Lipase-Catalyzed Kinetic Resolution of (±)-1-(2-Furyl) Ethanol in Nonaqueous Media

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ABSTRACT S-1-(2-Furyl) ethanol serves as an important chiral building block for the preparation of various natural products, fine chemicals, and is widely used in the chemical and pharmaceutical industries. In this work, lipase-catalyzed kinetic resolution of (R/S)-1-(2-furyl) ethanol using different acyl donors was investigated. Vinyl esters are good acyl donors vis-à-vis alkyl esters for kinetic resolution. Among them, vinyl acetate was found to be the best acyl donor. Different immobilized lipases such as *Rhizomucor miehei* lipase, *Thermomyces lanuginosus* lipase, and *Candida antarctica* lipase B were evaluated for this reaction, among which *C. antarctica* lipase B, immobilized on acrylic resin (Novozym 435), was found to be the best catalyst in n-heptane as solvent. The effect of various parameters was studied in a systematic manner. Maximum conversion of 47% and enantiomeric excess of the substrate (ee_s) of 89% were obtained in 2 h using 5 mg of enzyme loading with an equimolar ratio of alcohol to vinyl acetate at 60°C at a speed of 300 rpm in a batch reactor. From the analysis of progress curve and initial rate data, it was concluded that the reaction followed the ordered bi–bi mechanism with dead-end ester inhibition. Kinetic parameters were obtained by using nonlinear regression. This process is more economical, green, and easily scalable than the chemical processes. *Chirality 00:000-000, 2014.*

KEY WORDS: 1-(2-Furyl) ethanol; *Candida antarctica* lipase B; kinetic resolution; ordered bi–bi mechanism; vinyl acetate

INTRODUCTION

The production of optically active single enantiomers has not only gained great demand in pharmaceutical industries but it is also required for the synthesis of fine chemicals in fragrance, flavor, agro, and chemical industries.[1,2] The chemical methods used for the synthesis of optically active molecules are beset with many drawbacks such as the requirement for protection and deprotection steps, sluggish reaction rates, and longer reaction time, low yield, and byproduct formations.[3] In this regard, biocatalytic processes are favored for the synthesis of enantiomeric compounds, in view of their incredible advantages such as high chemo-, stereo-, regioselectivity, high efficacy, mild temperature protocols, suppression of byproduct formation, ease of scale-up, and low cost.[4,5]

Enantiomerically pure alcohols are one of the most attractive building blocks for active pharmaceutical intermediate (API) syntheses and are also used as chiral auxiliaries. In biocatalysis, both whole cell and isolated enzymes are used as catalysts.[6] Highly pure enantiomer of secondary alcohol can be prepared by adopting different methods such as resolution of existing racemic mixtures via enzymatic kinetic resolution, reduction of prochiral compounds by asymmetric reduction using whole-cell catalysts, and C-C bond formation with lyases.[7–9] Among them, the enzymatic method has been successfully applied. Especially, lipase-catalyzed kinetic resolution has been well adapted for preparation of single isomeric compounds such as secondary alcohol, acid, and amine through esterification/transesterification and hydrolytic reactions, respectively. Lipases offer high chemo-, regio-, and stereoselectivity, accept a repertoire of compounds, function without expensive cofactors, and are commercially prepared from different species like microbes, animals, and plants.[9–11] Further, the use of organic and neoteric solvent as reaction media for the lipase-catalyzed reaction widens the scope, including greater solubility of organic molecules, enhanced stability of enzymes, and ease of recovery of product and catalyst as compared to aqueous media.[12,13]

The optically active S-enantiomer of 1-(2-furyl) ethanol is used as an important building block for the synthesis of various natural products such as flavonoids, polyketide antibiotics, and carbohydrate derivatives.[14–16] Kobayashi et al. reported the enantioselective synthesis of natural product macrosphelides A and B, which act as anti-adhesion compounds and effectively inhibit the adhesion of human leukemia HL-60 cells to human-umbilical-vein endothelial cells, using S-1-(2-furyl) ethanol as the starting precursor.[17] There are a few studies reported on the synthesis of the single enantiomer of 1-(2-furyl) ethanol by asymmetric reduction using baker's yeast, asymmetric chemical catalysts, or lipase-catalyzed kinetic resolution.[18–21] All these reported processes require a longer time and there is no information on the kinetics of the reaction, which is required for reactor design and to scale up of the process. In the current work, lipase-catalyzed transesterification of (R/S)-1-(2-furyl) ethanol with various acyl donors was conducted in nonaqueous media (Scheme 1). The effect of various parameters such as different commercially available immobilized lipases, acyl donors, solvents, agitation speed, temperature, catalyst loading, and acyl donor concentration was studied systematically by varying one parameter at a time. Finally, the mechanism

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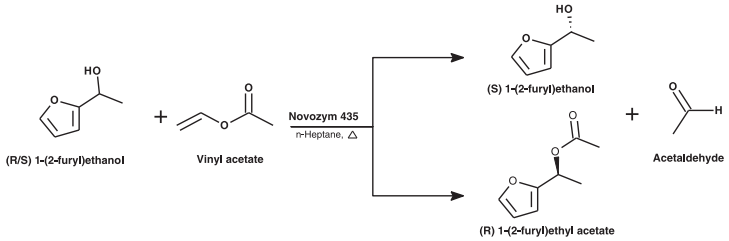


Scheme 1. Lipase-catalyzed kinetic resolution of (±)-1-(2-furyl) ethanol.

and kinetics were also developed. The results are new and have potential for scale up and industrial application.

MATERIALS AND METHODS

Enzymes

The following enzymes were received as gift samples from M/s Novozymes A/S (Bagsvaerd, Denmark): 1) Novozym 435: Lipase B from *Candida antarctica*, supported on a macroporous acrylic resin with a water content of 1–2% (w/w) and enzyme activity 10,000 PLU/g; 2) Lipozyme RM-IM: Lipase from *Rhizomucor miehei*, supported on a macroporous anion exchange resin with a water content of 2–3% (w/w) and enzyme activity 6 BAU/g; 3) Lipozyme TL IM: Lipase from *Thermomyces lanuginosus*, supported on porous silica granulates with water content 1–2% and enzyme activity 175 IU/g.

Chemicals. Iso-octane, n-heptane, toluene, cyclohexane, vinyl acetate, methyl acetate, acetic anhydride, ethyl acetate, and other analytical and high-performance liquid chromatography (HPLC)-grade reagents were purchased from M/s S.D. Fine Chemicals, Mumbai, India. (±)-1-(2-Furyl) ethanol, vinyl butyrate, and vinyl laurate were purchased from Sigma- Aldrich, Bangalore, India. All chemicals and enzymes were used without any further modification/purification.

Experimental Setup

The experimental setup consisted of a 3-cm i.d. mechanically agitated glass reactor of 50 cm^3 capacity, equipped with four baffles and a six-bladed turbine impeller. The entire reactor assembly was immersed in a thermostatic water bath, which was maintained at a predetermined temperature with an accuracy of ±1°C. A typical reaction mixture consisted of 0.001 mol racemic alcohol and 0.001 mol vinyl ester diluted to 20 cm^3 with n-heptane as a solvent. The reaction mass was agitated at 60°C for 15 min at a speed of 300 rpm and then 5 mg of enzyme was added to start the reaction.

Analysis

The reaction progress and enantiomeric excess (ee) were monitored by periodical withdrawal of clear liquid samples from the reaction mixture which were analyzed by HPLC (1260 infinity series, Agilent Technologies, Palo Alto, CA) equipped with Chiralpak- IB analytical column (250 × 4.6 mm ID) (Daicel, Japan; particle size 5 μm). Samples (10 μl) were injected via autosampler. The mobile phase consisted of n-hexane and isopropyl alcohol (99:1) and the flow rate was maintained at 0.75 $ml.min^{-1}$. A DAD detector was used at a wavelength of 230 nm. The retention time of (R) and (S) alcohol were 23.1 and 23.9 min, respectively.

The enantioselectivity ratio (E) and conversion (c,%), were calculated from the enantiomeric excess of the substrate (ee_s,%) and product (ee_p,%) based on the following equation:

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

Where,

$$c = 1 - \frac{B_{(R)} + B_{(S)}}{B_{(R_0)} + B_{(S_0)}} \tag{2}$$

$$ees = \frac{B(S) - B(R)}{B(S) + B(R)} \text{ and} \tag{3}$$

$$eep = \frac{Q(S) - Q(R)}{Q(S) + Q(R)} \tag{4}$$

where, $B_{(R_0)}, B_{(S_0)}, B_{(R)}, B_{(S)}, Q_{(R)}$ and $Q_{(S)}$ denote area under the curve of *(R)*-1-(1-furyl) ethanol, *(S)*-1-(1-furyl) ethanol, and their corresponding esters.

RESULTS AND DISCUSSION

Effect of Catalyst

Lipases from different origins such as *Candida antarctica* lipase B (Novozym 435), *Rhizomucor miehei* lipase (Lipozyme RMIM), and *Thermomyces lanuginosus* lipase (Lipozyme TLIM) were evaluated for resolution of (±)-1-(2-furyl) ethanol under similar conditions. The reactions were carried out using n-heptane as solvent. At the end of 2-h reaction time, lipase B from *C. antarctica* (Novozym 435) had given the maximum conversion compared to other immobilized catalysts. With Novozym 435, conversion of 47% was obtained compared to Lipozyme RMIM (1.8%) and Lipozyme TLIM (1.5%). The order of activity was as follows: Novozym 435 > Lipozyme RMIM Lipozyme TLIM. It is reported that both Lipozyme RMIM and Lipozyme TLIM are mainly used to interesterify bulk molecules such as fatty acid derivatives and they have less activity in the resolution of racemic molecules.[22] However, Novozym 435 is a well-known and commercially available lipase for both chiral resolution and synthesis of small molecules esters.[23] Hence, further study was carried out using Novozym 435 as catalyst.

Effect of Acyl Donor

The selection of a suitable acyl donor is primarily important for the lipase-catalyzed kinetic resolution of secondary alcohols. It has been well reported that the type of acyl donor and its chain length can influence both the reaction rate and enantioselectivity of enzyme in transesterification/interesterification reactions.[24–27] In order to examine the effect of acyl donor on kinetic resolution of (±) 1-(2-furyl) ethanol, initially we had taken different alkyl and vinyl esters such as acetic anhydride, methyl acetate, ethyl acetate, and vinyl acetate as model acylating agents (Fig. 1). The reactions were performed under similar conditions. At the end of 2 h, it was observed that vinyl acetate gave higher conversion (47%) and enantiomeric purity (89% ee) with E > 200 compared to the other alkyl esters. Among alkyl esters, acetic anhydride had given good conversion (39%) and enantiomeric purity (65% ee) than other esters. It has been reported that vinyl esters are better acylating agents than alkyl esters.[28] The liberated coproduct is vinyl alcohol (enol form), which is unstable and easily tautomerized to acetaldehyde (aldehyde form) and it is no longer a substrate for lipase. So the reaction equilibrium shifts to the forward direction, which leads to the formation of the chiral ester. Further, the effect of the acyl chain length was evaluated using three different vinyl esters such as vinyl acetate, butyrate, and laurate. Increasing the chain length led to a decrease in

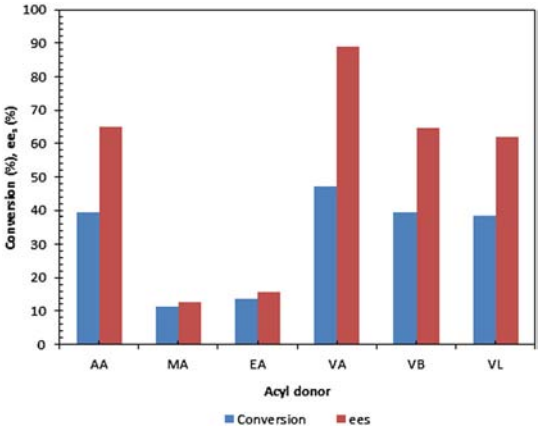


Fig. 1. Effect of acyl donor. Reaction condition: 1-(2-furyl) ethanol, 0.001 mol; acyl donor, 0.001 mol; n-heptane up to 20 cm^3; speed of agitation, 300 rpm; catalyst loading, 5 mg; temperature, 60°C, ■ee$_s$, ■Conversion, AA, acetic anhydride; EA, ethyl acetate; MA, methyl acetate; VA, vinyl acetate; VB, vinyl butyrate, VL, vinyl laurate.

enantioselectivity and conversion. The highest conversion (47%) with ee$_s$ of 89% was obtained with vinyl acetate as the acyl donor, whereas low conversion (38%) with ee$_s$ of 62% was obtained with vinyl laurate. Hence, further study was carried out using vinyl acetate as the acyl donor.

Effect of Organic Media

Solvent plays a significant role in any enzymatic reaction. The important criteria for proper choice of organic media are substrate solubility, product recovery, and enzyme stability.[29] It has been reported in the literature that solvent can alter the enzyme activity, reaction rate, and enantioselectivity by modifying the aqueous layer around the enzyme particle or changing the enzyme conformational structure.[30] Several experiments were performed to evaluate the effect of chosen solvents such as iso-octane (logP – 4.5), n-heptane (logP – 4), cyclohexane (logP – 3.2) and toluene (logP –2.5) on transesterification reaction (Fig. 2). The experiments were carried out using 1:1 mole ratio of alcohol to ester (each 1 mmol), with 5 mg enzyme loading at 60° C and the liquid phase volume was made up to 20 cm^3. Both conversion and enantioselectivity were found to increase as logP value decreased from -2.5 to -4 (toluene to n-heptane). The highest conversion (47%) and ee$_s$ (89%) were obtained when n-heptane was employed as solvent, whereas low conversion (22%) and ee$_s$ (29%) were obtained with toluene. Due to the low solubility of racemic alcohol in iso-octane, moderate conversion and enantiomeric purity were observed. This clearly correlated with the reported literature that solvents having high-log P are hydrophobic in nature but do not strip the water layer around enzyme particle, which is essential for maintaining the conformation structure.[31] Thus, they show high enzyme activity. Further work was carried out using n-heptane as the solvent.

Effect of Speed of Agitation

In immobilized catalysts, reactants have to pass from the bulk liquid phase to the enzyme particle's surface, and then diffuse from the external surface to the enzyme active site. The external mass transfer resistance and intraparticle diffusion rate play significant roles during the reaction. It must be overcome to study the intrinsic kinetics of lipase-catalyzed transesterification reaction. Several experiments were performed in the range of 100 to 400 rpm by taking 0.001 mol racemic alcohol and vinyl acetate each at 60° C with 5 mg Novozym 435 and volume made up to 20 cm^3 using n-heptane as solvent. From the progress curve (Fig. 3), the conversion is seen to increase with increase in agitation speed from 100 to 300 rpm and beyond this value; there was no significant difference in conversion. This implies that there is no external mass transfer resistance.

The values of solid-liquid mass transfer coefficients for both reactants with n-heptane as solvent were calculated using the correlations developed for polymer supported catalyst which took into account the effects of the Reynolds and Schmidt numbers.

$$\frac{k_{SL}d_P}{D_e\psi} = 2 + 0.4\left(\frac{ed_p^4\rho_L^3}{\mu^3{}_L}\right)^{1/4}\left(\frac{\mu_L}{\rho_L D_e}\right)^{1/3} \quad (5)$$

Where, $e = \frac{P}{\rho_L V_L}$ = Power consumption and $P = N_P N^3 D_I^5 \rho_L$, N_P, the power number was calculated from the correlation for the impeller used in this work. Diffusivity of racemic alcohol (D_e) at 60° C was calculated using the Wilke-Chang equation as 4.837×10^{-5} $cm^2\,s^{-1}$.[32] The effective diffusivity is given by

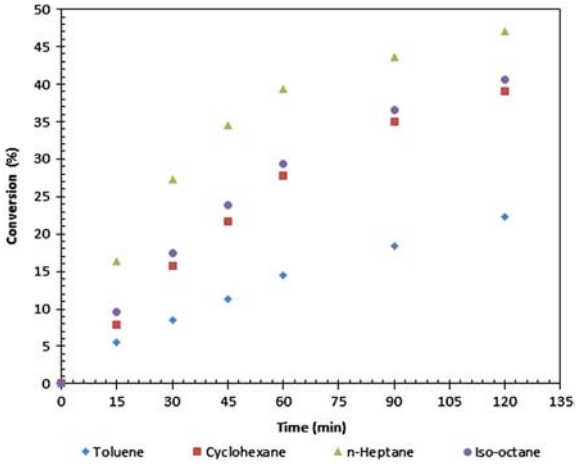


Fig. 2. Effect of solvent. Reaction condition: 1-(2-furyl) ethanol, 0.001; vinyl acetate, 0.001 mol; solvent up to 20 cm^3; speed of agitation, 300 rpm; catalyst loading, 5 mg; temperature, 60°C, ◆Toluene, ■Cyclohexane, ▲n-Heptane ×Iso-octane.

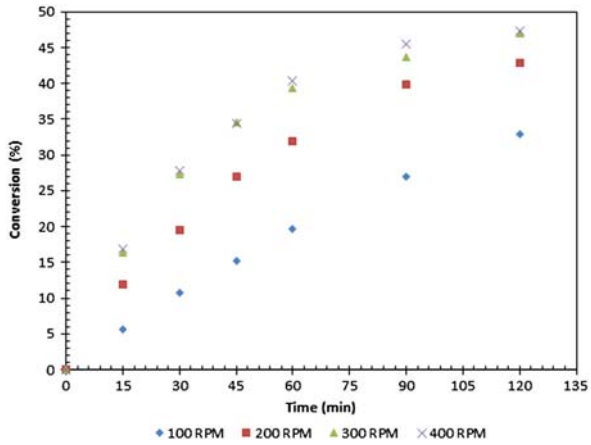


Fig. 3. Effect of agitation speed. Reaction condition: 1-(2-furyl) ethanol, 0.001; vinyl acetate, 0.001 mol; n-heptane up to 20 cm^3, 100–400 catalyst loading, 5 mg; temperature, 60°C, ◆100 RPM, ■200 RPM, ▲300 RPM ×400 RPM.

$De = \frac{D\varepsilon}{\tau}$ where the porosity (ε) and tortuoisity (τ) were taken as conservative values of 0.38 and 3, respectively. The average diameter of the support particle (D_p) was taken as 0.06 cm, since the particle size ranged between 0.03 and 0.09 cm. The value of mass transfer coefficient of liquid phase (k_{SL}) was calculated as 5.2×10^{-3} cm.s^{-1} by using a standard correlation for organic phase.[31] From this value, the calculated Sherwood number $Sh = \frac{k_{SL}D_p}{D}$ was 640, indicating that there was no external mass transfer resistance above 300 rpm.[33]

Further, it is necessary to rule out the intraparticle diffusion. This could be done by assessing the Thiele modulus (φ), which is defined as the ratio of reaction rate to internal diffusion rate. If the Thiele's modulus value is less than 0.3, then the effectiveness factor of the reaction approaches unity. Then it can be concluded that the reaction rate would be kinetically controlled and internal diffusion effect can be neglected.[33] Thiele modulus can be estimated using the following equation.

$$\phi = \frac{r(C_0)}{D_{eff}C_0}\left(\frac{R}{3}\right)^2 \quad (6)$$

Both C_0 and r (C_0) were determined experimentally and their values were 0.05 mol.dm^{-3} and 9.833×10^{-6} mol.dm^{-3}.s^{-1}. The effective diffusion of racemic alcohol is 6.127×10^{-6}. From these values, the Thiele modulus of the reaction is 3.21×10^{-3} and this value is less than 0.3, suggesting that there was no intraparticle diffusion limitation. Thus, the reaction was controlled by intrinsic enzyme kinetics. Therefore, further experiments were performed at a speed of 300 rpm.

Effect of Temperature

Temperature is one of the important parameters that affects the reaction rate and enantioselectivity in lipase-catalyzed kinetic resolution of secondary alcohols.[34,35] In order to study the effect of temperature, a number of experiments was performed in the range of 40°C to 70°C under similar conditions (Fig. 4). It was observed that reaction rate, enantioselectivity, and conversion increased with an increase in temperature up to 60°C. Above this value, there was no significant change in conversion. It has been reported that the enzyme is thermally stable at 60°C.[9] This would further confirm the previous finding that the reaction is intrinsically kinetically controlled. The elevated temperature reduces the viscosity of the reaction mixture and increases the collusion frequency between reactants and enzyme particles that lead to increase in reaction rate. Both conversion and enantioselectivity were found to be maximum at 60°C and also maintained the enzyme activity. Therefore, the temperature of 60°C was selected as the optimum and, further experiments were conducted at 60°C. The Arrhenius plot was made on the basis of ln(initial rates) vs. the reciprocal of temperature (Fig. 5). The apparent activation energy was calculated from the observed initial rates at different temperatures under otherwise similar conditions. The activation energy was found to be 8.57 kcal/mol. This value lies within the typical value for an enzyme catalytic reaction.[36]

Effect of Catalyst Loading

The addition of enzyme loading has a considerable effect on reaction rate and enantioselectivity.[12] In order to study the effect of catalyst loading, several experiments were performed by keeping the mole ratio constant while enzyme loading was changed in the range from 1.75×10^{-4} to 4×10^{-4} g.cm^{-3} under similar reaction conditions (Fig. 6). It was observed that the reaction rate had a linear dependence on enzyme loading. The overall conversion was increased with increase in enzyme loading from 1.75×10^{-4} to 2.5×10^{-4} g.cm^{-3}. Above this value, there was no significant change in substrate conversion. The increase of enzyme loading increases the number of active sites proportionately and facilitates the interaction between enzyme active sites and reactant that further enhance the reaction rate and conversion. However, at high enzyme concentration, the available enzyme sites are much larger than the required amount for reaction and it will not further improve the conversion. Thus, 2.5×10^{-4} g.cm^{-3} was taken as optimum and further studies were carried out with this loading.

Effect of Acyl Donor Concentration

In order to study the effect of acyl donor concentration on reaction rate and conversion of kinetic resolution of (±)-1-(2-furyl) ethanol, a number of experiments were performed at 60°C with 5 mg of enzyme loading in n-heptane as solvent. The concentration of (±)-1-(2-furyl) ethanol was kept constant (0.001 mol), and vinyl acetate concentration was varied from

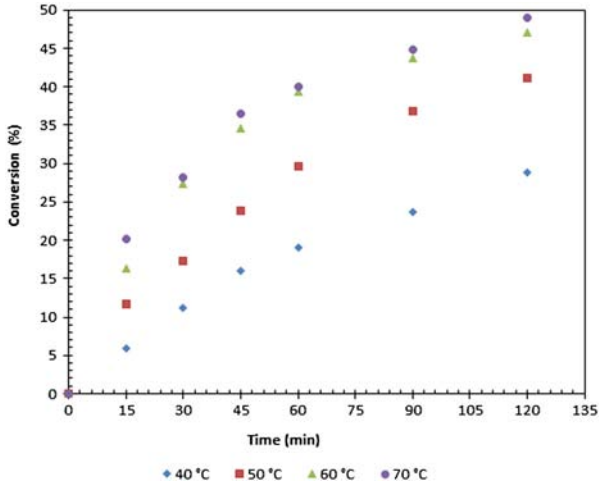


Fig. 4. Effect of temperature. Reaction condition: 1-(2-furyl) ethanol, 0.001; vinyl acetate, 0.001 mol; n-heptane up to 20 cm^3; speed of agitation, 300 rpm; catalyst loading, 5 mg; temperature, 40–70°C, ◆40°C, ■50°C, ▲60°C,×70°C.

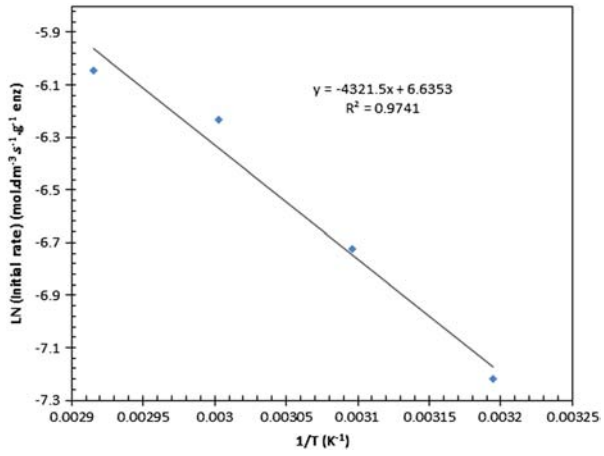


Fig. 5. Arrhenius plot. Reaction condition: 1-(2-furyl) ethanol, 0.001; vinyl acetate, 0.001 mol; n-heptane up to 20 cm^3; speed of agitation, 300 rpm; catalyst loading, 5 mg; temperature, 40–70°C.

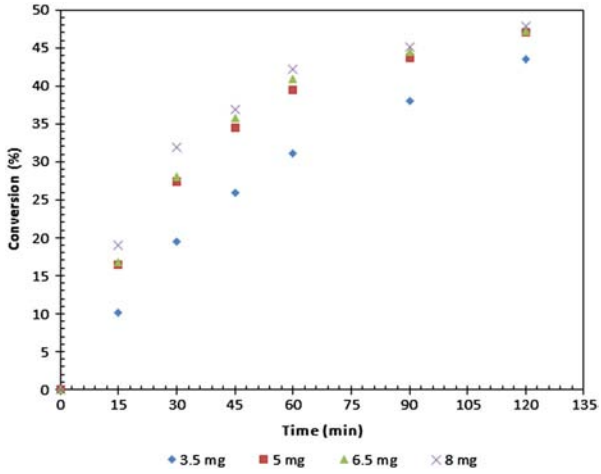


Fig. 6. Effect of catalyst loading. Reaction condition: 1-(2-furyl) ethanol, 0.001; vinyl acetate, 0.001 mol; n-heptane up to 20 cm^3; speed of agitation, 300 rpm; catalyst loading, 3.5–8 mg; temperature, 60°C, ◆3.5 mg, ■5 mg, ▲6.5 mg,×8 mg.

0.001 to 0.004 mol; the mixture volume was kept constant at 15 cm^3 by adjusting the addition of n-heptane (Fig. 7). It was found that the conversion and rate of reaction had decreased with an increase in the concentration of vinyl acetate. The overall conversion decreased from 47% to 41.7%. At high concentration, vinyl acetate acts as an inhibitor and this will be explained later.

Catalyst Reusability

Catalyst reusability is very important in order to make the process more viable at the industrial level and also to examine the stability of the catalyst after each run. Initial experiments were carried out using 5 mg of catalyst loading under similar conditions. After completion of each run, the catalyst particles were filtered by a membrane filter. Multiple washes were given to the catalyst with fresh solvent-heptane, dried at room temperature for 12 h, and reused. It was found that there was a marginal decrease in conversion from 47 to 44.5% after three reuses, which was due to loss of enzyme during filtration and drying. No make-up quantity was added.

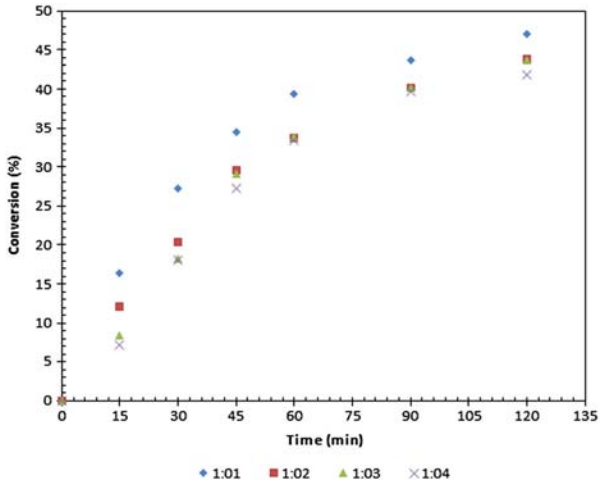


Fig. 7. Effect of acyl donor concentration. Reaction condition: 1-(2-furyl) ethanol, 0.001; vinyl acetate, 0.001–0.004 mol; n-heptane up to 20 cm^3; speed of agitation, 300 rpm; catalyst loading, 5 mg; temperature, 60°C, ◆1:1, ■1:2, ▲1:3,×1:4.

Kinetic Model

Two models have been proposed for two substrate-based enzymatic reaction, the first is the random model, where the order of binding of substrate to the enzyme active site is random and the second model is the bi–bi model, where the first step is formation of an acyl-enzyme complex.[37] It has been well reported that the first step of a lipase-catalyzed reaction involves the formation of an acyl enzyme complex with the acyl donor and will rules out a random mechanism.[38] As a result, it can only be the bi–bi model, wherein two mechanisms have been proposed, namely, the ordered bi–bi mechanism or ternary complex mechanism and the ping–pong bi–bi mechanism. In the former mechanism, both reactants bind to the enzyme and form a ternary complex and subsequently the products are released. In the latter mechanism, the product is released between the additions of substrates.[38–40]

An intricate kinetic analysis was carried out in order to develop the appropriate mechanism for the kinetic resolution of (±)-1-(2-furyl) ethanol. A number of experiments were performed to investigate the effect of substrate concentration on the rate of reaction over a wide range using 5 mg Novozym 435 under similar conditions and the volume was made up to 20 cm^{-3} with n-heptane. For determination of initial rates, the concentration of vinyl acetate (0.025–0.1 M) was varied over a wide range of (±)-1-(2-furyl) ethanol concentration (0.025–0.1 M).

The initial rates were calculated systematically from the linear portion of the concentration–time profiles. It was observed that the rate increased with increasing concentration of alcohol. However, the rate of reaction decreased with increasing concentration of vinyl acetate. Hara et al.[41] carried out the lipase-catalyzed kinetic resolution of furan-based alcohols in diisopropyl ether as solvent. They observed the formation of acetic acid / butyric acid as a byproduct which affected the course of the kinetic resolution. The possible reason for this behavior is that diisopropyl ether is polar solvent and has low log P value (logP -1.4).[41] It might have stripped out the essential (residual) water layer around the enzyme particles. In the presence of released water molecules, lipase catalyze hydrolysis of acylating agent (vinyl acetate or vinyl butyrate), which led to the formation of acids as byproducts. Contrary to this,

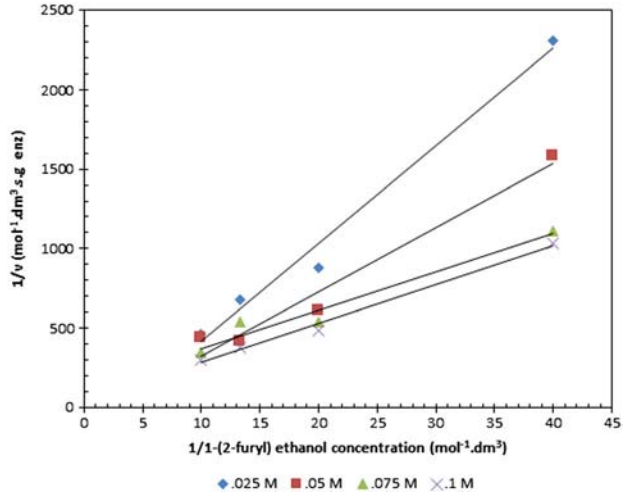


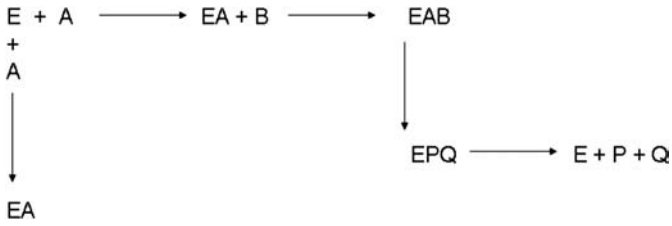
Fig. 8. Lineweaver-Burk plot. Reaction condition: 1-(2-furyl) ethanol, 0.025- 0.1 M; vinyl acetate, 0.025–0.1 M; n-heptane up to 20 cm^3; speed of agitation, 300 rpm; catalyst loading, 5 mg; temperature, 60°C, ◆0.025 M, ■0.05 M, ▲0.075 M,×0.1 M.

TABLE 1. Comparison with previous results

Lipase	Temperature (°C)	Enzyme loading (mg)	Mole ratio (alcohol: ester)	Time (h)	Conversion (%)	ee_p (%)	E	Ref.
Pesudomonas cepacia lipase	40	100	1:2	21	48	>99	>300	21
Porcine Pancreas lipase	R.T	100	1:5	24	53	>99	64	41
Burkholderia cepacia lipase	R.T	50	1:2	24	53	>99	>200	42
Candida antarctica lipase B	60	5	1:1	2	47	>99	>200	This work

we carried out the same reaction in n-heptane which has a high log P value [4] and found that there is no formation of acetic acid and loss of enzyme activity during the reaction. This indicated that the residual water layer around the enzyme was intact. Based on these observations, it is concluded that there would be reversible competition between vinyl acetate and alcohol at the active site of the enzyme which led to decreasing the reaction rate at high concentration of vinyl acetate.

The Lineweaver–Burk plot (Fig. 8), with double reciprocal of the initial rate and concentration of alcohol, shows that the lines are not parallel, ruling out the possibility of a ping–pong bi–bi mechanism. The slope and intercept changed linearly with increased concentration of the acyl donor. The lines were intersecting at certain points suggesting the ternary complex mechanism. According to this, the lipase (E) will react with acyl donor (A) to form a complex (EA). The second reactant 1-(2-furyl) ethanol (B) then reacts to form a ternary complex (EAB). This ternary complex then isomerizes to another ternary complex EPQ, which releases the product ester (P) and vinyl group (Q) and frees the enzyme E. At higher concentrations of vinyl acetate, a reversible deadend complex is formed. A typical reaction sequence is shown below.



The equation obtained with the above mechanism is:

$$v = \frac{v_m[A][B]}{K_{iA}K_{mB} + K_{mA}[B] + K_{mB}[A] + [A][B]} \quad (7)$$

where v is the rate of reaction, v_m the maximum rate of reaction, *[A]* the initial concentration of vinyl acetate, *[B]* the initial concentration of 1-(2-furyl) ethanol, K_{mA} the Michaelis constant for vinyl acetate, K_{mB} the Michaelis constant for 1-(2-furyl) ethanol, and K_{iA} the inhibition constant for vinyl acetate. The initial rate data were used to determine the kinetic parameters of the above mechanism by nonlinear regression analysis using the software package Polymath 5.1 and their values are V_m - 4.67×10^{-3} mol.dm^{-3}.s^{-1}, K_{mA} - 0.0261 mol.dm^{-3}, K_{mB} - 0.0313 mol.dm^{-3} and K_i - 0.0585 mol.dm^{-3}. The rates of reaction for different reactant concentrations were simulated using the above equation to verify the proposed kinetic model. The simulated and experimental rate had good agreement, which suggested that the proposed mechanism was valid for this reaction. As compared to previously reported processes (Table 1), we achieved good conversion (47%) and enantiomeric purity (ee_p>99.9) and E>200 with low enzyme loading in a very short time.[21,42,43] Considering the industrial implementation or scale up, Novozym 435 catalyzed kinetic resolution of (±)-1-(2-furyl) ethanol is the most suitable process compared to other reported lipases.

CONCLUSION

In this work, the kinetic resolution of (±)-1-(2-furyl) ethanol was carried out using different immobilized catalysts. Among which, *C. antarctica* lipase B (Novozym 435) was found to be the best catalyst in n-heptane as solvent. The effect of the acyl donor was compared by employing both alkyl and vinyl esters. The highest conversion and initial rate were obtained with vinyl acetate as the acyl donor. The effect of kinetic parameters on reaction conversion and initial rate was analyzed systematically over a wide range. Maximum conversion of 47% was obtained in 2 h using 5 mg enzyme loading with equimolar concentration of alcohol and ester at 60°C. The progress curve and initial rate data were used to predict the suitable model and then various kinetic parameters were estimated using nonlinear regression. The ordered bi–bi mechanism with acyl donor inhibition was found to fit the initial rate data. This model was used to simulate the rate data, which were in excellent agreement with the experimental values. The optimized values and kinetic mechanism for resolution of (±)-1-(2-furyl) ethanol are very useful and give new insight for scaling up the enzymatic process at industrial level.

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NOMENCLATURE

t_r	time constant for reaction, s
t_d	time constant for diffusion, s
C_O	initial concentration of limiting reactant, mol.dm^{-3}
$r_{(CO)}$	initial rate of reaction, mol.dm^{-3}.s^{-1}
D_S	diffusivity of limiting reactant, cm^2. s^{-1}
k_{SL}	liquid side mass transfer coefficient, m.s^{-1}
d_p	diameter of supported particle, cm
R_p	radius of supported particle, cm
φ	phase volume ratio
a	interfacial area per unit volume of organic phase
[A]	initial concentration of vinyl acetate, mol.dm^{-3}
[B]	initial concentration of 1-(2-furyl) ethanol, mol.dm^{-3}
E	Enzyme

EA	enzyme-acyl complex with A
EAB	ternary complex
EB	enzyme-alcohol complex with B
K_{iA}	inhibition constant for vinyl acetate, mol.dm^{-3}
K_{mA}	Michaelis constant for vinyl acetate, mol.dm^{-3}
K_{mB}	Michaelis constant for 1-(2-furyl) ethanol, mol.dm^{-3}
P	Ester
Q	Acetaldehyde
v	rate of reaction, mol.dm^{-3}.s^{-1}.g^{-1}-enz
v_{max}	maximum rate of reaction, mol.dm^{-3}.s^{-1}.g^{-1}-enz

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