Optimization and kinetic modeling of lipase mediated enantioselective kinetic resolution of (±)-2-octanol

Jyoti B. Sontakke, Ganapati D. Yadav[*]

Department of Chemical Engineering, Institute of Chemical Technology, Mumbai, India;
[*]Corresponding Author: gd.yadav@ictmumbai.edu.in, gdyadav@yahoo.com

ABSTRACT

Chiral 2-octanol is one of the key intermediates for preparation of liquid crystal materials, as well as many optically active pharmaceuticals. Lipase catalyzed kinetic resolution has proved to be an efficient technique for synthesis of enantiomerically enriched compounds. In the present study, optimization and kinetic modeling of kinetic resolution of (±)-2-octanol was done by using vinyl acetate as an acyl donor in *n*-heptane as a solvent. Response surface methodology (RSM) and four-factor-five-level Centre Composite Rotatable Design (CCRD) were employed to evaluate the effect of various parameters such as speed of agitation, enzyme loading, temperature and acyl donor/alcohol molar ratio on conversion, enantiomeric excess (ee), enantioselectivity and initial rate of reaction. Acylation of 2-octanol with vinyl acetate catalyzed by Novozyme 435 follows the ternary complex mechanism (ordered bi-bi mechanism) with inhibition by 2-octanol.

Keywords: Immobilized Lipase; Novozyme 435; 2-Octanol; Response Surface Methodology; Kinetic Modeling; Enantioselectivity

1. INTRODUCTION

Enzymatic catalysis in non-aqueous media has been greatly pursued these days for the synthesis of a wide variety of pharmaceuticals, agrochemicals, perfumes, flavors and other fine-chemicals [1-4]. In this regard, our group has contributed extensively to mechanistic studies, kinetic modeling and separation of enantiomers, covering several industrially relevant classes of reactions such as epoxidation/oxidation [5,6], hydrolysis [7], esterification [8-10], transesterification [11-13], amidation [14] and hydrazinolysis [15]. The synergism with microwave irradiation in immobilized lipase catalysis [16-20] and scope of non-aqueous systems in pharmaceutical industries [21] have been embraced. Optimization of process parameters by using statistical methods has been reported in a number of cases in literature and the current investigation in an effort in that direction.

Kinetic resolution of chiral compounds using enzymes especially lipases has proven to be an effective technique vis-a-vis chemical methods. The main consideration for adding biotransformation in a synthetic route is the regio- and stereo-control that can be achieved elegantly using enzyme-catalyzed step(s) [4]. Thus, chemo-enzymatic processes will see commercial utility in future. For instance, chiral aliphatic alcohols, which are important active pharmaceutical intermediates (API), have been obtained through lipase catalyzed kinetic resolution of corresponding racemic mixtures *via* esterification, transesterification or ester hydrolysis [21]. There are a number of ways to resolve the racemic mixtures by using enzymatic catalysis: dynamic kinetic resolution (DKR) with racemization catalysts [22], combination of DKR with double kinetic resolution [23], deracemisation [1], and sequential kinetic resolution [24]. Different lipases have been used for the kinetic resolution of aliphatic alcohols [23,25-30]. Various immobilization techniques for lipase immobilization have been reported; for instance, hexagonal mesoporous silica (HMS) [12], magnetic nanoparticles, Diaion HP20, ultrastable-Y molecular sieve [27], SBA 15 [29], and Sol-gel method [31].

Process optimization has a great relevance in complex reaction and has been done by two ways: one-factor-at-a-time method and statistical analysis such as Response Surface Methodology (RSM). RSM is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes in which a

response of interest is influenced by several variables and the objective is to optimize this response [32]. To avoid the disadvantages of the one-factor-at-a-time method since it does not illustrate interaction effect among various factors and gives only local optima of the reaction, in this work we have used the RSM for process optimization. The Centre Composite Rotatable Design of RSM has been previously been successfully applied in food technology [33,34], microbiology [35], biotechnological [36-41] and chemical processes [42]. To the best of our knowledge, there is a dearth of literature on RSM for kinetic resolution of chiral compounds using enzymatic catalysis [40-44].

In the present study, *Candida antartica* lipase B, *Thermomyces lanuginosus* lipase and *Rhizomucor meihei* lipase, were employed for the kinetic resolution of (±)-2-octanol by using vinyl acetate as an acylating agent. Optimization of reaction parameters has been done by RSM and CCRD using four factors, each at five variables.

2. MATERIALS AND METHODS

2.1. Enzymes and Chemicals

All chemicals were procured from firms of repute and used without any further purification: Novozyme 435 (*Candida arctica* lipase B immobilized on a macroporous polyacrylic resin, activity 10 PLU/g; (1 μmol propyl laurate formed/min/g-enzyme)), Lipozyme RM IM (*Mucor miehei* lipase immobilized on anionic resin, activity 6 BAUN (Acidolysis Unit Novo) and Lipozyme TL IM (*Thermomyces lanuginosus* immobilized on silica, activity 75 IUN/g) were procured as gift samples from Novo Nordisk, Denmark. (±)-2-Octanol was procured from Merck, India. Vinyl acetate and *n*-heptane were procured from SD Fine Chemicals Pvt. Ltd., Mumbai, India.

2.2. Experimental Setup

The experimental setup consisted of a 3 cm internal diameter (ID), fully baffled mechanically agitated reactor of 50 cm^3 capacity, which was equipped with four equispaced baffles and 1 cm diameter four bladed-pitched-turbine impeller. The entire reactor assembly was immersed in a thermostatic water bath which was maintained at a desired temperature with an accuracy of ±1˚C.

2.3. Kinetic Resolution of (R,S)-2-Octanol by Immobilized Lipase

The resolution was performed in the above reactor containing (±)-2-octanol, catalyst and solvent. When the set temperature was reached, vinyl acetate was added in the reactor, and agitation started. Samples were withdrawn periodically at regular time intervals, and the reso-

lution process was monitored by GC. The total reaction mixture volume was 25 cm^3 which was made up with *n*-heptane as a solvent. The total reaction time was 6 h.

2.4. Determination of Enantiomeric Excess (*ee*) and Enantioselectivity (*E*)

Clear liquid samples were withdrawn periodically from the reaction mass and analyzed by using Ceres 800 *plus* GC instrument equipped with flame ionization detector (FID) and *β*-Dex 120 (30 m × 0.25 mm × 0.25 μm) chiral capillary column. The analytical conditions were: injector temperature 220˚C; FID temperature 220˚C; oven temperature held at 65˚C for 30 min, then increased at 10˚C·min^{-1} to a final temperature of 130˚C, which was thereafter maintained for 10 min. The enantioselectivity (*E*) was calculated from the enantiomeric excess of the substrate (*ee_s* %) at a certain conversion (*c*, %) based on the following equations.

$$E = \frac{\ln\left[(1-c)(1-ee_s)\right]}{\ln\left[(1-c)(1+ee_s)\right]} \tag{1}$$

where,

$$c = 1 - \frac{\left[A_{(R)}\right]+\left[A_{(S)}\right]}{\left[A_{(R_0)}\right]+\left[A_{(S_0)}\right]} \tag{2}$$

and

$$ee_s = \frac{\left[A_{(S)}\right]-\left[A_{(R)}\right]}{\left[A_{(S)}\right]+\left[A_{(R)}\right]} \tag{3}$$

where, $A_{(R)}$ and $A_{(S)}$ denote (*R*)-2-octanol and (*S*)-2-octanol, respectively.

2.5. Design of Experiments and Statistical Analysis

RSM was used to optimize the process of resolution of (±)-2-octanol and to study the effect of different process variables on reaction along with the interactions among them. The experimental design applied to this study was CCRD (four factors, each at five levels). Compared with one-factor-at-a-time design, which has been adopted most often in the literature, the combination of RSM and four-factor-three-level CCRD employed in this study allowed us to reduce the number of experiments and time. The independent variables were: speed of agitation, catalyst loading, reaction temperature, and ester to alcohol molar ratio. Whereas the responses (dependent variables) chosen were 1) conversion of (±)-2-octanol; 2) ee of remaining alcohol; 3) *E* of the enzyme and 4) initial rate of reaction. **Table 1** shows the independent variables and their levels. The responses were then analyzed using numerical tools provided by Design Expert, Version 6.0.10

Table 1. Independent variables and their levels.

Independent variable	Coded symbol	−2	−1	0	1	2
Catalyst loading (g)	A	0.02	0.04	0.06	0.08	0.1
Reaction Temperature (°C)	B	10	25	40	55	70
Ester to alcohol molar ratio	C	1	2	3	4	5
Speed of agitation (rpm)	D	100	200	300	400	500

(Stat Ease, Minneapolis, MN, USA). The second order polynomial coefficients were calculated and analysis of variance (ANOVA) was conducted by using analytical tools of Design Expert. Contour and response surface plots were obtained after analysis of each response. After each response had been analyzed, multiple response optimizations were done by numerical tools provided by the Design Expert. Separate experiments at the optimum process conditions were performed for validation of the response models.

3. RESULTS AND DISCUSSION

Lipase catalyzed kinetic resolution of (±)-2-octanol with vinyl acetate as an acyl donor in *n*-heptane as a solvent produces ester and acetaldehyde is given in **Scheme 1**.

3.1. Effect of Different Catalysts

The activity and selectivity of Novozyme 435, Lipozyme RM IM and Lipozyme TL IM were evaluated towards the acylation of (±)-2-octanol under otherwise similar conditions. **Figure 1** shows the average conversion of three experiments at the end of 6 h for each enzyme. In the case of Novozyme 435, the conversion was 41.8% compared with 18.9% for Lipozyme RM IM and 9.6% for Lipozyme TL IM. The higher activity of Novozyme 435 is probably due to its stability in the presence of acetaldehyde which is liberated during the reaction. Novozyme 435 was selected for all further experiments as it gave highest conversion as compared to other catalysts.

3.2. Process Optimization

The major objective of this work was the development and evaluation of a statistical approach to better understand the relationship between the independent and dependent variables of a lipase catalyzed acylation of (±)-2-octanol. The experiments were performed as per design of experiments data. The order in which reactions were performed was randomized to minimize errors due to possible systematic trends in the variables. Six experiments were carried out at the center point, coded as "0", to minimize experimental error.
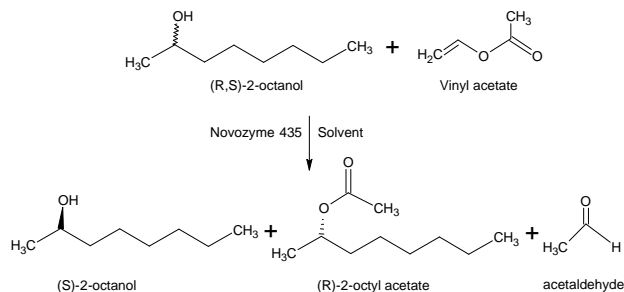
Scheme 1. Kinetic resolution of (±)-2-octanol with vinyl acetate.
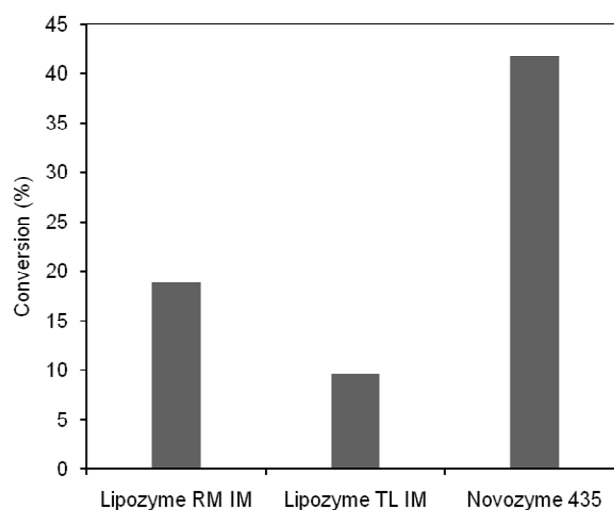
Figure 1. Effect of different catalysts.

Different models (linear, two factor interaction, quadratic and cubic) were tested for fitting experimental data. Based on p-value, the quadratic model was used for conversion, two-factor interaction for enantioselectivity and linear for initial rate (**Table 2**). The mean of the enantiomeric excess (ee) was taken into account as the values obtained were much closer and no model can be fitted to the experimental data. Correlation regression coefficients were obtained for all the response models indicating that second order polynomial model fitted well to the experimental data and was adequate to represent the relationship between the responses and significant variables with very small p value and a satisfactory coefficient of determination. The second order polynomial equations for conversion, enantioselectivity and initial rate of reaction are as follows,

$$\text{Conversion} = 45.00 + 2.12A + 1.46B - 0.71C + 3.71D$$
$$+0.20A^2 - 1.05B^2 - 0.55C^2 - 1.05D^2 + 0.44AB \qquad (4)$$
$$+0.69AC - 0.19AD + 0.44BC + 0.81BD - 0.44CD$$

$$\text{Enantioselectivity} = 84.13 + 32.67A + 21.58B - 27.67C$$
$$-29.08D + 58.75AB - 47.13AC - 70.25AD - 56.50BC \quad (5)$$
$$-53.88BD + 45.25CD$$

Table 2. ANOVA table for response variables[a].

| Source of variation	Conversion (%)		Enantioselectivity		Initial rate (M·min^{-1})	
	Sum of squares	p value	Sum of squares	p value	Sum of squares	p value
Linear	501.50	<0.0001	75461.7	0.5015	5.891×10^{-5}	<0.0001
2 Factor Interaction	27.88	0.5573	3.00×10^5	0.0114	3.879×10^{-6}	0.6028
Quadratic	62.51	0.0065	14606.30	0.9148	1.584×10^{-6}	0.7963
Cubic	35.00	0.0480	1.149×10^5	0.5953	1.225×10^{-5}	0.0230
Residual	8.08		1.191×10^5		2.121×10^{-6}	
Lack of fit	41.08		2.144×10^5		1.924×10^{-5}	
pure error	2.00		34139.33		5.950×10^{-7}	

[a]Reaction tme: 6 h, 2-octanol: 0.015 mol limiting reactant, volume: 25 cm^3.

$$\text{Initial rate} = +5.333 \times 10^{-3} + 3.750 \times 10^{-4} A + 7.917 \times 10^{-4} B - 4.167 \times 10^{-5} C + 1.458 \times 10^{-3} D \quad (6)$$

ANOVA was performed for the model fitted to the experimental data. The mean squares, F values and p values for the response surface models are given in **Table 3**. Low p-value indicates that the model term is significantly affecting the process. If it is a single order term, it indicates that process parameter is significantly affecting whereas if it is a second order term, it shows that the interaction between the process parameters is significant. Temperature, catalyst loading and mole ratio are the significantly affecting parameters for conversion; whereas for the initial rate, catalyst loading and mole ratio are the significantly affecting parameters. Temperature and mole ratio also show interaction amongst them to affect enantioselectivity. For initial rate, there were no interacting parameters as the model fitted to these responses was a linear model.

The lack of fit test is a measure of failure of a model to represent data in the experimental domain at which points were not included in the regression [45]. The analysis of lack of fit was performed on all the dependent variables and it was insignificant for all the models. Correlation regression coefficients greater than 0.9 for conversion showed that models gave satisfactory predicttion for experimental data; whereas for enantioselectivity and initial rate correlation regression coefficients are less than 0.9 which showed no model could gave satisfactory prediction for experimental data (**Table 3**).

The second order polynomial equations were used to generate surface response plots and then finally to arrive at the optimum reaction conditions to maximize conversion and enantiomeric excess. Response surface and contour plots were generated for interacting parameters for each response. **Figure 2** shows the surface response plot for enantioselectivity, as a function of the interacting parameters, i.e. temperature and mole ratio. Temperature and mole ratio were investigated in the range of 10˚C - 70˚C and 1:1 - 5:1, respectively, at a catalyst loading of 0.06 g and speed of agitation at 300 rpm. At a molar ratio of 2:1, enantioselectivity increased with an increase in
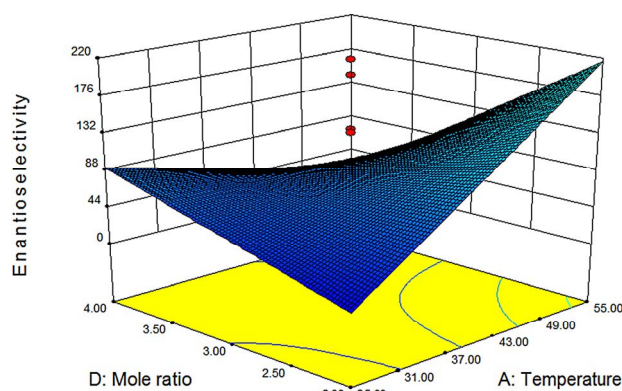
Figure 2. Effect of temperature, mole ratio and their mutual interaction on enantioselectivity.

temperature; however, as the mole ratio was increased, there was a decrease in enantioselectivity at higher temperatures. **Figure 3** shows the response surface plot for conversion, as a function of catalyst loading and temperature. Catalyst loading and temperature were investigated in the range of 1:1 - 5:1 and 10˚C - 70˚C, respectively. As the temperature and catalyst loading were increased, the conversion increased. **Figure 3** shows that at higher temperature, as the catalyst loading increased, conversion was increased.

A plot of distribution of residuals values, defined as the difference between calculated and observed values over the predicted values, shows that the quality of fit is good because the distribution does not follow the trend with respect to the predicted values. An optimum resolution reaction for (±)-2-octanol represents conditions which would give high enantiomeric excess, high enantioselectivity, higher initial rate and 50 % conversion. Numerical tools provided by Design Expert were used to find out the optimum conditions. The optimum reaction conditions thus obtained for the desired isomer, (R)-2-octanol, were, mole ratio of vinyl acetate: (±)-2-octanol of 4:1, temperature of 25˚C, 0.05 g of catalyst loading and 400 rpm as speed of agitation with conversion: 43.1%, ee of remaining enantiomer: 71.8%, enantioselectivity: 203 and Initial rate: 0.0060 M·min^{-1}.

Table 3. ANOVA for Response Surface Models[a].

Source	Conversion (%)			Enantioselectivity			Initial rate (M·min^{-1})		
	Mean Square	F value	p value	Mean Square	F Value	p value	Mean Square	F Value	p value
Model	42.28	14.72	<0.0001[b]	37545.72	2.87	0.023	1.47×10^{-5}	18.57	<0.0001[b]
$\beta 1$	108.37	37.73	<0.0001[b]	25610.67	1.96	0.178	3.60×10^{-6}	4.54	0.0431[c]
$\beta 2$	51.04	17.77	0.0007[c]	11180.17	0.85	0.367	1.55×10^{-5}	19.57	0.0002[c]
$\beta 3$	12.04	4.19	0.0585	18370.67	1.40	0.251	4.17×10^{-10}	5.25×10^{-4}	0.9819
$\beta 4$	330.04	114.9	<0.0001[b]	20300.17	1.55	0.228	3.98×10^{-5}	50.16	<0.0001[b]
$\beta 11$	1.07	0.37	0.5500	-	-	-	-	-	-
$\beta 22$	30.36	10.57	0.0054	-	-	-	-	-	-
$\beta 33$	8.36	2.91	0.1086	-	-	-	-	-	-
$\beta 44$	30.36	10.57	0.0054	-	-	-	-	-	-
$\beta 12$	3.06	1.07	0.3182	55225.00	4.22	0.054	-	-	-
$\beta 23$	3.06	1.07	0.3182	51076.00	3.90	0.063	-	-	-
$\beta 14$	0.56	0.20	0.6644	78961.00	6.04	0.024	-	-	-
$\beta 34$	3.06	1.07	0.3182	32761.00	2.50	0.130	-	-	-
$\beta 13$	7.56	2.63	0.1255	35532.25	2.72	0.116	-	-	-
$\beta 24$	10.56	3.68	0.0744	46440.25	3.55	0.075	-	-	-
R^2	0.93			0.60			0.74		

[a]Reaction time: 6 h. 2-octanol: 0.015 mol limiting reactant. Volume: 25 cm^3. β1, 2, etc. are model constants. [b]is significantly affecting at 99% level. [c]is significantly affecting at 95% level.
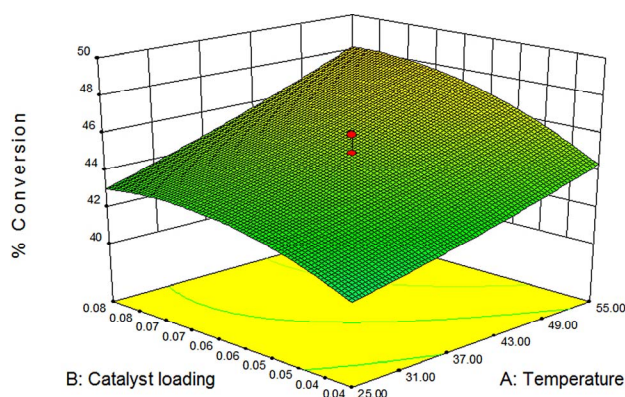
Figure 3. Effect of catalyst loading and temperature on conversion (%).

3.3. Model Validation

The validity of the predicted model was examined by carrying out additional independent experiments at the suggested optimum reaction conditions and three centre points. **Table 4** shows the predicted and observed values for the responses at optimum conditions for resolution of (±)-2-octanol using vinyl acetate as an acyl donor. The experimental values were averages of three values and were close to the predicted values indicating that the second order polynomial models generated were acceptable.

3.4. Operational Stability of Enzyme

The operational stability study was conducted under the optimum reaction conditions obtained from the RSM.

Table 4. Predicted and observed values for the response variables at optimum conditions.

Response variable	Predicted value	Experimental value ± SD
Conversion (%)	42.06	43.1
ee (%)	72.96	71.8
Enantioselectivity	234	203
Initial rate (M·min^{-1})	0.0061	0.0060

After each run, the enzyme was allowed to settle and the supernatant liquid was removed. Then, *n*-heptane was added to the solid particles, and the mixture was shaken to wash away the remaining substrate and product. The washing was carried out thrice. Then the enzyme was filtered, air dried and used for the next run. To investigate the effect of substrate on the stability of the enzyme, the reusability study was carried out under otherwise similar conditions. It was found that there was a decrease in conversion from 42% to 36% after third reuse (**Figure 4**). There was no make-up catalyst added and there was loss of catalyst of 3% - 4% during handling. Thus, the reusability of the enzyme also confirmed that acetaldehyde did not deactivate the enzyme.

3.5. Kinetic Modeling

The effect of concentration of both the reactants on the rate of reaction was investigated systematically over a wide range. For the determination of initial rates, two sets of experiments were conducted by using 0.05 g Novozyme 435 with appropriate quantities of (±)-2-octanol and vinyl acetate and the total volume was made up to 25
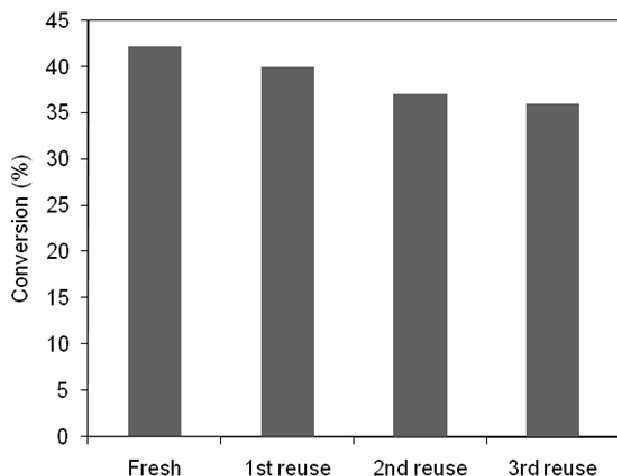
Figure 4. Reusability studies.

Figure 5. Lineweaver-Burk plot.

cm^3 with *n*-heptane. In one set of experiments, (±)-2-octanol amount was varied from 0.0075 - 0.06 mol at a fixed quantity of vinyl acetate (0.06 mol) and in another set, the amount of vinyl acetate was varied from 0.015 - 0.06 mol at a fixed quantity of (±)-2-octanol (0.015 mol). The conversions were quantified by using synthetic mixtures. The initial rates were determined from the quantified data.

When the concentration of (±)-2-octanol (A) was increased, by keeping the concentration of vinyl acetate (B) constant, the initial rate of reaction (r_0) increased proportionally and reached a maximum at a critical concentration. A subsequent increase in 2-octanol concentration immediately led to a decrease in the initial rate. Increasing concentrations of vinyl acetate under otherwise similar conditions increased the rate and conversion. There was no evidence of inhibition by vinyl acetate (B) at all the concentration tested. The Lineweaver-Burk plot of $1/r_0$ versus $1/[A]$ showed that at lower concentration of (±)-2-octanol, there was an increase in initial rates (**Figure 5**). Increase in the (±)-2-octanol concentration resulted in decrease in initial rates. It suggested that (±)-2-octanol acts as a dead-end inhibitor of enzyme whereas vinyl acetate does not inhibit the reaction.

In the case of lipase-catalyzed reactions, it has been established that the lipase first forms an acyl-enzyme complex with the acyl donor, ruling out the random mechanism [46]. As a consequence, it can only be the ordered bi-bi mechanism. Considering it as bi-bi reaction, two models were proposed, namely, the ternary complex mechanism with inhibition by (±)-2-octanol, and the ping-pong bi-bi mechanism with inhibition by (±)-2-octanol. The synthesis of isoamyl acetate by transesterification of ethyl acetate with isoamyl alcohol in *n*-hexane using lipozyme for which they had found a ping-pong bi-bi mechanism with competitive inhibition by substrates and product ethanol [47]. Since there was no reverse
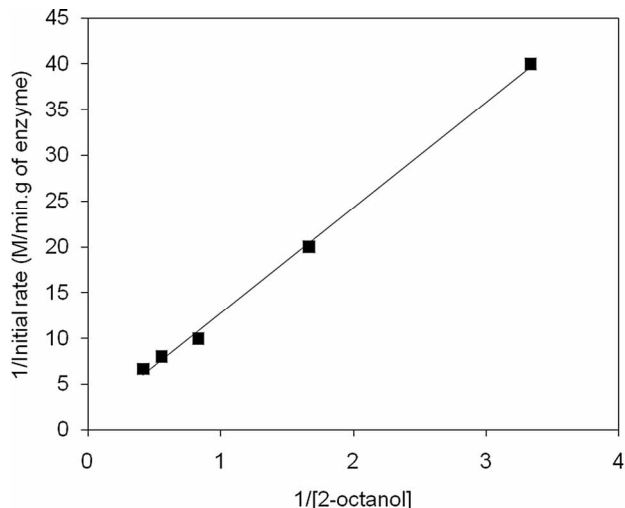
reaction in the current case, a possible inhibition by vinyl acetate at higher concentration was also considered whereby ping-pong bi-bi mechanism with inhibition by both (±)-2-octanol and vinyl acetate was also considered. The rate equation for ping-pong bi-bi mechanism with inhibition by (±)-2-octanol, for initial conditions [48], is as follows:

$$r = \frac{r_{\max}[A][B]}{K_{mA}[B]\left(1 + \dfrac{[B]}{K_{iA}}\right) + K_{mB}[A] + [A][B]} \quad (7)$$

The rate equation for ping-pong bi-bi mechanism with inhibition by (±)-2-octanol and vinyl acetate is as follows:

$$r = \frac{r_{\max}[A][B]}{K_{mA}[B]\left(1 + \dfrac{[B]}{K_{iB}}\right) + K_{mB}[A]\left(1 + \dfrac{[A]}{K_{iA}}\right) + [A][B]} \quad (8)$$

The rate equation for the ternary complex mechanism, for initial conditions, is as follows:

$$r = \frac{r_{\max}[A][B]}{K_{iA}K_{mB} + K_{mA}[B] + K_{mB}[A] + [A][B]} \quad (9)$$

where, r is the rate of reaction, $r_{\max}$, maximum rate of reaction, $[A]$, initial concentration of (±)-2-octanol, $[B]$, initial concentration of vinyl acetate, K_{mA}, Michaelis constant for (±)-2-octanol, K_{mB}, Michaelis constant for vinyl acetate, K_{iA}, inhibition constant for (±)-2-octanol, and K_{iB} is the inhibition constant for vinyl acetate.

Initial rates were calculated from the linear portion of the concentration-time profiles and the kinetic constants were obtained by non-linear regression analysis for the above models (**Table 5**). It is observed that the sum of the squared residuals was minimum in the case of ternary complex model with inhibition by (±)-2-octanol above

Table 5. Kinetic parameters for kinetic resolution of 2-octanol[a].

| Kinetic parameter	Ternary complex mechanism	Ping-pong mechanism with substrate inhibition	Ping-pong bi-bi model with both substrate inhibition
r_{max} (M·min^{-1}·g·enzyme^{-1})	1.16	0.18	0.17
K_{mA} (M·g·enzyme^{-1})	9.97	0.14	0.08
K_{mB} (M·g·enzyme^{-1})	0.87	−1.09	−0.08
K_{iA} (M·g·enzyme^{-1})	9.98	0.22	−66.83
SSE	2.56×10^{-11}	7.75×10^{-5}	1×10^{9}

[a]A: 2-octanol, B: vinyl acetate.

certain concentration. In the other two cases, some of the estimated parameters were found to be negative and unrealistic. It is thus concluded that the reaction sequence follows the ternary complex mechanism with inhibition by (±)-2-octanol. The sequence is as follows:

According to the ordered bi-bi mechanism, the acyl donor (B) first binds with the enzyme and forms an acyl-enzyme complex (EB). The second reactant (A) then combines with (EB) to form ternary complex EBA. This ternary complex then isomerizes to another ternary complex, which releases the first product vinyl alcohol. This vinyl alcohol is highly unstable and therefore it irreversibly tautomerizes to acetaldehyde and the binary complex of (±)-2-octanol and enzyme which subsequently releases (±)-2-octyl acetate. However, at high concentrations of (±)-2-octanol the dead-end binary complex between (±)-2-octanol and enzyme is formed instead of vinyl acetate and enzyme. The reaction mechanism may be depicted in **Scheme 2**.

Where E, enzyme; A, (±)-2-octanol; B, vinyl acetate; EA, enzyme-(±)-2-octanol dead-end complex; Q, acetaldehyde; P, (±)-2-octyl acetate; EBA, ternary complex; and EPQ is the isomer of EBA. The theoretical (simulated) initial rates were calculated by using the parameters in **Table 5** for the ternary model and are compared against the experimental values for different (±)-2-octanol concentrations (**Figure 6**). There is an excellent match between theory and experiment, proving the validity of the ternary model.

4. CONCLUSION

In the present study, three commercial lipases, *Candida antartica* lipase B (Novozyme 435), *Thermomyces lanuginosus* and *Rhizomucor meihei*, were employed for the kinetic resolution of (±)-2-octanol by using vinyl acetate as an acylating agent. The process of synthesis of (R)-2-octyl acetate using immobilized lipase, Novozyme 435 was optimized applying RSM with CCRD. Second order polynomial equations have been obtained for the conversion of alcohol, enantioselectivity of enzyme and initial rate of reaction. It was possible to predict the reac-
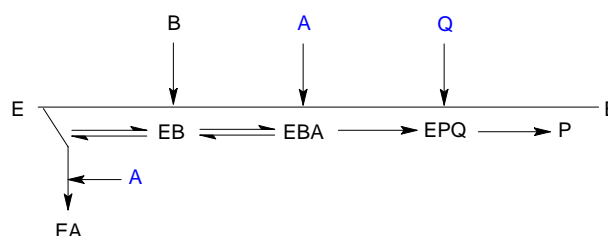
Scheme 2. Ternary complex mechanism with inhibition by A.
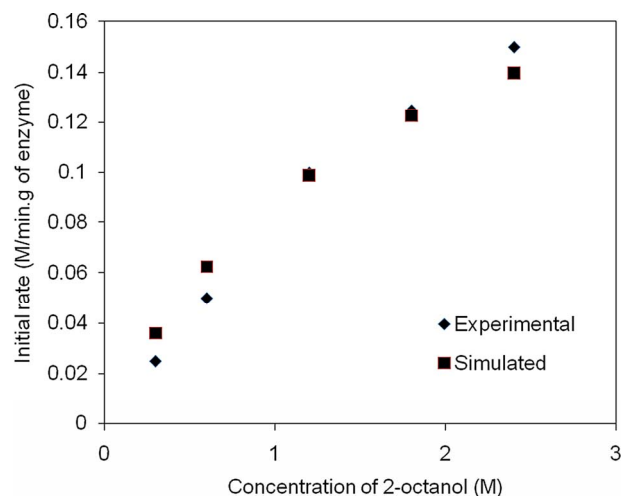
Figure 6. Parity plot.

tion conditions required to obtain well-defined amount of acetate, enantiomeric excess of remaining alcohol, enantioselectivity of enzyme and initial rate of reaction. These models are useful to determine the optimum operating conditions for the resolution reaction using the minimal number of experiments with the consequent economical benefit. The analysis of the kinetic data showed that the acylation of (±)-2-octanol with vinyl acetate catalyzed by Novozyme 435 follows the ternary complex mechanism (ordered bi-bi mechanism) with 2-octanol inhibition providing support for one of the two proposed mechanisms. The optimum reaction conditions thus obtained for the desired isomer, (R)-2-octanol, were, mole ratio of vinyl acetate: (±)-2-octanol of 4:1, temperature of 25°C, 0.05 g of catalyst loading and 400 rpm as speed of agitation

with conversion, 43.1%; ee, 71.8%; enantioselectivity, 203; and Initial rate, 0.0060 M·min^{-1}.

5. ACKNOWLEDGEMENTS

REFERENCES

[1] Turner, N.J. (2003) Controlling chirality. *Current Opinion in Biotechnology*, **14**, 401-406. doi:10.1016/S0958-1669(03)00093-4

[2] Huisman, G. and Gray, D. (2002) Towards novel processes for the fine chemical and pharmaceutical industries. *Current Opinion in Biotechnology*, **13**, 352-358. doi:10.1016/S0958-1669(02)00335-X

[3] Patel, R.N. (2001) Biocatalytic synthesis of intermediates for the synthesis of chiral drug substances. *Current Opinion in Biotechnology*, **12**, 587-604. doi:10.1016/S0958-1669(01)00266-X

[4] Loughlin, W.A. (2000) Biotransformations in organic synthesis. *Bioresource Technology*, **74**, 49-62. doi:10.1016/S0960-8524(99)00145-5

[5] Yadav, G.D. and Devi, K.M. (2002) Enzymatic synthesis of perlauric acid using Novozym 435. *Biochemical Engineering Journal*, **10**, 93-101. doi:10.1016/S1369-703X(01)00164-4

[6] Yadav, G.D. and Borkar, I.V. (2006) Kinetic modeling of microwave assisted chemo-enzymatic epoxidation of styrene to styrene oxide. *American Institute of Chemical Engineers Journal*, **52**, 1235-1247. doi:10.1002/aic.10700

[7] Yadav, G.D. and Devi, K.M. (2004) Kinetics of hydrolysis of tetrahydrofurfuryl butyrate in a three phase system containing immobilized lipase from *Candida antartica*. *Biochemical Engineering Journal*, **17**, 57-63. doi:10.1016/S1369-703X(03)00125-6

[8] Yadav, G.D. and Devi, K.M. (2004) Immobilized lipase-catalyzed esterification and transesterification reactions in non-aqueous media for synthesis of tetrahydrofurfuryl butyrate: Comparison and kinetic modelling. *Chemical Engineering Science*, **59**, 373-383. doi:10.1016/j.ces.2003.09.034

[9] Yadav, G.D. and Lathi, P.S. (2006) Intensification of enzymatic synthesis of propylene glycol monolaurate from 1,2-propanediol and lauric acid under microwave irradiation: Kinetics of forward and reverse reactions, *Enzyme and Microbial Technology*, **38**, 814-820. doi:10.1016/j.enzmictec.2005.08.013

[10] Yadav, G.D. and Dhoot, S.B. (2009) Immobilized lipase-catalysed synthesis of cinnamyl laurate in non-aqueous media. *Journal of Molecular Catalysis B*: *Enzymatic*, **57**, 34-39. doi:10.1016/j.molcatb.2008.06.013

[11] Yadav, G.D. and Jadhav, S.R. (2005) Synthesis of reusable lipases by immobilization on hexagonal mesoporous silica and encapsulation in calcium alginate: Transesterification in non-aqueous medium. *Microporous and Mesoporous Materials*, **86**, 215-222. doi:10.1016/j.micromeso.2005.07.018

[12] Yadav, G.D. and Devendran, S. (2012) Lipase catalyzed synthesis of cinnamyl acetate via transesterification in non-aqueous medium. *Process Biochemistry*, **47**, 496-502. doi:10.1016/j.procbio.2011.12.008

[13] Yadav, G.D. and Borkar, I.V. (2008) Kinetic modeling of immobilized lipase catalysis in synthesis of *n*-butyl levulinate. *Industrial and Engineering Chemistry Research*, **47**, 3358-3363. doi:10.1021/ie800193f

[14] Yadav, G.D. and Borkar, I.V. (2009) Novelties of synthesis of n-butyl acetamide over immobilized lipase. *Journal of Chemical Technology and Biotechnology*, **84**, 420-426. doi:10.1002/jctb.2056

[15] Yadav, G.D. and Borkar, I.V. (2010) Lipase-catalyzed hydrazinolysis of phenyl benzoate: Kinetic modeling approach. *Process Biochemistry*, **45**, 586-592. doi:10.1016/j.procbio.2009.12.005

[16] Yadav, G.D. and Borkar, I.V. (2009) Kinetic and mechanistic investigation of microwave-assisted lipase catalyzed synthesis of citronellyl acetate. *Industrial and Engineering Chemistry Research*, **48**, 7915-7922. doi:10.1021/ie800591c

[17] Yadav, G.D., Dhoot, S.B. and Sajgure, A.D. (2008) Insight into microwave irradiation and enzyme catalysis in enantioselective resolution of RS-(±)methyl mandelate. *Journal of Chemical Technology and Biotechnology*, **83**, 1145-1153. doi:10.1002/jctb.1975

[18] Yadav, G.D. and Thorat, P.A. (2012) Microwave assisted lipase catalyzed synthesis of isoamyl myristate in solvent-free system. *Journal of Molecular Catalysis B*: *Enzymatic*, **83**, 16-22. doi:10.1016/j.molcatb.2012.06.011

[19] Yadav, G.D. and Shinde, S.D. (2012) Synergism of microwave irradiation and immobilized lipase catalysis in synthesis of 4,8-dimethylnon-7-en-1yl (2E)-3-phenylpro-2-enolate. *International Reviews in Chemical Engineering*, **4**, 589-596.

[20] Yadav, G.D. and Pawar, S.V. (2012) Synergism between microwave irradiation and enzyme catalysis in trans-esterification of ethyl-3-phenylpropanoate withn-butanol. *Bioresource Technology*, **109**, 1-6. doi:10.1016/j.biortech.2012.01.030

[21] Yadav, G.D., Sajgure, A.D. and Dhoot, S.D. (2007) Enzyme catalysis in fine chemical and pharmaceutical industries. In: Bhattacharya, S.K., Ed., *Enzyme Mixtures and Complex Biosynthesis*, Landes Biosciences, Austin.

[22] Riermeier, T.H., Gross, P., Monsees, A., Hoff, M. and Trauthwein, H. (2005) Dynamic kinetic resolution of secondary alcohols with a readily available ruthenium-based racemization catalyst. *Tetrahedron Letters*, **46**, 3403-3406. doi:10.1016/j.tetlet.2005.03.074

[23] Cong, F.D., Wang, Y.H., Ma, C.Y., Yub, H.F., Han, S.P., Tao, J. and Cao, S.G. (2005) A way for resolution of (*R*, *S*)-2-octanol by combining dynamic kinetic resolution with double kinetic resolution. *Enzyme and Microbial Technology*, **36**, 595-599. doi:10.1016/j.enzmictec.2004.12.009

[24] Yu, D., Chen, P., Wang, L., Gu, Q., Li, Y., Wang, Z. and Cao, S. (2007) A chemo-enzymatic process for sequential kinetic resolution of (R,S)-2-octanol under microwave irradiation. *Process Biochemistry*, **42**, 1312-1318. doi:10.1016/j.procbio.2007.06.011

[25] Wang, Y., Wang, R., Li, Q., Zhanga, Z. and Feng, Y. (2009) Kinetic resolution of *rac*-alkyl alcohols *via* lipase-catalyzed enantioselective acylation using succinic anhydride as acylating agent. *Journal of Molecular Catalysis B*: *Enzymatic*, **56**, 142-145. doi:10.1016/j.molcatb.2008.02.002

[26] Xun, E.N., Lv, X.L., Kang, W., Wang, J.X., Zhang, H., Wang, L. and Wang, Z. (2012) Immobilization of pseudomonas fluorescens lipase onto magnetic nanoparticles for resolution of 2-octanol. *Applied Biochemistry Biotechnology*, **168**, 697-707. doi:10.1007/s12010-012-9810-9

[27] Ren, L., Xu, T., He, R., Jiang, Z., Zhou, H. and Wei, P. (2013) A green resolution-separation process for aliphatic secondary alcohols. *Tetrahedron*: *Asymmetry*, **24**, 249-253. doi:10.1016/j.tetasy.2013.01.018

[28] Zhao, L.F. and Zheng, L.Y. (2011) Resolution of 2-octanol via immobilized Pseudomonas sp. lipase in organic medium. *Biocatalysis and Biotransforamtion*, **29**, 47-53. doi:10.3109/10242422.2010.551189

[29] Yu, D., Ma, D., Wang, Z., Wang, Y., Pan, Y. and Fang, X. (2012) Microwave-assisted enzymatic resolution of (R,S)-2-octanol in ionic liquid. *Process Biochemistry*, **47**, 479-484. doi:10.1016/j.procbio.2011.12.007

[30] Wang, Y., Li, Q., Zhang, Z., Ma, J. and Feng, Y. (2009) Solvent effects on the enantioselectivity of the thermophilic lipase QLM in the resolution of (*R,S*)-2-octanol and (*R,S*)-2-pentanol. *Journal of Molecular Catalysis B*: *Enzymatic*, **56**, 146-150. doi:10.1016/j.molcatb.2008.01.010

[31] Ursoiu, A., Ungurean, M., Paul, C. and Peter, F. (2010) Optimization of 2-octanol kinetic resolution by selection of solgel immobilization precursors and reaction parameters. *Journal of Biotechnology*, **150S**, S1-S576. doi:10.1016/j.jbiotec.2010.09.493

[32] Bas, D. and Boyaci, I.H. (2007) Modeling and optimization I: Usability of response surface methodology. *Journal of Food Engineering*, **78**, 836-845. doi:10.1016/j.jfoodeng.2005.11.024

[33] Soto-Cruz, O., Saucedo-Castañeda, G., Pablos-Hach, J.L., Gutiérrez-Rojas, M. and Favela-Torres, E. (1999) Effect of substrate composition on the mycelial growth of *Pleurotus ostreatus*. An analysis by mixture and response surface Methodologies *Process Biochemistry*, **35**, 127-133. doi:10.1016/S0032-9592(99)00043-6

[34] Diniz, F.M. and Martin, A.M. (1996) Use of response surface methodology to describe the combined effects of temperature and E:S ratio on the hydrolysis of dogfish (*Squalus acanthias*) muscle. *International Journal of Food Science and Technology*, **31**, 419-426. doi:10.1046/j.1365-2621.1996.00351.x

[35] Guinard, J.X., Zoumas-Morse, C., Mori, L., Panyam, D. and Kilara, A. (1996) Effect of sugar and fat on the acceptability of vanilla ice cream. *Journal of Dairy Science and Technology*, **79**, 1922-1927.

[36] Hwang, S. and Hancen, C.L. (1997) Modeling and optimization in anaerobic bioconversion of complex substrates to acetic and butyric acids. *Biotechnology and Bioengineering*, **54**, 451-460. doi:10.1002/(SICI)1097-0290(19970605)54:5<451::AID-BIT5>3.0.CO;2-D

[37] Rastogi, N.K., Rajesh, G. and Shamala, T.R. (1998) Optimization of enzymatic degradation of coconut residue. *Journal of Science Food Agriculture*, **76**, 129-134. doi:10.1002/(SICI)1097-0010(199801)76:1<129::AID-JSFA909>3.0.CO;2-C

[38] Gokhale, S.V. and Lele, S.S. (2012) Optimization of convective dehydration of *Beta vulgaris* for color retention. *Food and Bioprocess Technology*, **5**, 868-878.

[39] Mahajan, P.M., Gokhale, S.V. and Lele, S.S. (2010) Production of nattokinase using *Bacillus natto* NRRL 3666: Media optimization, scale up and kinetic modeling. *Food Science Biotechnolology*, **19**, 1593-1603. doi:10.1007/s10068-010-0226-4

[40] Sontakke, J.B. and Yadav, G.D. (2011) Optimization and kinetic modeling of lipase catalyzed enantioselective *N*-acetylation of (±)-1-phenylethylamine under microwaves irradiation. *Journal of Chemical Technology and Biotechnology*, **86**, 739-748. doi:10.1002/jctb.2582

[41] Sontakke, J.B. and Yadav, G.D. (2011) Kinetic modeling and statistical optimization of lipase catalyzed enantioselective resolution of (R,S)-2-pentanol. *Industry Engineering and Chemistry Research*, **50**, 12975-12983. doi:10.1021/ie2012032

[42] Yadav, G.D. and Sontakke, J.B. (2011) Optimization of chiral resolution of (R,S)-1-phenylethanol by statistical methods. *International Journal of Chemical Reactor Engineering*, **9**, A77, 1-15.

[43] Dai, D.Z. and Xia, L.M. (2006) Resolution of (R,S)-2-octanol by *Penicillium expansum* PED-03 lipase immobilized on modified ultrastable-Y molecular sieve in microaqueous media. *Process Biochemistry*, **41**, 1455-1460. doi:10.1016/j.procbio.2006.01.015

[44] Zhang, D.H., Bai, S., Ren, M.Y. and Sun, Y. (2008) Optimization of lipase-catalyzed enantioselective esterification of (±)-menthol in ionic liquid. *Food Chemistry*, **109**, 72-80. doi:10.1016/j.foodchem.2007.12.020

[45] Montgomery, D.C. (1984) Design and analysis of experiments. 2nd edition, John Wiley and Sons, New York.

[46] Faber, K. and Riva, S. (1992) Enzyme-catalyzed ireversible acyl transfer. *Synthesis*, **10**, 895-910. doi:10.1055/s-1992-26255

[47] Rizzi, M., Stylos, P. and Reuss, M. (1992) A kinetic study of immobilized lipase catalysing the synthesis of isoamyl acetate by transesterification in *n*-hexane. *Enzyme Microbial Technology*, **14**, 709-714. doi:10.1016/0141-0229(92)90110-A

[48] Segel, I.H. (1975) Enzyme kinetics. Wiley/Interscience, New York.