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Novelty of immobilized enzymatic synthesis of 3-ethyl-1,3-oxazolidin-2-one using 2-aminoalcohol and dimethyl carbonate: Mechanism and kinetic modeling of consecutive reactions

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ABSTRACT

Oxazolidinones are multifunctional compounds possessing diverse biological and pharmacological activity. Enzymatic synthesis of oxazolidin-2-one was studied using 2-aminoalochol and dimethyl carbonate and synthesis of 3-ethyl-1,3-oxazolidin-2-one was chosen as the model reaction using a variety of immobilized lipases; among which *Candida antarctica* lipase B (Novozyme 435) was the best catalyst. The reaction leads to the final product oxazolidin-2-one via methyl ethyl (2-hydroxyethyl) carbamate as the intermediate. The parameters affecting rate of reaction and the conversion of both steps were studied systematically and covered effects of agitation speed, solvent, catalyst loading and reaction temperature. A reaction mechanism was proposed wherein the coproduct methanol is generated in the first step leading to the formation of methyl ethyl (2-hydroxyethyl) carbamate as the intermediate which rearranges itself leading to the final products 3-ethyl-1,3-oxazolidin-2-one and methanol. The kinetic constant and activation energy were determined for each step of the reaction. The study was further extended to other 2-aminoalochols under optimized reaction conditions to prepare different oxazolidinones. This is a first report of its kind describing kinetics and mechanism of bimolecular consecutive enzyme catalyzed reactions.

1. Introduction

Biocatalytic reactions and their applications in organic media have achieved a considerable importance in industry [1–3]. Lipases are the most important group of biocatalysts in nonaqueous enzymology; they do not require the labile cofactors for carrying out the reaction, able to recognize the chiral centers and have wide substrate specificity [4–8]. Lipases are widely present in different sources, which include bacteria and fungi and hence can be readily available for biotransformation of interest. Lipases are suitable catalysts in terms of catalytic activity and selectivity [9–11]. Lipase enzymes are widely known to catalyze various reactions in organic media, viz. amidation, transesterification, hydrolysis, esterification, thioesterification, epoxidation, and trans-thioesterification [1–3,12–16].

The heterocyclic compounds, particularly five and six membered rings, have gained importance for their diverse biological and pharmacological actions [17]. Oxazolidin-2-one ring is a well-liked scaffold in synthetic organic chemistry as well as in medicinal chemistry. Oxazolidinone and its derivatives have been included into a variety of therapeutically interesting compounds having antibacterial, antifungal, antiallegry, psychotropical and immunomodulatory activity [18–21]. Oxazolidin-2-ones, in particular enatiomerically pure 4-substituted ring, have been used as chiral auxiliaries in asymmetric synthesis of a wide range of compounds [22]. By far, production of oxazolidinone and its derivatives has been studied extensively using various conventional routes. The conventional path for synthesis of 2-oxazolidinones includes phosgenation of corresponding aminoalcohol with toxic phosgene or its derivatives, and this results in serious environmental pollution [23]. Several non-toxic procedures have been reported including (a) oxidative carbonylation using CO/O_2 and aminoalochols [24], (b) preparation by dialkyl carbonates and aminoalcohols, (c) direct synthesis of 2-oxazolidinones from carbon dioxide and aminoalcohol [25], and (d) environmentally benign and atom economic process for synthesis of 2-oxazolidinones from epoxides and carbon dioxide [26].

Since the enzymatic methodologies constitute an important alternative to conventional synthetic methods, the present study

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reports an alternative method for the synthesis of 3-ethyl-1,3-oxazolidin-2-one under mild conditions over immobilized lipase in nonaqueous medium. There has been no report on synthesis of 3-ethyl-1,3-oxazolidin-2-one using aminoalcohol and dialkyl carbonate over immobilized lipase. The study aims at development of environment friendly lipase catalyzed biosynthesis of 2-oxazolidinone including optimization of reaction parameters, and determination of kinetic constants. This is the first report on kinetics of consecutive enzymatic reactions without any substrate or product inhibition. It is further extended to synthesis of oxazolidin-2-ones derivatives from different 2-aminoalcohol substrates.

2. Materials and methods

2.1. Enzymes

Novozyme 435, Lipozyme RM IM and Lipozyme TL IM were procured as gift samples from Novo Nordisk, Denmark. Lipase AYS "Amano", Lipase AS "Amano", Lipase AK "Amano" and Lipase PS "Amano" were procured as gift samples from Amano Enzyme Inc. Japan. Porcine pancreatic lipase (crude extract) was purchased from Sigma–Aldrich, India. Novozyme 435 is *Candida antarctica* lipase immobilized on a macroporous polyacrylic resin beads (bead size 0.3–0.6 mm, bulk density 0.430 g/cm^3, water content 3%, activity of 7000 PLU g^{-1}); Lipozyme RM IM is *Mucor miehei* immobilized on an ion resin with activity of 5–6 BAUN while Lipozyme TL IM is *Thermomyces lanuginosus* immobilized on silica. Lipase AYS "Amano" is *Candida rugosa* lipase in form of lyophilized powder (activity ≥ 30,000 U/g); Lipase PS "Amano" is *Burkholderia cepacia* lipase immobilized on diatomaceous earth (activity ≥ 500 U/g); Lipase AK "Amano" is powdered *Pseudomonas fluoroscens* lipase (activity ≥ 20,000 U/g); Lipase AS "Amano is *Aspergillus niger* lipase in powder form (activity of 12,000–15,000 U/g).

2.2. Chemicals

All chemicals used were of AR grade obtained from the firms of repute: ethanolamine; diethanolamine; 2-(ethylamino)-ethanol; 2-(methyl amino)-ethanol; 2-amino-2-methyl-1-propanol; tetrahydrofuran; 1,4-dioxane; acetonitrile; ethanol; isopropyl alcohol (S. D. Fine Chemicals Pvt. Ltd., Mumbai, India); bis-2-(hydroxypropyl) amine (Sigma–Aldrich, India).

2.3. Experimental setup

The setup consisted of a 3 cm i.d. glass reactor of 50 cm^3 capacity which was fully baffled, mechanically agitated, and provided with a six bladed pitched-turbine impeller. The thermostatic water bath was maintained at the desired temperature with an accuracy of ±1 °C and the entire reactor assembly was immersed in it. The reaction mixture in a typical experiment contained 3 mmol of 2-(ethylamino)-ethanol and 6 mmol of dimethyl carbonate which was diluted to 15 mL with 1,4-dioxane as solvent. The resulting reaction mixture was then agitated at 60 °C at 600 rpm over a specified period of time. Effect of various reaction parameters was studied by varying one parameter at a time. Clear samples were taken out regularly for analysis ensuring no enzyme particles were entrapped within.

2.4. Analytical method

The concentration of reaction components was determined by Ceres 800, a high resolution GC equipped with FID. A 30 m × 0.32 mm BPX-5 capillary column packed with 5% phenyl polysilphenylene-siloxane was used for analysis. The product was confirmed by using GCMS.

^{1}H NMR and ^{13}C NMR were obtained with Bruker DPX 300 (^{1}H 300 MHz and ^{13}C 75.5 MHz) spectrometers using $CDCl_3$. Chemical shifts are expressed in parts per million (ppm) using tetramethylsilane as an internal standard. ^{1}H NMR and ^{13}C NMR ($CDCl_3$) for the desired product are as follows: 3-ethyl-1,3-oxazolidin-2-one: colorless oil, yield – 85%; ^{1}H NMR ($CDCl_3$): δ 4.28–4.24 (m, 2H), 3.52–3.48 (m, 2H), 3.25 (q, J = 7.2, 2H), 1.10 (t, J = 7.1 3H); ^{13}C (CDCl3): δ 158.41, 61.79, 44.09, 39.01, 12.69.

Table 1
Lipase catalyzed synthesis of 3-ethyl-1,3-oxazolidin-2-one using various lipases.

Lipase	Conversion %
Novozyme 435 (*Candida antarctica* lipase B)	83.15 ± 0.98
Lipozyme RMIM (*Rhizomucor miehei*)	0
Lipozyme TMIM (*Thermomyces lanuginosus*)	0
Lipase Amano PS (*Burkholderia cepacia*)	0
Lipase Amano AYS (*Candida rugosa*)	17.27 ± 0.72
Lipase Amano AS (*Aspergillus niger*)	0
Lipase Amano AK (*Pseudomonas fluoroscens*)	0
Porcine pancreatic lipase (crude extract)	12.63 ± 1.20

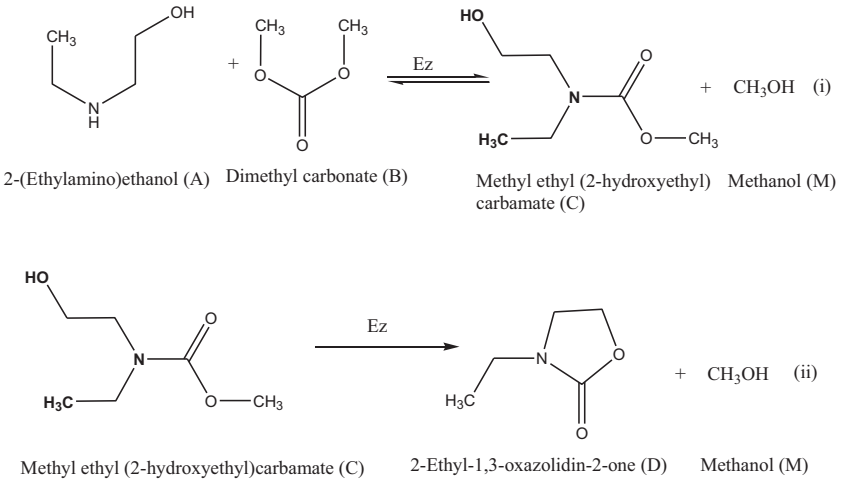
Reaction conditions: 2-(ethylamino)-ethanol – 3 mmol; dimethyl carbonate – 6 mmol; enzyme – 0.016 g/cm^3; temperature – 60 °C; speed of agitation – 400 rpm; 1,4-dioxane upto 15 ml.

3. Result and discussion

The two-step consecutive enzyme catalyzed reaction is given in Scheme 1.

3.1. Effect of various biocatalysts

Biocatalytic synthesis of 3-ethyl-1,3-oxazolidin-2-one by using 2-(ethylamino)-ethanol and dimethyl carbonate was chosen as the model reaction. Initially eight lipases were screened for investigating their activity for the production of the desired product (Table 1). However, five of them did not catalyze the reaction, two (Lipase AYS Amano and porcine pancreatic lipase) showed low activity whereas Novozyme 435 possessed the highest activity (Table 1, Entry 1). The conversions with Novozyme 435, Lipase AYS Amano and porcine pancreatic lipase were 83.15, 17.27 and 12.63% respectively. *C. antarctica* lipase B (Novozym 435) is a thermostable lipase which has been reported as a versatile enzyme from a unique origin having very broad substrate specificity, both with respect to *regio*- and *enantio*-selectivity [27]. Between the two functional groups present in 2-(ethylamino)-ethanol, the amine is presumably more reactive than the hydroxyl group. Therefore, the intermediate methyethyl (2-hydroxyethyl) carbamate is formed as the first step, and subsequently it results in production of 3-ethyl-1,3-oxazolidin-2-one (Scheme 1). Fig. 1 shows the concentration profiles for the limiting reactant (2-ethylaminoethanol), the intermediate (methyethyl (2-hydroxyethyl) carbamate) and the final product (3-ethyl-1,3-oxazolidin-2-one). The control experiment in the absence of Novozyme 435 did not show any conversion. In another set of study, when substantial concentration of carbamate was formed the biocatalyst was filtered out of reaction mixture after 90–120 min and the reaction was allowed to continue for next three hours in absence of enzyme. The resulting reaction did not proceed to form the oxazolidinone which proved that entire consecutive reaction occurs in the presence of enzyme only and rules out possibility of non-enzymatic reaction. *C. antarctica* lipase B (Novozyme 435) was found as the most active and suitable catalyst which led to the highest conversion and it was considered for all further studies.



Scheme 1. Synthesis of 3-ethyl-1,3-oxazolidin-2-one using 2-(ethylamino)-ethanol and dimethyl carbonate.

3.2. Effect of speed of agitation

An optimum speed of agitation and low enzyme loading of the optimum size are important to carry out the reaction to overcome the limitations of external mass transfer and internal diffusion. The reactants diffuse from the bulk liquid to the external surface of the particle and from there into the interior pores of the immobilized biocatalyst. Therefore, the effect of agitation speed was studied on conversion in the range of 200 rpm – 800 rpm (Fig. 2). The initial rates of reaction were obtained from conversion profiles and initial concentration. The conversion and reaction rate were found to increase as the speed of agitation increased from 200 to 600 rpm. However, there was a slight variation in conversion and reaction rate at speed of agitation of 600 and 800 rpm; which indicates that the reaction was not mass transfer controlled. In order to ascertain that the reaction was kinetically controlled within the chosen experimental parameters, it was essential to compare the time constants for reaction (t_r) and diffusion (t_d) to determine the influence of mass transfer resistance and intra-particle diffusion control. The time constants are defined as: $t_r = C_0/r(C_0)$ and $t_d = D_s/(k_{SL})^2$, where C_0 is the substrate concentration in bulk organic phase (mol cm^{-3}), $r(C_0)$ is the rate of reaction (mol cm^{-3} s^{-1}), D_s is the diffusivity of substrate in organic phase (cm^2 s^{-1}) and k_{SL} is the solid–liquid mass transfer coefficient in organic phase (cm s^{-1}). C_0 and $r(C_0)$ could be obtained from the experimental results. The Sherwood number is also useful to determine mass transfer coefficient and thus $k_{SL} = 2D_s/d_p$, where d_p is the diameter of the support particle. If t_r is greater than t_d, it means that the reaction is not influenced by the mass transfer resistance . The Shiebel equation plays an important role to determine the diffusivity of the substrate in organic solvent [28]. It was seen that when C_0 was 0.2×10^{-3} mol cm^{-3}, $r(C_0)$ was 9.0×10^{-7} mol cm^{-3} s^{-1}. Diffusivity of 2-(ethylamino)-ethanol in 1,4-dioxane was calculated as 1.07×10^{-5} cm^2 s^{-1}. As the particle size of catalyst ranged in between 0.03 and 0.09 cm, the average diameter of the support particle was considered as 0.06 cm. The value of $k_{SL} = 3.56 \times 10^{-4}$ cm s^{-1} was calculated from the Sherwood number correlation. Using these results, t_r and t_d, were calculated as 222 s and 84 s respectively, which indicated that t_r is greater than t_d. Thus, the reaction was kinetically controlled. In another case it was equally important to compare the substrate diffusion rate per unit interfacial area ($k_{SL}C_0$) with reaction rate per unit area $\varphi r(C_0)/a$. φ represents the phase volume ratio and a is interfacial area per volume of organic phase. As the catalyst particles are spherical, $\varphi/a = R/3$, here R is the radius of the particle, which was 0.03 cm as mentioned earlier. From calculation $k_{SL}C_0$ was found to be 7.13×10^{-8} mol cm^{-2} s^{-1} and $\varphi r(C_0)/a$ was found as 9.0×10^{-9} mol cm^{-2} s^{-1}. It was observed that substrate diffusion rate per unit area is much higher compared to rate of reaction per unit area. Hence, it is evident that reaction rate is not influenced by mass transfer and intrinsic enzyme kinetics controls it.

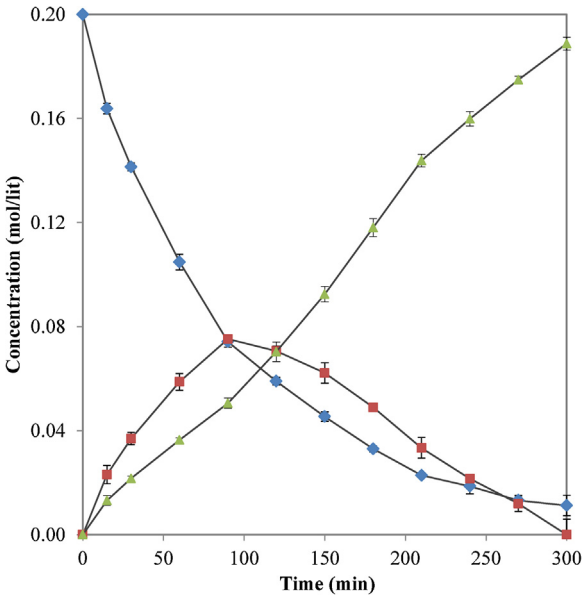


Fig. 1. Reaction profile for synthesis of 3-ethyl-1,3-oxazolidin-2-one. (◆) Concentration of 2-(ethylamino)-ethanol, (▲) concentration of methyethyl(2-hydroxyethyl) carbamate, (■) concentration of 3-ethyl-1,3-oxazolidin-2-one.

3.3. Effect of different solvents

The prerequisite for a successful biocatalytic reaction is the selection of a suitable reaction medium and it is important because most of nonaqueous solvents are known to denature the enzyme. The enzyme activity is strongly affected by the varied functional groups as well as molecular structure of the solvent [29]. To preserve its biological activity in organic media, monolayer of water is essential for an enzyme. In general, enzymes are more stable when suspended in non-polar solvents that have low solubility for water than in polar solvents [30]. A number of

Table 2
Effect of different solvents on biosynthesis of 3-ethyl-1,3-oxazolidin-2-one.

Solvents	log *p*	Conversion %
Tetrahydrofuran	0.49	43.63 ± 0.25
Acetonitrile	−0.33	5.30 ± 1.04
1,4-Dioxane	−1.1	82.32 ± 0.59
Ethanol	−0.24	9.70 ± 1.24
Isopropyl alcohol	0.8	10.25 ± 0.63

Reaction conditions: 2-(ethylamino)-ethanol – 3 mmol; dimethyl carbonate – 6 mmol; Novozyme 435 – 0.016 g/cm^3; temperature – 60 °C; speed of agitation – 600 rpm; solvent upto 15 ml.

solvents such as tetrahydrofuran (THF), acetonitrile (ACN), ethanol, isopropyl alcohol and 1,4-dioxane were used for the reaction (Table 2). The conversion of 2-(ethylamino)-ethanol was very low in ethanol (log *P* = −0.24) whereas, in 1,4-dioxane (log *P* = −1.1), higher conversion of 82.32% was observed. However, acetonitrile (log *P* = −0.33), isopropyl alchol (log *P* = 0.8) and tetrahydofuran (log *P* = 0.49) showed conversion of 5.30, 10.25 and 43.6.3%, respectively. Conversion and log *P* value of reaction medium as parameters do not show any systematic pattern in the current case. With change of environment of reaction medium from hydrophilic to hydrophobic, the overall enzyme efficiency changes dramatically. It has been established that solvents having log *P* greater than 4 do not distort the essential water layer around the particle, thereby leaving the biocatalyst in an active state [31]. The solubility of 2-aminoalcohol limited the study to multiple solvents. Among all solvents, 1,4-dioxane was found to be the suitable reaction medium and further experiments were carried out by using 1,4-dioxane.

3.4. Effect of catalyst loading

The study of effect of catalyst loading was carried out on synthesis of 3-ethyl-1,3-oxazolidin-2-one under otherwise similar conditions. The catalyst loading was varied from 0.013 g/cm^3 to 0.023 g/cm^3 keeping the molar ratios of the reactants constant. It was observed that as the catalyst loading increased the reaction rate increased and an overall increase in conversion was observed from 71% to 93% (Fig. 3). There was a slight increase in the rate of reaction and conversion beyond the catalyst loading of 0.02 g/cm^3, which indicated that the catalyst loading was higher than that required for reaction. The enzyme loading of 0.02 g/cm^3 was found to be optimum and used in all further experiments.

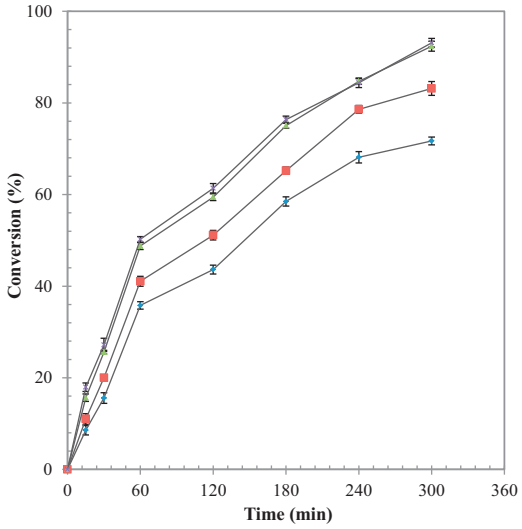


Fig. 3. Effect of catalyst loading on synthesis of 3-ethyl-1,3-oxazolidin-2-one. Reaction condition: 2-(ethylamino)-ethanol – 3 mmol; dimethyl carbonate – 6 mmol; temperature – 60 °C; speed of agitation – 600 rpm; reaction time – 5 h; 1,4-dioxane upto 15 ml. (◆) 0.013 g/cm^3, (▲) 0.016 g/cm^3, (■) 0.02 g/cm^3, (×) 0.023 g/cm^3.

3.5. Effect of temperature

The enzymatic reactions are sensitive to temperature and elevated temperature increases the rate of enzymatic reactions but up to a certain temperature beyond which the enzyme could be unstable. With increase in reaction temperature chance of collision between enzyme and substrate molecule increases which results in formation of enzyme substrate complexes and this leads to increased enzyme activity [7]. The effect of temperature on activity of Novozyme 435 was studied in the range of 40–70 °C for the synthesis of 3-ethyl-1,3-oxazolidin-2-one. It was found that the initial rate of reaction increased from 0.9×10^{-6} to 1.7×10^{-5} mol/L min g of enzyme and the conversion increased from 77 to 94% as the

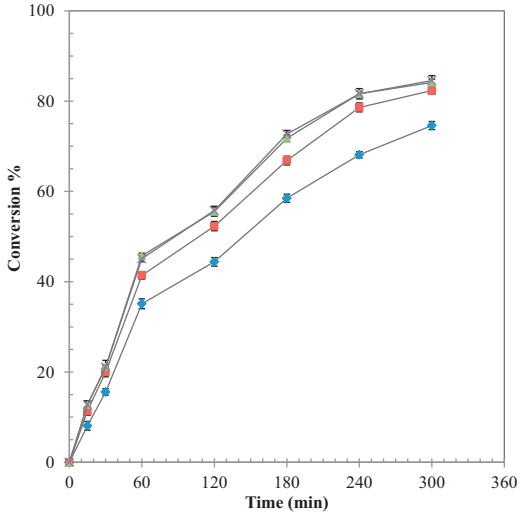


Fig. 2. Effect of speed of agitation on synthesis of 3-ethyl-1,3-oxazolidin-2-one. Reaction conditions: 2-(ethylamino)-ethanol – 3 mmol; dimethyl carbonate – 6 mmol; Novozyme 435 – 0.016 g/cm^3; temperature – 60 °C; reaction time – 5 h; 1,4-dioxane upto 15 ml. (◆) 200 rpm, (▲) 400 rpm, (■) 600 rpm, (×) 800 rpm.

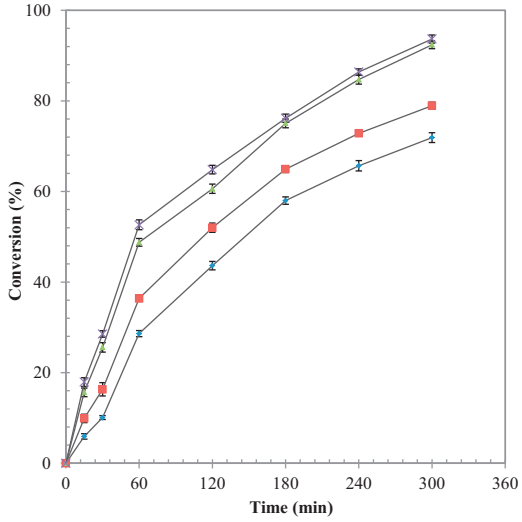
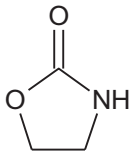
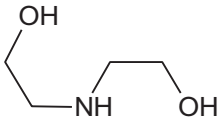
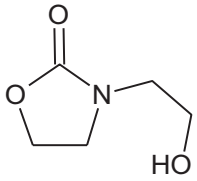
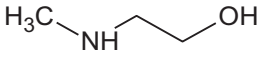
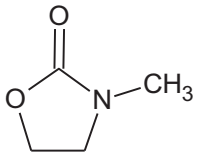
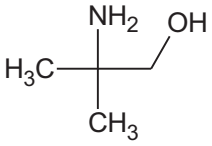
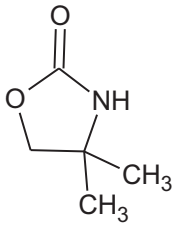
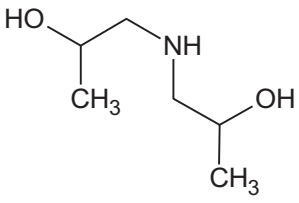
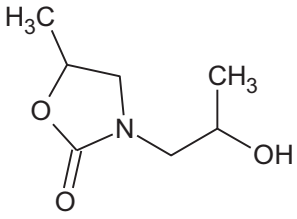


Fig. 4. Effect of temperature on synthesis of 3-ethyl-1,3-oxazolidin-2-one. Reaction conditions: 2-(ethylamino)-ethanol – 3 mmol; dimethyl carbonate – 6 mmol; Novozyme 435 – 0.02 g/cm^3; speed of agitation – 600 rpm; reaction time – 5 h; 1,4-dioxane upto 15 ml. (◆) 40 °C, (▲) 50 °C, (■) 60 °C, (×) 70 °C.

Table 3
Synthesis of oxazolidin-2-ones from different 2-aminoalochols.

Sr. no.	Substrate	Product	Conversion %
1			83 ± 0.73
2			74 ± 1.17
3			89 ± 1.32
4			77 ± 0.65
5			61 ± 0.96

Reaction conditions: 2-aminoalochol – 3 mmol; dimethyl carbonate – 6 mmol; Novozyme 435 – 0.02 g/cm^3; temperature – 60 °C; speed of agitation – 600 rpm; 1,4-dioxane upto 15 ml.

reaction temperature was varied in range of 40–70 °C in 5 h(Fig. 4). This aspect is further discussed later in Section 3.9.

3.6. Effect of concentration of substrate

The amount of 2-(ethylamino)-ethanol was varied from 3 mmol to 12 mmol, keeping the other parameters constant: dimethyl carbonate (3 mmol), Novozyme 435 (0.02 g/cm^3), 1,4-dioxane (up to 15 ml). The increasing concentration of 2-(ethylamino)-ethanol caused decrease in conversion and reaction rate. It may be due to inhibition of enzyme by excess substrate which gets adsorbed on the active site of enzyme.

The effect of varying molar concentration of dimethyl carbonate was studied by changing its concentration in the range of 3 mmol to 12 mmol under otherwise similar conditions: 2-(ethylamino)-ethanol (3 mmol), Novozyme 435 (0.02 g/cm^3), 1,4-dioxane (up to 15 ml). It was found that with increase in the concentration of dimethyl carbonate, the reaction rate and overall conversion increased at all concentrations.

3.7. Effect of different aminoalochols

Under the optimized reaction conditions (2-aminoalochol – 3 mmol; dimethyl carbonate – 6 mmol; novozyme – 0.02 g/cm^3; temperature – 60 °C; speed of agitation – 600 rpm; 1,4-dioxane upto 15 ml), the method was applicable to the synthesis of corresponding 2-oxazolidinones from different 2-aminoalochols and dimethyl carbonate (Table 3). It can be seen that excellent conversion was achieved for all employed 2-aminoalochols for the synthesis of a variety of oxazolidinones.

3.8. Reusability of catalyst

Recycling of the catalyst is an important factor in chemical industry due to the cost management. The stability of enzyme was determined by carrying out reusability study of biocatalyst. The biocatalyst was filtered and washed with reaction solvent 1,4-dioxane after each use. Then it was kept for drying at room temperature and reused for subsequent studies. It can be concluded from Fig. 5 that there was a slight decrease in conversion after three cycles of reuse and enzyme was not denatured after

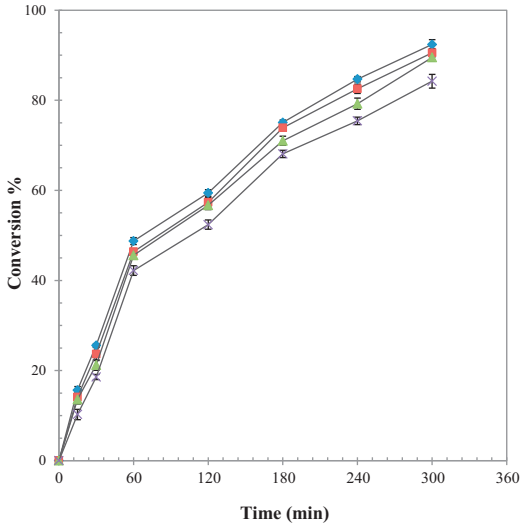


Fig. 5. Effect of reusability of biocatalyst on synthesis of 3-ethyl-1,3-oxazolidin-2-one. Reaction conditions: 2-(ethylamino)-ethanol – 3 mmol; dimethyl carbonate – 6 mmol; Novozyme 435 – 0.02 g/cm^3; temperature – 60 °C; speed of agitation – 600 rpm; 1,4-dioxane upto 15 ml. (◆) fresh enzyme, (▲) first reuse, (■) second reuse, (×) third reuse.

repeated use. The slight decrease in conversion might have resulted due to loss of catalyst particles during its use during repeated cycles.

3.9. Reaction kinetics

Scheme 2 illustrates the reaction mechanism. For sake of convenience the enzyme is colored differently when there is an interaction with it at each step. Also the inhibition substrates is assumed to be absent under the experimental conditions.

Table 4
Kinetic constants for bimolecular consecutive reaction.

Temp. (°C)	k_1	k'_1	k_2	k'_2	k_3
40	1.3×10^{-4}	1.5×10^{-8}	3.4×10^{-4}	5.7×10^{-8}	8.7×10^{-5}
50	1.8×10^{-4}	7.1×10^{-8}	4.9×10^{-4}	8.2×10^{-8}	1.2×10^{-4}
60	2.9×10^{-4}	1.1×10^{-7}	6.4×10^{-4}	1.1×10^{-7}	1.4×10^{-4}
70	3.7×10^{-5}	2.7×10^{-7}	8.1×10^{-4}	2.7×10^{-7}	2.2×10^{-4}

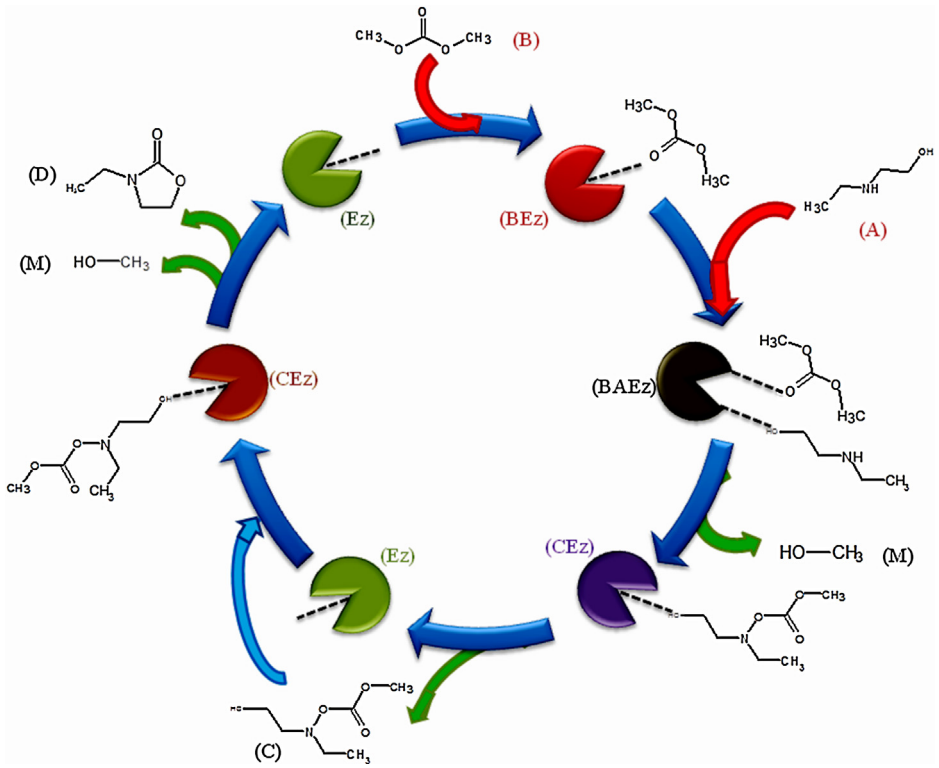
k_1, k_2, k_3 – L g-enz^{-1} min^{-1}; k'_1 – L^2 mol^{-1} g-enz^{-1} min^{-1}.

The first step involves the adsorption of the species **B** (dimethyl carbonate) into the enzyme (**Ez**) followed by formation of a complex **BEz** which thereafter accommodates species **A** (2-ethylaminoethanol) to form a complex **BAEz**. There is internal molecular arrangement and a mole of methanol is released to form **CEz**, namely, methyethyl(2-hydroxyethyl) carbamate complex with the enzyme. Some free C is seen in the reaction mass which is ultimately consumed as shown in the Scheme and no C was finally left at the end of reaction. Thus, there is further rearrangement of the complex CEz which leads to the formation of **D** (3-ethyl-1,3-oxazolidin-2-one) and **M** (methanol). The enzyme is released for the next cycle. Steady-state assumption is made for simplifying the kinetic equation.

The synthesis of 3-ethyl-1,3-oxazolidin-2-one is achieved through two consecutive reactions:

$$\mathrm{B} + \mathrm{Ez} \underset{k'_1}{\overset{k_1}{\rightleftharpoons}} \mathrm{BEz} \quad (1)$$

$$\mathrm{BEz} + \mathrm{A} \underset{k'_2}{\overset{k_2}{\rightleftharpoons}} \mathrm{CEz} + \mathrm{M} \quad (2)$$



Scheme 2. Reaction mechanism of consecutive enzyme catalyzed reaction. Different colors are used for the enzyme complexes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 5
Activation energy for consecutive reaction in series.

Reaction step	Activation energy	kcal/mol
1	E_1	5.8
1′	E'_1	19.6
2	E_2	6.14
2′	E'_2	10.52
3	E_3	6.3

In the above equation, the following ternary complex is included $BEz + A \underset{k'_{21}}{\overset{k_{21}}{\rightleftharpoons}} BAEz$ as shown in Scheme 2.

$$CEz \xrightarrow{k_3} D + Ez + M \tag{3}$$

$$\frac{-dC_B}{dt} = k_1 C_B C_{EZ} - k'_1 C_{BEZ} \tag{4}$$

$$\frac{dC_{BEZ}}{dt} = k_1 C_B C_{EZ} - k'_1 C_{BEZ} - k_2 C_{BEZ} C_A + k'_2 C_{CEZ} C_M \tag{5}$$

$$\frac{dC_{CEZ}}{dt} = k_2 C_{BEZ} C_A - k'_2 C_{CEZ} C_M - k_3 C_{CEZ} \tag{6}$$

Total enzyme concentration (C_{Et}) is

$$C_{Et} = C_{EZ} + C_{CEZ} + C_{BEZ} \tag{7}$$

At steady state,

$$\frac{dC_{BEZ}}{dt} = 0 \tag{8}$$

And

$$\frac{dC_B}{dt} = 0 \tag{9}$$

Using Eqs. (7)–(9), we can obtain expressions for C_{BEz}, C_{CEz} and C_{Ez}.

$$C_{BEz} = \frac{k_1}{k'_1} C_B C_{Ez} \tag{10}$$

$$C_{CEz} = \frac{k_1 k_2}{k'_1 k'_2} \frac{C_B C_A C_{Ez}}{C_M} \tag{11}$$

$$C_{Ez} = \frac{C_{Et}}{\left[1 + \frac{k_1}{k'_1} C_B + \frac{k_2 k_1}{k'_1 k'_2} \frac{C_A C_B}{C_M}\right]} \tag{12}$$

Thus the rate equation, assuming reaction (3) is rate controlling, becomes:

$$-\frac{dC_A}{dt} = k_3 C_{CEz}$$

On substituting the values, we get,

$$-\frac{dC_A}{dt} = \frac{k_3 k_2 k_1}{k'_2 k'_1} \frac{C_A C_B}{C_M} \frac{C_{Et}}{\left[1 + \frac{k_1}{k'_1} C_B + \frac{k_2 k_1}{k'_1 k'_2} \frac{C_A C_B}{C_M}\right]} \tag{13}$$

The rate constants (k_1, k'_1, k_2, k'_2, and k_3) for the consecutive reaction were obtained at different temperatures (Table 4). The Arrhenius plots were used to determine the activation energy for each step of the reaction (Table 5). The activation energy for enzyme catalysis falls within a range of 5–15 kcal/mol. The activation energies E_1, E_2 and E_3 for forward reactions and E'_1 and E'_2 for corresponding backward reactions were calculated (Table 5). The last step is thus the rate-controlling step.

4. Conclusion

The present study reports an alternate method for synthesis of oxazolidin-2-one under mild conditions over immobilized lipase (Novozyme 435) as the best biocatalyst among eight different lipases. The mechanism shows reactions in series which include 2-(ethylamino)-ethanol and dimethyl carbonate which forms (methyethyl (2-hydroxyethyl) carbamate) with coproduct methanol in the first step. The intermediate subsequently forms the desired product 3-ethyl-1,3-oxazolidin-2-one with methanol as coproduct. Effects of various reaction parameters including agitation speed, solvent, loading of catalyst, reaction temperature and mole ratio of reactants were discussed systematically. The kinetic constants and activation energy for reaction were determined from reaction kinetics. The enzymatic synthesis of corresponding 2-oxazolidinones was studied using different 2-aminoalochols. This is a first report of its kind describing consecutive enzyme catalyzed reaction. The results are novel both from the system and modeling perspectives.

Conflict of interest statement

The authors declare no conflict of interest.

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References

[1] G.D. Yadav, K.M. Devi, J. Am. Oil Chem. Soc. 78 (2001) 347–351.
[2] G.D. Yadav, K.M. Devi, Biochem. Eng. J. 10 (2002) 93–101.
[3] G.D. Yadav, K.M. Devi, Chem. Eng. Sci. 59 (2004) 373–383.
[4] G.D. Yadav, P. Sivakumar, Biochem. Eng. J. 19 (2004) 101–107.
[5] G.D. Yadav, I.V. Borkar, Ind. Eng. Chem. Res. 48 (2009) 7915–7922.
[6] G.D. Yadav, S.V. Pawar, Bioresour. Technol. 109 (2012) 1–6.
[7] G.D. Yadav, S.V. Pawar, Appl. Microbiol. Biotechnol. 96 (2012) 69–79.
[8] K.P. Dhake, K.M. Deshmukh, Y.P. Patil, R.S. Singhal, B.M. Bhanage, J. Biotechnol. 56 (2011) 46–51.
[9] P.B. Juhl, K. Doderer, F. Hollmann, O. Thum, J. Pleiss, J. Biotechnol. 150 (2010) 474–480.
[10] A. Acosta, M. Filice, G. Fernandez-Lorente, J.M. Palomo, J.M. Guisan, Bioresour. Technol. 102 (2011) 507–512.
[11] L. Banoth, M. Singh, A. Tekewe, U.C. Banerjee, Biocatal. Biotransform. 27 (2009) 263–270.
[12] G.D. Yadav, A.H. Trivedi, Enzyme Microb. Technol. 32 (2003) 783–789.
[13] G.D. Yadav, P.S. Lathi, Biochem. Eng. J. 16 (2003) 245–252.
[14] G.D. Yadav, P.S. Lathi, J. Mol. Catal. A: Chem. 223 (2004) 51–56.
[15] Z. Jin, J. Ntwali, S.Y. Han, S.P. Zheng, Y. Lin, J. Biotechnol. 159 (2012) 108–114.
[16] K.C. Badgujar, B.M. Bhanage, Enzyme Microb. Technol. 57 (2014) 16–25.
[17] N. Sampath, R. Mathews, M.N. Ponnuswamy, J. Chem. Crystallogr. 40 (2010) 1105–1109.
[18] S.K. Bhattacharya, A. Clow, A. Przyborowska, J. Halket, V. Glover, M. Sandler, Neurosci. Lett. 132 (1991) 4–46.
[19] S. Grabley, H. Kluge, H.U. Hoppe, Angew. Chem. Int. Ed. 26 (1987) 664–665.
[20] H. Kakeya, M. Morishita, K. Kobinata, M. Osono, M. Ishizuka, H. Osada, J. Antibiot. 51 (1998) 1126–1128.
[21] K. Danielmeier, E. Steckhan, Tetrahedron: Asymmetry 6 (1995) 1181–1190.
[22] G. Zappia, E. Gacs-Baitz, G. Delle Monache, D. Misiti, L. Nevola, B. Botta, Curr. Org. Synth. 4 (2007) 81–135.
[23] F. Li, C. Xia, J. Catal. 227 (2004) 542–546.

[24] B. Gabriele, R. Mancuso, G. Salerno, M. Costa, J. Org. Chem. 68 (2003) 601–604.
[25] C.J. Dinsimore, S.P. Mercer, Org. Lett. 6 (2004) 2885–2888.
[26] L.F. Xiao, L.W. Xu, C.G. Xia, Green Chem. 9 (2007) 369–372.
[27] T. Tanino, T. Aoki, W.Y. Chung, Y. Watanabe, C. Ogino, H. Fukuda, A. Kondo, Appl. Microbiol. Biotechnol. 82 (2009) 59–66.
[28] D.W. Green, Perry's Chemical Engineers Handbook, McGraw-Hill Book Company, New York, 1984, pp. 226–289.
[29] D. Yu, L. Tian, D. Ma, H. Wu, Z. Wang, L. Wang, X. Fang, Green Chem. 12 (2010) 844–850.
[30] G.D. Yadav, I.V. Borkar, J. Chem. Technol. Biotechnol. 84 (2009) 420–426.
[31] G.D. Yadav, I.V. Borkar, AIChE J. 52 (2006) 1235–1247.