Microwave and Conventional Heating Protocols. *J. Org. Chem.* **2009**, 74, 6157–6162.
20. Mehmetoğlu, Ü.; Bayraktar, E.; Babaarslan, Ç. Production of Enantiomerically Pure Pharmaceutical Compound Using Biocatalyst. In *Enzyme Mixture and Complex Biosynthesis*; Bhattacharya, S.; Ed.; Landes Bioscience: Austin, TX, **2007**; 65–78.
21. Huang, W.; Xia, Y.M.; Gao, H.; Fang, Y.J.; Wang, Y.; Fang, Y. Enzymatic Esterification Between *n*-Alcohol and *n*-Caprylic Acid in Non-Aqueous Medium Under Microwave Irradition. *J. Mol. Catal. B Enzymol.* **2005**, 35, 113–116.
22. Reetz, M.T. Lipases as Practical Biocatalysts. *Curr. Opin. Chem. Biol.* **2002**, 6, 145–150.
23. Ottosson, J.; Hult, K. Influence of Acyl Chain Length on the Enantioselectivity of *Candida antartica* Lipase B and Its Thermodynamic Components in Kinetic Resolution of sec-Alcohols. *J. Mol. Catal. B Enzymol.* **2001**, 11, 1025–1028.
24. Bayraktar, E. Response Surface Optimization of the Separation of DL-Tryptophan Using an Emulsion Liquid Membrane. *Process. Biochem.* **2001**, 37, 169–175.
25. Murthy, M.S.R.C.; Swaminathan, S.K.; Rakshit, S.K.; Kosugi, Y. Statistical Optimization of Lipase Catalyzed Hydrolysis of Methyloleate by Response Surface Methodology. *Bioprocess Eng.* **2000**, 22, 35–39.
26. Leadbeater, N.E.; Stencel, L.M.; Wood, E.C. Probing the Effects of Microwave Irradiation on Enzyme-Catalysed Organic Transformations: The Case of Lipase-Catalysed Transesterification Reactions. *Org. Biomol. Chem.* **2007**, 5(7), 1052–1055.