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Enhanced synthesis of isoamyl acetate using an ionic liquid–alcohol biphasic system at high hydrostatic pressure

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ABSTRACT

Isoamyl acetate has a banana flavor that can be considered a "natural" ingredient when synthesized using a lipase-catalyzed reaction. Production of isoamyl acetate was up to 10-fold higher with free lipase *versus* immobilized *Candida antarctica* lipase B (CALB) after 3 h at 300 MPa and 80 °C in 1-butyl-3-methylimidazolium hexafluoro-phosphate, isoamyl alcohol biphasic system. Rate of catalysis by free CALB was 15-fold greater at 500 MPa, 40 °C than at 0.1 MPa, 40 °C and 14-fold at 500 MPa, 80 °C than at 0.1 MPa, 80 °C. Activation energy of free lipase calculated between 40 and 80 °C at 0.1 MPa (55.6 ± 4.2 kJ mol^{-1}) or 300 MPa (56.2 ± 4.6 kJ mol^{-1}) was not significantly different. Similarly, activation volume ($\Delta V^{\ddagger}$) of free lipase calculated between 0.1 and 500 MPa at 40 °C (-16.1 ± 1.5 cm^3 mol^{-1}) or 80 °C (-16.7 ± 1.4 cm^3 mol^{-1}) was not significantly different. After treatment at high pressure and upon pressure release, the free lipase was temporarily suspended in a semi-solid IL phase. This study is the first to combine the use of a room temperature ionic liquid (RTIL) and high hydrostatic pressure (HHP) for enhanced enzyme catalysis.

1. Introduction

Isoamyl acetate is a commonly used ingredient with a characteristic banana flavor with a production of about 74,000 kg/yr [1]. Enzymatic syntheses of this and other aroma active esters such as geranyl acetate [2] are relevant to the food industry as these processes are typically more efficient that conventional chemical synthesis and enzyme-synthesized products in some cases can be labeled as "natural"

Room temperature ionic liquids (RTILs) are liquids that are composed completely of ions and are liquid at or near room temperature. They can have very low vapor pressure, high thermal stability, and widely tunable properties (by changing the cation/anion combination) such as polarity, hydrophobicity, and solvent miscibility [3]. RTILs are attractive reaction media because their use can enhance enzyme stability [4–7], selectivity [8], and reaction rates [5,9]. Also, as compared to monophasic systems, RTILs utilized in a biphasic system can reduce downstream processing and facilitating separation when using free enzymes thus improving production efficiency [3,10,11]. Enzymes in RTILs have been termed as "carrier-free" immobilized as they are not required to be bound to a solid support or crosslinked. Cao et al. [12] described how carrier-free immobilized enzymes generally exhibit 10–1000 times higher volumetric activity (U/g) *versus* carrier-bound enzymes and are also highly stabilized against heating and use of organic solvents. Carrier-free immobilized *Candida antarctica* lipase B (CALB) can also be used and recycled in both batch [7] and continuous [13] processes for ester synthesis with excellent operational stability, even under harsh conditions (e.g. SC-CO_2 at 150 °C and 10 MPa) [9,14]. The combination of these attributes along with the ease of recyclability (ability to easily separate using simple decanting and purify using distillation techniques due to their high boiling point) add to their growing potential application in diverse processes and make them useful alternatives to organic solvents.

In an effort to determine the optimal RTIL for CALB synthesis of butyl butyrate, Lozano et al. [15] tested six imidazolium-type RTILs. All the assayed RTILs proved adequate media for enzyme-catalyzed trans-esterification and activity was increased with respect to that obtained in organic solvents of similar polarity. For example, 1-ethyl-3-methylimidazolium tetrafluoroborate (emimBF_4) enhanced five times the synthetic activity and four times half-life time of lipase in comparison to 1-butanol. Lipase half-lime time in (emimBF_4) was about two to three times that of all the other RTILs [15]. Lou et al. [8] also found that 1-butyl-3-methylimidazolium tetrafluoroborate (bmimBF_4) can serve as an excellent co-solvent with buffer in place of traditional organic solvents for CALB mediated hydrolysis of D,L-phenylglycine methyl

Abbreviations: HHP, High hydrostatic pressure; RTIL, Room temperature ionic liquid; SC-CO_2, Supercritical carbon dioxide; CALB, *Candida antarctica* lipase B.

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ester with the advantage of markedly improved enantioselectivity and activity.

RTILs have been previously studied in combination with enzyme enhancement techniques such as immobilization, microwave heating, and biphasic media. Combining the use of RTILs with different immobilizations resulted in increasing the half-life of CALB up to 6-fold in hexane at 95 °C and increased synthetic activity up to 6-fold in SC-CO_2 [16]. Recently, a microfulidic reactor with a 1-butyl-3-methylpyridinium dicyanamide (bmpyrdca) n-heptane biphasic system produced rates of isoamyl acetate production three times greater than regular batch systems and highest productivity with respect to the initial acyl donor concentration [17]. Combining microwave heating with 20 different RTILs with different polarity, viscosities, hydrophobicity, and water content revealed that RTIL stabilization and activation effects on CALB were greatest with dried RTILs and non-dried enzymes [18]. Using six imidazolium RTILs in a biphasic media for the synthesis of isoamyl acetate resulted in a stabilized CALB which allowed the enzyme to be reused after 7–10 cycles with optimum conditions yielding nearly 100% isoamyl acetate conversion [19]. Lastly, we recently reported that HHP stabilized and activated CALB in hexane [20]. However, the effects of combining HHP and an RTIL–alcohol biphasic system on any enzyme-catalyzed reaction have yet to be documented.

The objectives of this research are: using the most effective RTIL and optimized biphasic conditions reported by Feher et al. [19], (1) determine the effect of HHP on the production of isoamyl acetate from isoamyl alcohol and acetic acid by free and immobilized lipase in an RTIL–alcohol biphasic system, (2) quantify the effect of HHP on the activation energy (E_a) of free lipase in an RTIL–alcohol biphasic system, and (3) quantify the effect of temperature on the activation volume ($\Delta V^{\ddagger}$) of free lipase in an RTIL–alcohol biphasic system.

2. Experimental

2.1. Materials

2.1.1. Enzyme and reagents

Lipase was from *C. antarctica* lipase B (CALB) expressed in *Aspergillus oryzae* and either immobilized on a macroporous acrylic resin (Novozyme 435®, 13.10 U/mg) or free (BioChemika # 65986, 1.51 U/mg) and obtained from Sigma–Aldrich (St. Louis, MO, USA). Isoamyl alcohol, glacial acetic acid, hexane, hexanol, toluene, and the RTIL 1-butyl-3-methylimidazolium hexafluoro-phosphate (bmimPF6) were obtained from Fisher Scientific (Pittsburgh, PA, USA). All solvents and substrates were held at −10 °C or on ice while preparing for assay.

2.1.2. HHP system

The HHP system consisted of a high pressure reactor (model U111), a high pressure micropump (model MP5), and a pump controller (MP5 micropump control unit) all from Unipress Equipment (Warsaw, Poland). The reactor was temperature controlled with a water jacket alternatively fed by two water baths (Isotemp 3016D); one cooling (5 °C) and another heating (40–80 °C ± 0.1 °C) from Fisher Scientific (Pittsburgh, PA, USA) and controlled by an array of pinch valves. Computer programs were written in LabVIEW and a data acquisition board (DAQ Card 6062E) from National Instruments (Austin, TX, USA) were used to collect temperature and pressure data and to control the heating/cooling valve array. A depiction of the HHP system has been previously published [20]. Stirring inside the reaction vial was done by a magnetic stir bar inside the reaction vessel and controlled by external spinning neodymium magnets on an AC motor type NS1-12 (Bodine Electric Company, Chicago, IL, USA). Reaction vials were made using 3-mL syringes with Luer-Lock™ tips (BD Franklin Lakes, NJ, USA) as shown in Fig. 1, which allowed substrate insertion with an opposing plunger while preventing solvent, substrate, or enzyme leaching during pressurization.

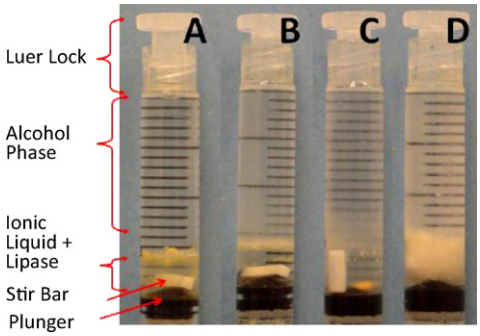

Fig. 1. Reaction cells filled with isoamyl alcohol, acetic acid free lipase and bmimPF6 (A) fresh, (B) after thermal treatment, (C) after incubation at 100 MPa, (D) after incubation at 400 MPa.

2.2. Methods

2.2.1. Reaction conditions

Enzyme was added (3.75 U/mL) into the reaction vial before adding isoamyl alcohol (76.5% v/v), ionic liquid (bmimPF6, 13.0% v/v), glacial acetic acid (9.45% v/v), toluene as a internal standard (0.39% v/v) and water (0.88% v/v), according to the optimized proportions presented by Feher et al. [19] who found that (bmimPF6) was the best among six tested imidazolium RTILs for the production of isoamyl acetate in the same biphasic system. The reaction vial plunger was moved into position to eliminate air bubbles then sealed with a Luer-Lock™ plug. The reaction vial was then placed in the high pressure reaction chamber being held at 5 °C. Polydimethylsiloxane silicone liquid (Accumetric, Inc., Elizabethtown, KY, USA) was added as hydraulic fluid to fill the reactor. The reactor was sealed and pressurized. After pressure reached the set-point, temperature was adjusted to the selected set-point. Upon completion of incubation, the temperature was returned to 5 °C, and the reactor was depressurized and opened. Adiabatic heating or cooling effects associated with HHP systems upon pressurization and depressurization were not significant factors because temperature controlled jacket limited temperature fluctuations to less than 2 °C. Controls run in the absence of enzyme with or without presence of RTIL at pressures up to 400 MPa and 80 °C for 2.5 h did not result in the production of any detectable amount of isoamyl acetate. This was similar to previous studies [19,21] where isoamyl acetate production was negligible in absence of a catalyst. Samples were treated and assayed in a randomized block design, blocked by temperature while pressure varied. Samples were blocked by temperature to reduce experimental time. Activation energies and volumes were calculated by linear regression of the linearized Arrhenius and Eyring equations respectively.

2.2.2. Quantitative analysis

At selected incubation times, the reactor was cooled, depressurized and the reaction vial was withdrawn. A 10-μL aliquot was drawn from the top (alcohol) phase through the Luer-Lock™ tip, diluted to 1 mL in hexane, and dried over excess ammonium sulfate anhydrous and sodium bicarbonate to remove remaining acetic acid and water. Then 10 μL of 7.9×10^{-5} mM hexanol in hexane was added as a second internal standard in preparation for GC–FID analysis. The toluene standard was added at the initiation of the reaction to account for error associated with transferring and sample loss, while the hexanol internal standard added prior to GC–FID injection accounts for error associated with quantita-

tive analysis. Reaction progress was monitored using GC–FID 5890 (HP, Palo Alto, CA, USA) with a ZB-5 column (30 m length × 0.53 mm ID × 1.5 μm thickness) at a gradient temperature from 50 to 90 °C at 5 °C min^{-1}. Injector temperature was held at 200 °C and FID detector at 250 °C. Peak identification and quantification was determined using pure standards and a calibration curve. Linear regression analysis was conducted to generate apparent initial reaction rates expressed as rate of isoamyl acetate formed per enzyme unit. Reaction stoichiometry has been recently reviewed and confirmed [22–24] which allowed kinetic analysis to be conducted by following isoamyl acetate formation.

2.2.3. Conversion using free and immobilized lipase

Free or immobilized lipase was incubated at either 0.1 or 300 MPa for 3 h at 42, 63.5, or 80 °C. After incubation was complete the reaction vial was withdrawn and an aliquot was analyzed as previously described. Reactions and analysis were both conducted in triplicate and error was reported as standard deviation.

2.2.4. Activation energy

Free lipase was incubated at either 0.1 or 300 MPa at 40.0, 49.4, 59.0, 69.3, or 80.0 °C. At 30-min intervals the reaction mixture was cooled, depressurized, and aliquots were taken and analyzed as described above to determine apparent initial rate. Linear regression analysis was conducted from five data points using Excel (Microsoft Office 2007) data analysis tool. Error was represented as standard error of the linear regression. Apparent E_a was determined using the Arrhenius equation (Eq. (1)).

$$\ln k = -\frac{E_a}{R} \cdot \frac{1}{T} + \ln k_{T_0} \quad (1)$$

where E_a is the activation energy, T is the absolute temperature, R is the ideal gas constant and k_{T_0} is the rate constant at a reference temperature T_0.

2.2.5. Activation volume

Free lipase was incubated at pressures from 0.1 to 500 MPa at 40 or 80 °C. At 30-min intervals the reaction mixture was cooled, depressurized, and aliquots were taken and analyzed as described above to determine apparent initial rate. Linear regression analysis was conducted using Excel (Microsoft Office 2007) data analysis. Error is represented as standard error of the linear regression. Activation volume ($\Delta V^{\ddagger}$) was determined using Eyring equation (Eq. (2)) [25],

$$\ln k = \frac{\Delta V^{\neq}}{RT} \cdot P + \ln k_P \quad (2)$$

where k is the rate constant P is the pressure and $\Delta V^{\neq}$ is the activation volume that represents the dependence of the reaction rate with pressure.

2.2.6. Ionic liquid recovery

After each reaction cycle, the biphasic mixture was decanted to separate the alcoholic and RTIL phase containing the lipase. The lipase and RTIL was then filtered through filter paper (type: P8) from Fisher Scientific (Pittsburgh, PA, USA) to remove protein aggregates. Then, to remove trace volatiles the RTIL was held at 70 °C under vacuum for 8 h.

3. Results and discussion

3.1. Qualitative observations

It was observed that the biphasic system's physical state appeared to change depending on the incubation conditions. As shown in Fig. 1A the native enzyme–RTIL mixture appears clear with yellow suspended enzyme. As shown in Fig. 1B, after incubation at 0.1 MPa the enzyme appears white and mostly accumulated at the RTIL–alcohol interface. Following incubations between 100 and 300 MPa (Fig. 1C), some enzyme appeared to be in a dispersed cloud above the RTIL–alcohol interface. After incubation at ≥400 MPa the enzyme–RTIL mixture appeared to be in a semi-solid white mass upon depressurization and cooling (shown in Fig. 1D). After several minutes at ambient pressure and temperature all treatments appeared as shown in Fig. 1B. This phenomenon was also observed with just the pure ionic liquid (data not shown). It remains unclear when this apparent phase change occurs; either during come-up, incubation, or come-down. Further analysis utilizing a high pressure optical cell is needed to determine accurately the conditions which induce this previously unseen phenomenon. This temporary immobilization shown in Fig. 1D has not been previously described, and may be useful in aiding the separation of phases between reaction cycles.

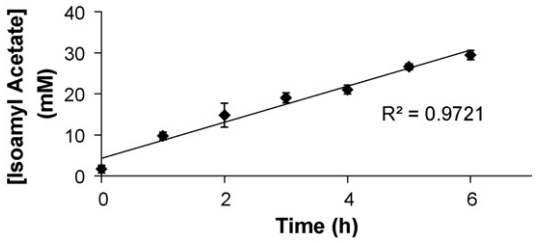

Fig. 2. Production of isoamyl acetate catalyzed by CALB (7.5 U/mL) in isoamyl alcohol bmimPF6 biphasic system at 40 °C. Error bars represent standard deviation of three replicates.

3.2. Conversion with free and immobilized lipase

Increasing temperature increased the production of isoamyl acetate for both free and immobilized lipase (which is within the linear range of conversion as shown in Fig. 2) as shown in Fig. 3A and B respectively. Production also increased at 300 MPa compared to at 0.1 MPa reaching a maximum relative increase of 4.9-fold at 63.5 °C with free lipase. Production was ~10-fold higher with free

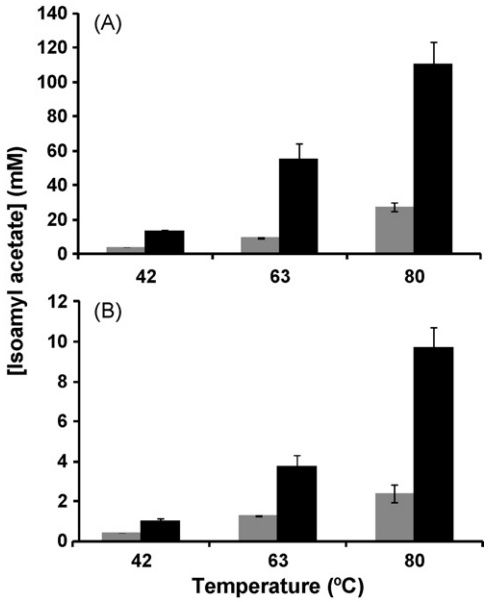

Fig. 3. Production of isoamyl acetate catalyzed by (A) free or (B) immobilized CALB after 3-h incubation per enzyme unit at (gray) 0.1 MPa or (black) 300 MPa. Error bars represent standard deviation of three replicates.

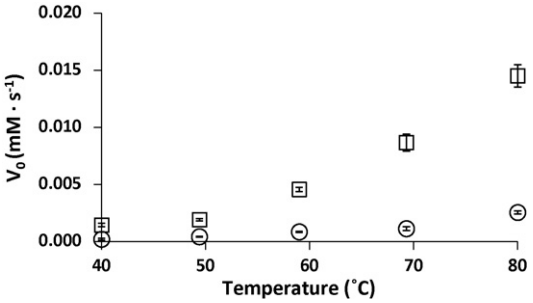

Fig. 4. Effect of temperature on apparent initial rate per enzyme unit of free CALB at (◯) 0.1 MPa or (□) 300 MPa. Error bars represent standard error of linear regression.

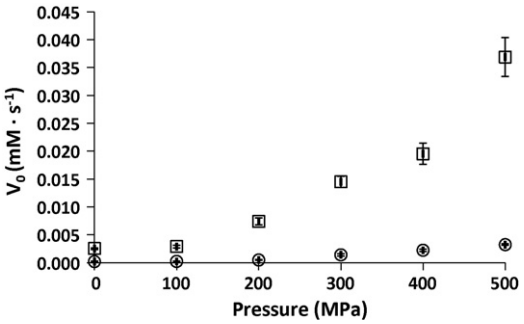

Fig. 6. Effect of pressure on apparent initial rate per unit enzyme of free CALB at (◯) 40 °C or (□) 80 °C. Error bars represent standard error of linear regression.

lipase compared to immobilized lipase. Based on these findings, the rest of the experiments used free lipase instead of immobilized lipase due to catalytic efficiency differences. Production may be higher with free lipase due to increased substrate-active site collisions associated with free lipases becoming more dispersed in the alcohol phase due to continuous stirring as discussed below.

Conversion of isoamyl alcohol and acetic acid to isoamyl acetate was more efficient in an RTIL–alcohol biphasic system than in the monophasic system previously described in hexane [20]. The maximal concentration after 95% conversion was about 60 mM after 1 h in hexane, while the maximal concentration after 95% conversion was about 1500 mM after 4 h in the RTIL–alcohol biphasic system. Furthermore, catalysis in an RTIL–alcohol biphasic system benefits from the advantage of having a potentially inhibitory substrate (such as acetic acid) in a different phase than the enzyme, thus reducing substrate inhibition [19]. For example, acetic acid was shown to have strong inhibitory effects on lipase at concentrations as low as 0.1 M in hexane [24]. High concentration of acetic acid may be affecting the pH of the aqueous mono-layer around the enzyme. However, acetic acid concentration can be increased up to 3.6 M in a "solvent free" (isoamyl alcohol based solvent) system before experiencing lipase inactivation [26]. Therefore, utilization of the RTIL–alcohol biphasic system benefits from the lipase being in a separate phase than the acetic acid and from the increased allowable concentrations of acetic acid due to being in the alcohol based "solvent free" system.

3.3. Determination of activation energy

Temperature effects on apparent initial rate of free lipase-catalyzed isoamyl acetate synthesis are shown in Fig. 4 and transformed into Arrhenius plots in Fig. 5. The apparent activation energy (E_a) derived from the slope of the lines in Fig. 5 is 55.6 ± 4.2 and 56.2 ± 4.6 at 0.1 and 300 MPa respectively. These E_a values are higher than similar previous studies in an RTIL-biphasic mixture (27.3 kJ mol^{-1}) [19], in a solvent free system (28.7 kJ mol^{-1}) [26], and in hexane (35.7–47.8 kJ mol^{-1}) at various pressures [20].

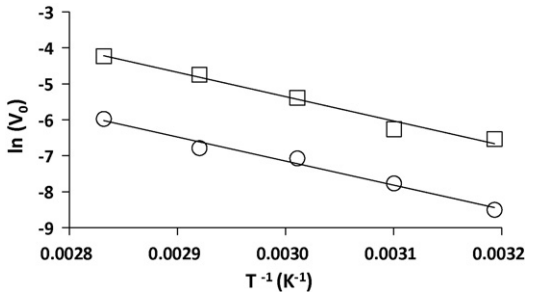

Fig. 5. Linearized effect of temperature on initial rate (Arrhenius plot) at (◯) 0.1 MPa and (□) 300 MPa.

With regards to E_a in RTIL-biphasic mixture, differences may be attributed to use of different immobilization techniques and temperatures ranges, since this study examined free lipase at five temperatures from 40 and 80 °C while Feher et al. [19] used immobilized lipase at only four temperatures between 30 and 60 °C with no mention of the error. Likewise, Guvenc et al. [26] only used three temperatures from 30 to 50 °C to determine E_a with no mention of error. Finally, the E_a results from isoamyl acetate synthesis in hexane [20] used the same lipase but it was immobilized, which may account for differences in E_a. Unlike previous results in hexane, this study in RTIL–alcohol biphasic system found a continuous increase in activity within the temperature range tested (up to 80 °C) at all pressures tested. This may be attributed to the stabilization effects of ionic liquid and/or high pressure on lipase. The possible mechanisms of pressure induced stabilization have been previously described [27]. Although the stabilization effects of RTILs have yet to be fully understood, they are thought to be related to the RTILs ability to; (1) negate substrate or product inhibitory action, (2) prevent essential water loss associated with thermal denaturation as organic solvents do, (3) remove excess water produced from esterification reactions also as organic solvents do, and (4) have electrostatic interactions with the enzyme. Furthermore, Lozano et al. [7] suggested that since RTILs form a strong ionic matrix, and added enzyme is considered dispersed or included but not dissolved, an optimal microenvironment may be formed for improved stability and activity. This last proposed mechanism is the most likely since organic solvents also have interactions with substrates and/or products, restrict essential water loss, facilitate excess water removal, and can have electrostatic interactions with the enzyme but do not possess the ability to disperse an enzyme like an RTIL. However, the activities are different when using the same enzyme and the same reaction but changing the solvent system. It is assumed that apparent initial rates are representative of the actual initial rates. It is also assumed that the substrate (acetic acid) is available to CALB in excess ($K_M \ll [S]$). Although the K_M of this reaction has not been determined in this RTIL-biphasic mixture, it has been determined in monophasic hexane [20] to be from 2 to 38 mM (acetic acid) depending on the reaction conditions. This reaction in the RTIL–alcohol biphasic system utilized acetic acid at 1589.5 mM which is much greater than the K_M determined in hexane.

3.4. Determination of activation volume

As shown in Fig. 6, increasing pressure increased the apparent initial reaction rate of free lipase at 40 and 80 °C from 0.1 to 500 MPa. Pressure effects resulted in a 15- and 14-fold increase in apparent initial rate from 0.1 to 500 MPa at 40 and 80 °C respectively. The activation volume ($\Delta V^{\ddagger}$) was -16.1 ± 1.5 and -16.7 ± 1.4 cm^3 mol^{-1} at 40 and 80 °C respectively and was derived

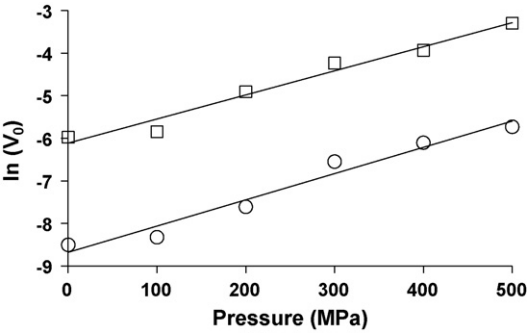

Fig. 7. Linearized effect of pressure on initial rate at (○) 40 °C and (□) 80 °C.

from the slope of the plots shown in Fig. 7. The $\Delta V^{\ddagger}$ was negative and unchanged by temperature, therefore increasing pressure continuously increased initial rates regardless of temperature. These results are different than in hexane where the negative $\Delta V^{\ddagger}$ of CALB catalyzed isoamyl acetate synthesis in hexane was decreased by increasing temperature from -21.6 ± 2.9 at 40 °C to -12.9 ± 1.7 at 80 °C [20]. This study found a negative $\Delta V^{\ddagger}$ throughout the entire pressure range tested (0.1–500 MPa) which is in contrast to CALB in hexane that found a negative $\Delta V^{\ddagger}$ from 0.1 to 200 MPa and positive $\Delta V^{\ddagger}$ from 300 to 500 MPa. These differences between temperature affects on $\Delta V^{\ddagger}$ indicate that the solvent (either hexane or RTIL) has an effect on pressure induced activation.

Similarly, the activity and selectivity of free *C. antarctica* lipase B (CALB)-catalyzed trans-esterification of butyl butanoate (a flavor ester) were both higher in water-immiscible but not water-miscible ionic liquids than in hexane [28]. This was explained by Rios et al. [28] as being due to the increasing hydrophobicity of the RTIL involves a better preservation of the essential water layer, reducing the direct protein-ion interactions. Likewise, CALB increased activity in all four RTILs tested in comparison with hexane and 1-butanol. Also, according to Lozano et al. [7], the lipase was "over-stabilized" in ionic liquids as evidenced when the reuse of free lipase in continuous operation cycles showed a half-life time 2300 times greater than that when the enzyme was incubated in the absence of substrate, and had selectivity higher than 90%. Interestingly, RTIL pretreated lipase also had higher activity and stability than untreated lipase for the hydrolysis of p-nitrophenol butyrate in phosphate buffer. According to Nara et al. [6], pretreated lipase maintained activity after seven days at 60 °C in phosphate buffer, while untreated lipase was fully inactivated after only 12 h at the same conditions. It was suggested that this may be caused by the RTIL-coated lipase having altered structure thus enhancing activity and stability of the lipase. More recently, Zhao studied [18] microwave heating effects on CALB activity in twenty different RTILs. Higher activity was observed compared to conventional thermal heating and varied depending on RTIL purity, polarity, hydrophobicity, and viscosity.

The combination of RTIL and HHP may be altering the tertiary structure of the lipase, thus activating and/or stabilizing the lipase. Stabilization allows the activation effects to be observed at a wider temperature–pressure range.

4. Conclusions

This is the first report of the effects of HHP on enzyme catalysis in an RTIL–alcohol biphasic system. Although the temperature range was narrow, it covered most commonly used enzyme reaction conditions. Up to 25-fold CALB activity increase was attributed to increased HHP for the synthesis of isoamyl acetate. The conversion of isoamyl alcohol and acetic acid to isoamyl acetate in an RTIL–alcohol biphasic system is 10 times more efficient with free than with immobilized lipase. Also, rate of conversion was higher at elevated pressure for both free and immobilized lipase. High pressure had no significant effect on the activation energy of free lipase and temperature had no significant effect on the activation volume, which was negative throughout the pressure range tested. Free lipase is better suited than immobilized lipase in this system which will allow future studies to proceed more effectively. Lastly, the observation of the temporary immobilization phenomenon is a new and previously undocumented occurrence. This unique phenomenon may aid in recycling during a batch process by increasing the ease of decanting procedures while minimizing enzyme and RTIL loss.

Acknowledgement

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References

[1] F.W. Welsh, R.E. Williams, K.H. Dawson, J. Food Sci. 55 (1990) 1679–1682.
[2] P. Mahapatra, A. Kumari, G.V. Kumar, R. Banerjee, A. Nag, Biocatal. Biotransform. 27 (2009) 124–130.
[3] F. van Rantwijk, R.A. Sheldon, Chem. Rev. 107 (2007) 2757–2785.
[4] E. Feher, B. Major, K. Belafi-Bako, L. Gubicza, Biochem. Soc. Trans. 35 (2007) 1624–1627.
[5] D.T. Dang, S.H. Ha, S.-M. Lee, W.-J. Chang, Y.-M. Koo, J. Mol. Catal. B: Enzym. 45 (2007) 118–121.
[6] S.J. Nara, J.R. Harjani, M.M. Salunkhe, Tetrahedron Lett. 43 (2002) 2979–2982.
[7] P. Lozano, T. De Diego, D. Carrie, M. Vaultier, J.L. Iborra, Biotechnol. Lett. 23 (2001) 1529–1533.
[8] W.-Y. Lou, M.-H. Zong, Y.-Y. Liu, J.-F. Wang, J. Biotechnol. 125 (2006) 64–74.
[9] M. Noel, P. Lozano, M. Vaultier, J.L. Iborra, Biotechnol. Lett. 26 (2004) 301–306.
[10] E. Feher, B. Major, K. Belafi-Bako, L. Gubicza, Desalination 241 (2009) 8–13.
[11] F.J. Hernández, A.P. de los Ríos, D. Gómez, M. Rubio, G. Víllora, Appl. Catal. B: Environ. 67 (2006) 121–126.
[12] L. Cao, L.v. Langen, R.A. Sheldon, Curr. Opin. Biotechnol. 14 (2003) 387–394.
[13] P. Lozano, A.B. Perez-Marin, T. De Diego, D. Gomez, D. Paolucci-Jeanjean, M.P. Belleville, G.M. Rios, J.L. Iborra, J. Membr. Sci. 201 (2002) 55–64.
[14] P. Lozano, T. De Diego, D. Carrie, M. Vaultier, J.L. Iborra, in: R.D. Rodgers, K.R. Seddon (Eds.), Enzymatic Catalysis in Ionic Liquids and Supercritical Carbon Dioxide, 2003, pp. 239–250.
[15] P. Lozano, T. De Diego, D. Carrie, M. Vaultier, J.L. Iborra, Enzymatic Ester Synthesis in Ionic Liquids, 2003, pp. 9–13.
[16] P. Lozano, T. De Diego, T. Sauer, M. Vaultier, S. Gmouh, J.L. Iborra, J. Supercrit. Fluid 40 (2007) 93–100.
[17] A. Pohar, I. Plazl, P. Znidarsic-Plazl, Lab Chip 9 (2009).
[18] H. Zhao, G.A. Baker, Z.Y. Song, O. Olubajo, L. Zanders, S.M. Campbell, J. Mol. Catal. B-Enzym. 57 (2009) 149–157.
[19] E. Feher, V. Illeova, I. Kelemen-Horvath, K. Belafi-Bako, M. Polakovic, L. Gubicza, J. Mol. Catal. B: Enzym. 50 (2008) 28–32.
[20] M.J. Eisenmenger, J.I. Reyes-De-Corcuera, Enzyme. Microb. Tech. 45 (2009) 118–125.
[21] L.-s. Lee, R.-g. Lin, Fluid Phase Equilib. 165 (1999) 261–278.
[22] M.D. Romero, L. Calvo, C. Alba, A. Daneshfar, H.S. Ghaziaskar, Enzyme Microb. Technol. 37 (2005) 42–48.
[23] A. Guvenc, N. Kapucu, E. Bayraktar, U. Mehmetoglu, Chem. Eng. Commun. 190 (2003) 948–961.
[24] M.D. Romero, L. Calvo, C. Alba, A. Daneshfar, J. Biotechnol. 127 (2007) 269–277.
[25] H. Eyring, J.L. Magee, J. Cell. Comp. Physiol. 20 (1942) 169–177.
[26] A. Guvenc, N. Kapucu, U. Mehmetoglu, Process Biochem. 38 (2002) 379–386.
[27] M.J. Eisenmenger, J.I. Reyes-De-Corcuera, Enzyme Microb. Technol. 45 (2009) 331–347.
[28] A. Rios, F.J. Hernandez-Fernandez, F. Tomas-Alonso, D. Gomez, G. Villora, Flavour. Frag. J. 23 (2008) 319–322.