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# Use of deep eutectic solvents in the enzyme catalysed production of ethyl lactate

Azime Arıkaya[1], Ayşe Ezgi Ünlü, Serpil Takaç*

*Ankara University Faculty of Engineering, Department of Chemical Engineering, Tandoğan, 06100 Ankara, Turkey*

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ABSTRACT

The use of deep eutectic solvents (DESs) in the lipase-catalysed esterification of lactic acid with ethanol was explored by screening several DESs. Choline chloride:glycerol (1:2) was the most effective DES and provided 28.7% yield of ethyl lactate under the following conditions: 10% (v/v) of water content in DES, 3 M of initial lactic acid concentration, 5 M of initial ethanol concentration, 30 mg/mL of enzyme concentration, 50 °C temperature and 200 rpm agitation rate. Individual and combined effects of the reaction medium components on the enzyme activity were investigated and it was discovered that DES stimulated the enzyme activity while reactants inhibited it. A kinetic model that obeys the Ping-Pong Bi-Bi mechanism with ethanol inhibition was suggested. The kinetics parameters were determined as $r_{max} = 0.401$ mol/L h, the Michaelis constants $K_A = 1.657$ mol/L and $K_B = 0.799$ mol /L, the inhibition constant $K_{iB} = 0.156$ mol/L. The model reasonably predicted the experimental data. The activation energy was found to be 43.28 kJ/mol. Mass transfer limitations in the reaction medium were negligible. The results are promising for further studies that will research on the use of green solvents in enzyme-catalysed lactic acid esterification reactions.

## 1. Introduction

Ethyl lactate (EL) is a bio-based and environmentally benign solvent that has gained considerable attention. This solvent is in accordance with at least eight of the "Twelve Principles of Green Chemistry" [1]. Although mainly used as a solvent, ethyl lactate is also used as a food additive and flavour chemical [1,2]. It is produced by the esterification of lactic acid (LA) with ethanol (EtOH), which is a reversible reaction with water as a co-product. Although esterification reactions are self-catalysed by the acid involved in the reaction, homogeneous (sulphuric acid) or heterogeneous (Amberlyst 15) catalysts are used to accelerate the reaction rate [1]. Reactive distillation [1,3,4] and membrane-based water separation processes [1,5–7] have also been used to overcome the equilibrium limitation in the reaction. On the other hand, enzyme-catalysed esterification reactions have the following advantages over acid-catalysed reactions such as higher substrate specificity, milder reaction conditions, and lower energy requirement [8]. Lipase enzyme has been successfully employed in several esterification reactions [9]. Therefore, lipase-catalysed esterification reactions appear to be a future alternative to the acid-catalysed processes.

Several scientists have studied the esterification of lactic acid by the catalytic effect of lipase. Hasegawa et al. [10] suggested that the use of polar solvents in enzymatic esterification of lactic acid was important as they suppressed the acidity of lactic acid presumably due to their basicity and miscibility with lactic acid. Huang et al. [11] examined different types of lipase enzymes for lactic acid esterification in *t*-butanol (*t*-ButOH) and found that Novozym 435 exhibited the highest activity. A 77% yield of ethyl lactate was obtained under the following conditions: molar ratio of LA:EtOH 1:8, temperature 60 °C, lactic acid concentration 0.3 M, revolutions per minute 200 rpm, lipase concentration 45 g/mol and reaction time 24 h. Sun et al. [12] studied the production of ethyl lactate and reaction kinetics in the presence of immobilised enzymes CALB on NKA-9 resin or Novozym 435 and *t*-ButOH medium. The EtOH:LA molar ratio, total substrate amount, temperature, reaction time, and agitation rate were optimized as 8.3:1, 0.4 g, 55 °C, 26.87 h and 150 rpm, respectively. Under optimum conditions, the yield of ethyl lactate was 24.32%. Major et al. [13] studied the synthesis of ethyl lactate using Novozym 435 in the presence of hexane, toluene, and ionic liquids. Cyphos 202, as ionic liquid, afforded the highest yield (95%) and less amount of enzyme was required in the presence of Cyphos 202 when compared to organic solvents. In another study conducted with Novozym 435, production of L-ethyl lactate from racemic lactic acid was investigated in Cyphos 104 ionic liquid medium [14]. The temperature and the excess alcohol influenced the

* Corresponding author.
*E-mail addresses:* azimearikaya@iyte.edu.tr (A. Arıkaya), Ezgi.Unlu@eng.ankara.edu.tr (A.E. Ünlü), Serpil.Takac@eng.ankara.edu.tr (S. Takaç).
[1] Present address: İzmir Institute of Technology, Department of Chemical Engineering, 35430, İzmir, Turkey.

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enantiomeric excess of ethyl lactate while water content and temperature significantly influenced the lactic acid conversion. The optimum values for initial water content (8%), molar excess of alcohol (11-fold) and temperature (30 °C) were obtained using a developed statistical model. Under optimum conditions, the highest conversion was 63.8% and the highest enantiomeric excess was 34.3%. Recently, Koutinas et al. [15] reported that chloroform and hexane were effective solvents with yields of 88% and 75%, respectively, for lipase-catalysed ethyl lactate production at low lactic acid concentrations. The yield of ethyl lactate increased as the solvent's hydrophobicity increased up to a value of Log P = 2.

Deep eutectic solvents (DESs) are a new class of green solvents and have gained enormous attention for their utilization in several fields such as catalysis, organic synthesis, electrochemistry, material chemistry as well as dissolution and extraction processes [16]. There is an increasing number of studies on the successful use of enzymes [17,18] and whole cells in DES [18–21]. Additionally, the cascade reactions in DES interfacing bio-catalysis with transition metal catalysis was also reported [22]. The use of DES in bio-transformations as a reaction medium provides many advantages arising from their easy preparation techniques, low price, biodegradability and non-toxicity. Recently, a considerable amount of research on the enzyme-catalysed reactions such as esterification, transesterification, polymerization, and hydrolysis in the presence of DESs has been conducted [17,23,24]. While the results obtained have been encouraging, no study in the literature has reported the utilization of DESs in the lipase-catalysed esterification of lactic acid to yield ethyl lactate.

In the present study, the use of DESs as reaction medium in the Novozym 435-catalysed esterification of lactic acid with ethanol was investigated in an attempt to search for an advantage of DES on the ethyl lactate production. Several DESs were screened and the esterification parameters in the presence of choline chloride:glycerol (1:2) that provided high reaction rate and ethyl lactate yield were presented.

## 2. Materials and methods

### 2.1. Materials

Novozym 435 (lipase B from *Candida antarctica*; immobilised on macro-porous polyacrylic resin beads) was kindly provided by Novozyms A/S (Bagsvaerd, Denmark). Lactic acid (88–92% total acid basis), ethyl lactate, ethanol, choline chloride (ChCl), glycerol (Gly), acetonitrile (HPLC grade), *o*-phosphoric acid and *p*-nitrophenyl acetate were purchased from Sigma-Aldrich; glucose (Glu) and sucrose (Suc) were purchased from Applichem; fructose (Fru), maltose (Malt), ethylene glycol (EG), urea, *t*-ButOH and *p*-nitrophenol were purchased from Merck.

### 2.2. DES preparation

Choline chloride was dried under vacuum over Silica gel in a desiccator for 24 h prior to use. Deep eutectic solvents were prepared in sealed flasks by heating and stirring the constituents at 80 °C for ChCl:Glu (1:1), ChCl:Glu (5:2), ChCl:Suc (1:1), ChCl:Malt (4:1), Fru:Suc (1:1), ChCl:Urea (1:2) and at 65 °C for ChCl:Gly (1:2), ChCl:Gly (1:1), ChCl:Gly (3:2), and ChCl:EG (1:2) until transparent colourless liquid form was obtained [25–27]. Unless otherwise noted, 10% (v/v) of distilled water was added into DES before being used in the esterification reaction.

### 2.3. Esterification

Esterification of lactic acid with ethanol by Novozym 435 in DES:water binary mixture was performed in sealed flasks with 10 mL of reaction volume (V) in an orbital air shaker (Shel Lab S16R2) under isothermal conditions. Samples were taken at 24 h intervals throughout 72 h and then analysed for lactic acid and ethyl lactate concentrations. The effects of water content in DES:water binary mixture, initial enzyme concentration ($C_{Eo}$), initial lactic acid concentration ($C_{Ao}$), initial ethanol concentration ($C_{Bo}$), temperature (T) and agitation rate (N) on the reaction rate and yield of ethyl lactate were investigated.

### 2.4. Analyses

#### 2.4.1. HPLC analysis

Samples from the reaction medium were analysed for LA and EL concentrations by HPLC (Waters Alliance 2695) using XTerra RP 18 column (Waters, 4.6 mm x 150 mm x5 μm) at 40 °C and 210 nm with a flow rate of 0.6 mL/min of isocratic elution. Mobile phase was composed of 20% acetonitrile:water (70:30% (v/v)), and 80% *o*-phosphoric acid:water (0.1:99.9% (v/v)) and injection volume was 10 μL. Lactic acid and ethyl lactate concentrations were calculated by using calibration curves prepared by standard samples. The coefficients of variation were 0.28 and 0.66% for LA and EL, respectively. Each analysis was conducted at least in duplicate. The yield of ethyl lactate was defined as moles ethyl lactate formed per initial moles of lactic acid and the reaction rate was defined as μmoles lactic acid consumed per g of enzyme per min. The initial rate of reaction was calculated by using the change in LA concentration over the first 24 h period.

#### 2.4.2. Lipase activity assay

The activity of Novozym 435 was measured by the enzymatic hydrolysis of *p*-nitrophenyl acetate at room temperature. Hydrolysis reaction was carried out in 4 mM *p*-nitrophenyl acetate solution dissolved in 20% acetonitrile and 80% potassium phosphate buffer (50 mM, pH 7). Absorbance of samples was measured using a UV-VIS spectrophotometry (Shimadzu 1601A) at 348 nm. One unit of enzyme activity was defined as the quantity of enzyme necessary to release 1 μmole *p*-nitrophenol per minute.

The effects of DES (ChCl:Gly (1:2)), substrates (EtOH, LA, 3 M LA + 5 M EtOH) and DES together with substrates (DES + 5 M EtOH, DES + 3 M LA, DES + 5 M EtOH + 3 M LA) on the enzyme activity were tested by incubating enzyme beads (30 mg/ml) within the media at 50 °C and 200 rpm for 24 h. The beads taken out from the media were filtered, washed with n-hexane and dried at 40 °C before measuring the activity. The change in activity after 24 h according to initial value was calculated and compared to the enzyme beads incubated in organic solvent (*t*-ButOH, *t*-ButOH + 3 M LA + 5 M EtOH).

#### 2.4.3. Physical properties of ChCl:Gly (1:2)

Electrical conductivity of ChCl:Gly (1:2) was measured using a Radiometer Ion Check 65; viscosity was measured using a Fungilab Viscometer, and density was measured using a pycnometer. The pH was measured using a Sartorius PP25 pH meter and the water activity ($a_w$) was determined using a Novasina Aw SPRINT TH 500 apparatus. All the properties were measured at room temperature.

## 3. Result and discussion

### 3.1. Effect of DES type on the esterification of lactic acid

Esterification of lactic acid with ethanol was carried out in the presence of different DESs including ChCl:Glu (1:1), ChCl:Glu (5:2), ChCl:Suc (1:1), ChCl:Malt (4:1), Fru:Suc (1:1), ChCl:Urea (1:2), ChCl:Gly (1:2), ChCl:Gly (1:1), ChCl:Gly (3:2), and ChCl:EG (1:2) to investigate the effect of DES type on the initial rate of reaction. Higher initial rates were obtained in the presence of ChCl:Glu (1:1), ChCl:Gly (1:2), ChCl:Glu (5:2) and Fru:Suc (1:1) compared to other DESs (Fig. 1). ChCl:Gly (1:2) was selected to be used in further experiments due to its ease of preparation and higher stability in comparison with the others. ChCl:Gly (1:2) was also reported as a good solvent for esterification and transesterification reactions in many other studies [28,29].

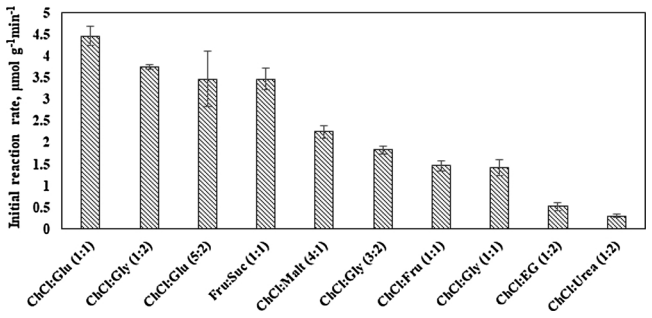


**Fig. 1.** Effect of deep eutectic solvents (DESs) on the esterification. DES:water (10%, v/v), 0.81 M lactic acid, 6.72 M ethanol, 30 mg/mL Novozym 435, T = 50 °C, N = 200 rpm, V = 10 mL.

ChCl:Gly (1:2) was prepared in the laboratory and characterised by measuring the following physical properties electrical conductivity - 1.189 mS/cm, viscosity - 351 cP, density - 1.181 g/L, pH - 3.91 and water activity - $a_w < 0.03$. These values are in accordance with those given in the literature by Garcia et al. [30], D'Agostino et al. [31], Abbott et al. [32], and Durand et al. [33], respectively. Lactic acid, ethanol, and ethyl lactate were easily dissolved in ChCl:Gly (1:2) since it is a polar mixture with the polarity comparable to short-chain alcohols [34].

The change of electrical conductivity of ChCl:Gly (1:2) with the addition of ethanol (0–100%, v/v) was investigated to determine whether ethanol disrupted the structure of DES. The electrical conductivity of ChCl:Gly (1:2) increased gradually from 1.181 to 6 mS/cm when the amount of alcohol added into the medium increased from zero to 60% (v/v) and then decreased. Therefore, ethanol concentrations below 60% (v/v) were used in the esterification reaction to ensure DES structure was conserved. The change in electrical conductivity of DES with the addition of water can be explained by the hole theory which indicates that the conductivity of DES depends on the interstitial space available between hydrogen bond donor/cation network [35]. In this study, very similar results were obtained with the addition of ethanol to DES and those obtained with that of water [35–37].

### 3.2. The change in enzyme activity

The effect of reaction medium components on the lipase activity is presented in Fig. 2. DES increased the enzyme activity by 3-fold, indicating that ChCl:Gly (1:2) has a stimulatory effect on the enzyme activity. *t*-Butanol also increased the enzyme activity (2-fold) although the value was less than DES provided. Lactic acid, ethanol, lactic acid + ethanol, as well as their combinations with DES and with *t*-butanol drastically decreased the enzyme activity. In the literature, the inhibition effects of lactic acid and ethanol on the lipase activity were

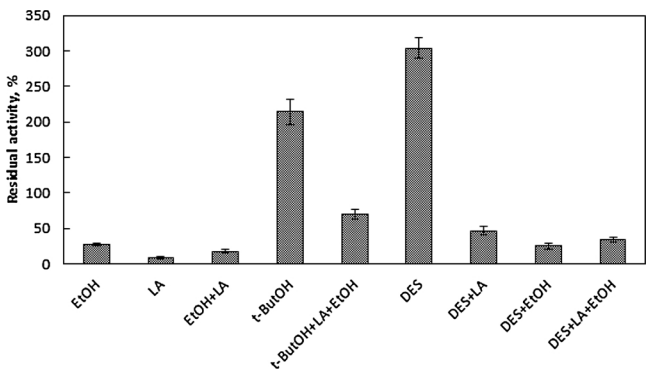


**Fig. 2.** Change in the activity of Novozym 435. T = 50 °C, N = 200 rpm, t = 24 h.

also reported [10,38]. On the other hand, the pH of the reaction medium involving ChCl:Gly (1:2) was about a unit lower (pH = 2.25) than the medium containing *t*-butanol (pH = 3.08). This also showed that inactivation of the enzyme by ethanol and lactic acid played an important role in the esterification medium in addition to the acidity. The presence of DES in the medium partially repressed the inhibition effects of lactic acid and ethanol on the enzyme activity. Residual activity of the enzyme was 34% in the reaction medium (DES + LA + EtOH) after 24 h of esterification. Recently, Elgharbawy et al. [39] reported that ChCl:Gly (1:2) activated and stabilized the lipase (CALB) activity which could be attributed to the strong hydrogen bond network and the water content in DES. Our results confirmed that ChCl:Gly (1:2) has an activation effect on the lipase activity.

### 3.3. Effect of water content of DES

Water is known to play a crucial role in the lipase-catalysed reactions for water limited environments [29]. It was reported that the lipase-catalysed reactions of dissolved substrates in DES were difficult to perform without the addition of water [33]. Therefore, different amounts of water (5, 10 and 20% (v/v)) were added into ChCl:Gly (1:2) to determine the effect of water on the esterification rate of lactic acid.

The initial rate of reaction considerably increased at 10% (v/v) water content ($a_w = 0.207$), indicating that a minimum 10% water is essential for enzyme activity (Fig. 3). However, at a higher water content (20% v/v), the reaction rate decreased to about zero presumably due to the reverse hydrolysis reaction becoming significant as suggested by Findrik et al. [14]. The effect of the water content in DES medium on the bio-transformations was explained by different mechanisms such as the three-dimensional organisation of enzyme molecules [33,39], the rupture of the hydrogen-bond network between the starting materials [17,40], and the decreased viscosity of DES solution [17]. The present work demonstrated that the reaction rate of a lipase-catalysed lactic acid esterification with ethanol in ChCl:Cly (1:2) was considerably dependent on the water content.

### 3.4. Effect of initial concentrations of lactic acid and ethanol

Esterification of lactic acid with ethanol was carried out at different concentrations of lactic acid (0.5, 1.0, 1.5, 2.0, 3.0 M) and ethanol (3.0, 5.0, 6.0 M). The initial rate of reaction increased with lactic acid concentration for all ethanol concentrations, indicating that there was no lactic acid inhibition on the enzyme activity under the conditions studied (Fig. 4a). On the other hand, when ethanol concentration increased, the initial rate of reaction first increased and then slightly

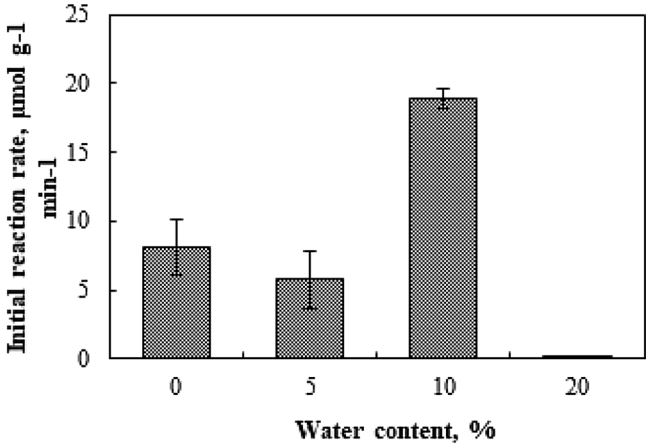


**Fig. 3.** Effect of water content of ChCl:Gly (1:2):water on the esterification. Ethanol/lactic acid molar ratio = 1, 30 mg/mL Novozym 435, T = 50 °C, N = 200 rpm, V = 10 mL.

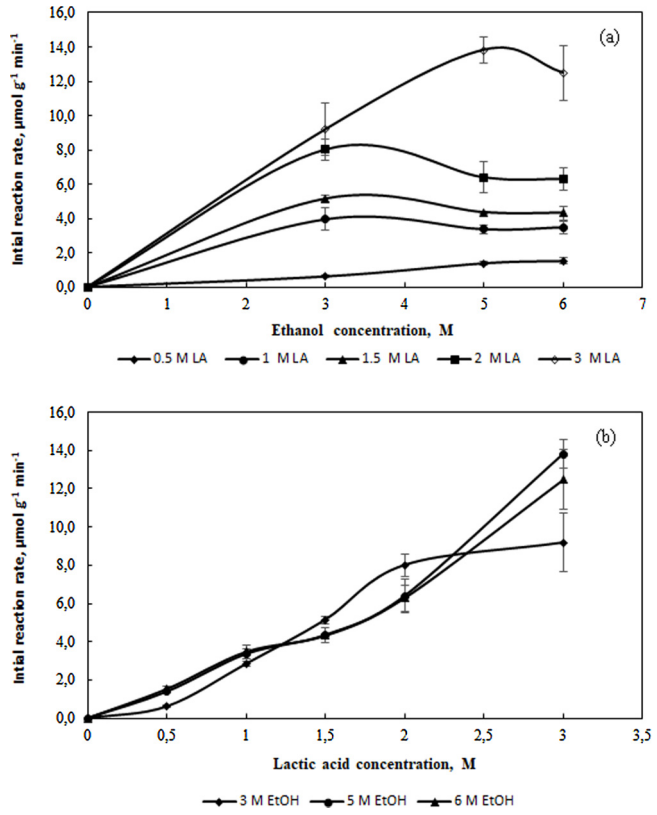


**Fig. 4.** (a) Effect of initial lactic acid concentration on the esterification at varying concentrations of ethanol (b) Effect of initial ethanol concentration on the esterification at varying concentrations of lactic acid. ChCl:Gly-water (10%, v/v), 30 mg/mL Novozym 435, T = 50 °C, N = 200 rpm, V = 10 mL.

decreased (Fig. 4b), where the inhibition by ethanol changed with lactic acid concentration. There was no ethanol inhibition at 0.5 M lactic acid concentration whereas different extents of ethanol inhibition were observed at higher amount of lactic acid. The highest yield of ethyl lactate obtained after 72 h of esterification was 28.7%, at concentrations of 5 M ethanol and 3 M lactic acid. These concentrations of the reactants that did not cause any inhibition were used in further experiments.

The significant effect of substrate concentrations on the conversion and reaction rate of lactic acid with ethanol was also reported in the literature. Wang et al. [41] stated that the initial rate of reaction first increased and then decreased with increasing initial concentrations of lactic acid and ethanol in the presence of *t*-butanol. The authors explained this phenomenon with the inhibition of lipase by the reactants. Additionally, Sun et al. [12] suggested that the substrate inhibition or strong adsorption of substrates on the active sites of the enzyme might cause a decrease in the reaction rate.

### 3.5. Effect of enzyme concentration

The effect of enzyme concentration on the initial rate of reaction of lactic acid esterification is presented in Fig. 5 for $C_{Eo}$ = 0, 10, 30, 50, 70 and 90 mg/mL. The reaction occurred even in the absence of Novozym 435 since esterification reactions are self-catalytic. The reaction rate of lactic acid with ethanol increased when 30 mg/mL enzyme was loaded in the medium, however, no change was observed at higher lipase concentrations. Huang et al. [11] explained a similar course with the difficulty of the maintenance of the uniform suspension of enzyme in the reaction medium.

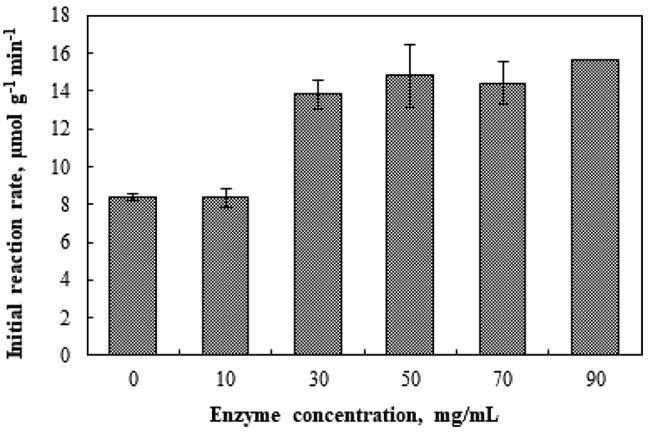


**Fig. 5.** Effect of enzyme concentration on the esterification. ChCl:Gly-water (10%, v/v), 3 M lactic acid, 5 M ethanol, T = 50 °C, N = 200 rpm, V = 10 mL.

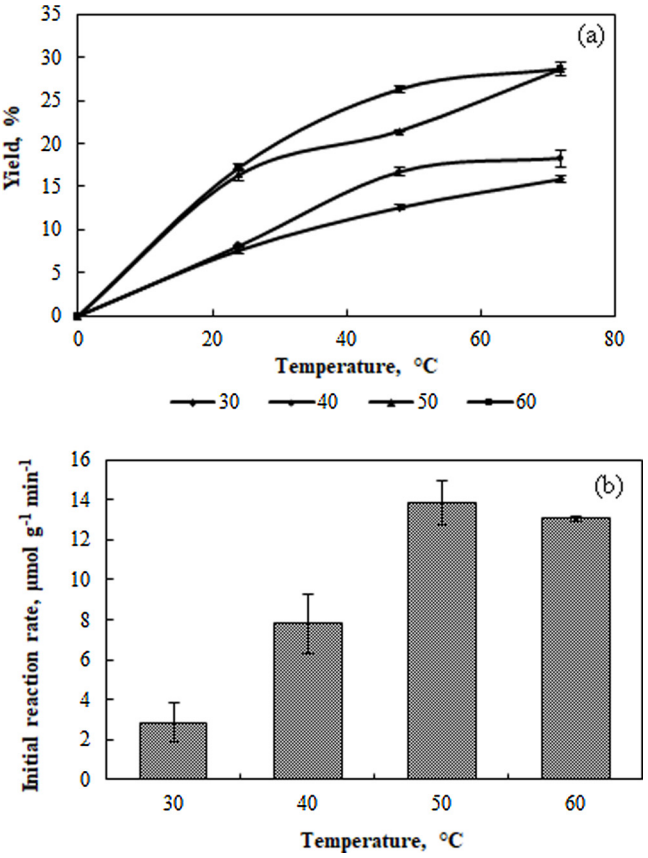


**Fig. 6.** Effect of temperature (a) on the yield of ethyl lactate (b) on the initial rate of esterification. ChCl:Gly-water (10%, v/v), 3 M lactic acid, 5 M ethanol, 30 mg/mL Novozym 435, N = 200 rpm, V = 10 mL.

### 3.6. Effect of temperature and reaction time

Esterification reaction of lactic acid with ethanol was carried out at 30, 40, 50 and 60 °C, respectively and the results are given as time courses of ethyl lactate yield in Fig. 6a. The yield increased with reaction time and temperature; additionally, the reaction at 50 °C and 60 °C provided almost the same yield at 72 h of esterification. Temperature affects not only the reaction rate but also the mass transfer rate of the substrates in the medium. The viscosity of ChCl:Gly (1:2) is considerably influenced by temperature [42]. Therefore, the increase in the yield of ethyl lactate can be attributed both to the increased reaction rate with temperature and the enhanced mass transfer rate with the

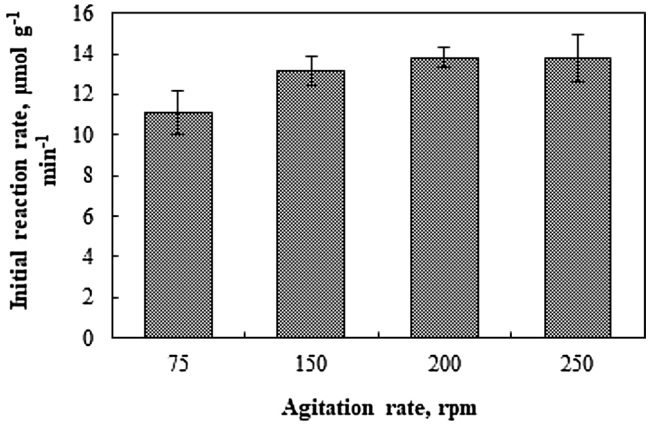


**Fig. 7.** Effect of agitation rate on the esterification. ChCl:Gly-water (10%, v/v), 3 M lactic acid, 5 M ethanol, 30 mg/mL Novozym 435, T = 50 °C, V = 10 mL.

reduction in viscosity with temperature.

The initial rates of reaction of lactic acid esterification at different temperatures are illustrated in Fig. 6b. According to the data, using Arrhenius equation, the activation energy for lactic acid esterification with ethanol by Novozym 435 in the presence of ChCl:Gly (1:2) was found to be 43.28 kJ/mol.

### 3.7. Mass transfer effects and kinetic model

The degree of good mixing that eliminates external mass transfer limitations in the system was investigated by carrying out the reaction at 75, 150, 200 and 250 rpm agitation rates, respectively. As shown in Fig. 7, there was no considerable change in the initial rate of reaction after 150 rpm. Therefore, external mass transfer limitations could be eliminated at 150 rpm and higher agitation rates.

The level of internal mass transfer resistance was revealed by calculating the Thiele modulus. Eq. (1) expresses the observable Thiele modulus, Φ, which represents the ratio of the reaction rate to the internal diffusion rate [43]:

$$\Phi = \frac{r_o}{D_E C_{so}} \left( \frac{V_p}{A_p} \right)^2 \quad (1)$$

where $r_o$ is the initial rate of reaction (mol/Lh), $D_E$ is the effective diffusivity coefficient ($cm^2/h$), $C_{so}$ is the initial concentration of the substrate (mol/L), $V_p$ is the volume of enzyme particle ($cm^3$), and $A_p$ is the surface area of the particle ($cm^2$). For spherical particles with the radius of $R_p$ (cm), the observable Thiele modulus can be written as in Eq. (2) [43]:

$$\Phi = \frac{r_o}{D_E C_{so}} \left( \frac{Rp}{3} \right)^2 \quad (2)$$

Here;

$$D_E = D_S \frac{\varepsilon_p}{\tau} \sigma \quad (3)$$

where $D_s$ is the substrate diffusivity coefficient in liquid ($cm^2/h$), $\varepsilon_p$ is porosity of the particle, τ is tortuosity factor of the particle, and σ is the constriction factor [44]. The values of $\varepsilon_p$, τ and $R_p$ were taken as 0.5, 6 and 0.045, respectively [45]. σ was neglected since the radius of pore was assumed to be larger than the solute molecule [45]. $D_s$ was estimated through Scheibel equation (Eq. (4)) [46].

$$D_s = \frac{k\,T}{\mu V_S^{0.33}} \quad (4)$$

Here, k is the equation constant, T is the reaction temperature (323.13 K), μ is the viscosity of the reaction medium (cP), and $V_S$ is the molar volume of substrate ($cm^3$). The value of k was taken as $17.5 \times 10^{-8}$ [46]. The viscosity of the reaction medium was estimated to be 1.11 cp using Refutas equation ASTM D7152 [47–49] and medium density (1.048 g/$cm^3$). The molar volume (Vs) of EtOH and LA were 60.30 and 76.87 $cm^3$, respectively [50,51]. $D_S$ and $D_E$ for EtOH were calculated as $1.32 \times 10^{-5}$ $cm^2/s$ and $6.91 \times 10^{-6}$ $cm^2/s$, respectively. These values were $1.21 \times 10^{-5}$ $cm^2/s$ and $1.01 \times 10^{-6}$ $cm^2/s$ for LA, respectively. The observable Thiele modulus values were found to be $\Phi = 2.83 \times 10^{-4}$ and $\Phi = 5.12 \times 10^{-4}$, respectively, for the optimum concentrations of EtOH (5 M) and LA (3 M), indicating that the internal diffusion limitation was negligible according to the Weisz's criteria ($\Phi < 0.3$) [52].

Lipase-catalysed esterification of lactic acid with ethanol obeys the Ping-Pong Bi-Bi mechanism [12]. According to this mechanism, enzyme reacts with the acyl donor LA to form an enzyme-acyl complex. This complex produces water as the first product. Enzyme-acyl complex binds with the second substrate EtOH to form enzyme-acyl-alcohol complex, which then releases the product EL and the enzyme. In this mechanism, irreversible bonds between the enzyme-acyl complex and lactic acid or between the enzyme and ethanol complex could cause inhibition [53].

In the kinetic study, the data given in Fig. 4b was used where ethanol inhibition was clearly detected. The kinetic model for the Ping-Pong Bi-Bi mechanism with alcohol inhibition can be expressed by Eq. (5) [12]:

$$r = \frac{r_{max} {}^* C_A {}^* C_B}{C_A {}^* C_B + K_A {}^* C_B {}^* \left(1 + \frac{C_B}{K_{IB}}\right) + K_B C_A} \quad (5)$$

Here r is the initial rate of reaction (mol/Lh), $r_{max}$ is the maximum reaction rate (mol/Lh), $C_A$ is the initial LA concentration (mol/L), $C_B$ is the initial EtOH concentration (mol/L), $K_A$ is Michaelis constant for LA (mol/L), $K_B$ is Michaelis constant for EtOH (mol/L), and $K_{IB}$ is inhibition constant for EtOH (mol/L).

The kinetic parameters were calculated using MATLAB Curve Fitting tool as $r_{max} = 0.401$ mol/L h, $K_A = 1.657$ mol/L, $K_B = 0.799$ mol/L, and $K_{iB} = 0.156$ mol/L. The compatibility of the experimental data with the proposed model (Eq. 5) is shown in Fig. 8. The model provided a considerably good correlation with the data. The inhibition by ethanol under the reaction conditions was confirmed. The model can be used to make quantitative predictions for the esterification rate of lactic acid with ethanol within the reaction conditions used.

In summary, the results of this study showed that DES may be a promising reaction medium for the lipase-catalysed esterification of lactic acid with ethanol to yield ethyl lactate. The use of DES in the

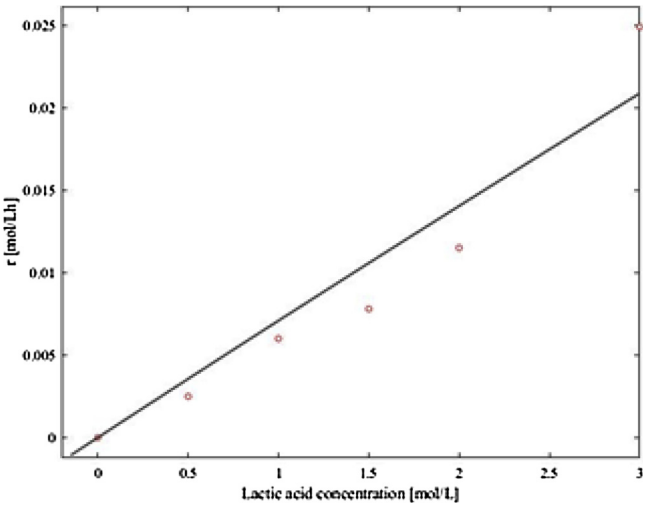


**Fig. 8.** Comparison of model prediction (-) and experimental data (-o-) of the kinetic model for the esterification. ChCl:Gly-water (10%, v/v), 5 M ethanol, 30 mg/mL Novozym 435, T = 50 °C, V = 10 mL.

enzyme-catalysed reactions has diverse effects on the reaction course that may lead to advantageous or disadvantageous outputs. Basically, DES can change the functional configuration of the enzyme [17]. The hydrogen bonds between DES components and amino acid residues at the active site of the enzyme may decrease the enzyme activity. On the other hand, the hydrogen bonds between DES components and surface residues of the enzyme, instead of denaturation, may even increase the enzyme stability. The strong intermolecular hydrogen-bonding network between DES components can limit the solvent diffusion to the protein chains [54]. It was also pointed out that the DES components and water percentage in the eutectic mixture were critical and a selective inhibition of some enzymes was observed in the bio-catalytic reduction of prochiral ketones as a result of a subtle interplay of solvation of whole cells [40]. The occupation of immobilized enzyme pores with DES which prevents the substrates from reaching the active sites can also be a difficulty. In this study, we demonstrated that DES itself did not have any inhibitory effect on Novozym 435; moreover, it enhanced the activity. The only challenge in the study was the inhibition of enzyme by the substrates; however, it was partially repressed by the presence of DES. Other challenges addressed above were overcome by optimising the process parameters.

The recovery of ethyl lactate from the reaction mixture is another question that should be considered. Vu et al. [55] successfully used a catalytic extractive reaction system to recover ethyl lactate where fatty acid methyl ester was the extractive phase. For the present work, an extractive reaction technology that uses a hydrophobic solvent -in which ethyl lactate has a greater solubility than in DES phase - can be a promising route to recover ethyl lactate.

## 4. Conclusions

Solvent engineering favours a biotransformation towards synthesis, enhances the solubility of substrates, and modifies the enzyme activity. Green solvents are in a trend of replacing organic solvents and the deep eutectic solvents have been successfully used in several lipase-catalysed reactions. In this study, we showed that DESs were effective in lactic acid esterification. A 28.7% yield of ethyl lactate was obtained by using ChCl:Gly (1:2) as solvent which is reasonably compatible with those reported in the literature. Molar ratio of the substrates was the most crucial parameter, which was followed by the enzyme concentration. A kinetic model that obeys the Ping-Pong Bi-Bi mechanism with ethanol inhibition that reasonably predicted the experimental data was suggested. This study may provide an insight for future studies on the production of ethyl lactate with a green biocatalytic esterification processes using DESs. Additionally, a study on the enantiomeric ethyl lactate production in the presence of DES followed by a hydrolysis reaction will also provide new aspects on the production of high purity L- or D- lactic acid for further production of lactic acid biopolymers.

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# Update


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Corrigendum

# Corrigendum to "Use of deep eutectic solvents in the enzyme catalysed production of ethyl lactate" [Process Biochem. 84 (2019) 53–59]

Azime Arıkaya[1], Ayşe Ezgi Ünlü, Serpil Takaç*

*Ankara University Faculty of Engineering, Department of Chemical Engineering, Tandoğan, 06100 Ankara, Turkey*

The unit of the density of DES ChCl:Gly (1:2) was written wrong unintentionaly.

On page 3, first paragraph 'density - 1.181 g/L' should be as 'density - 1.181 g/mL'.

The authors would like to apologize for any inconvenience caused.

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* Corresponding author.

*E-mail addresses:* azimearikaya@iyte.edu.tr (A. Arıkaya), Ezgi.Unlu@eng.ankara.edu.tr (A.E. Ünlü), Serpil.Takac@eng.ankara.edu.tr (S. Takaç).

[1] Present address: İzmir Institute of Technology, Department of Chemical Engineering, 35430, İzmir, Turkey.

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