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Effects of physicochemical properties of ionic liquids on butyl acetate synthesis using *Candida antarctica* lipase B

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Abstract−Butyl acetate synthesis in imidazolium ring-based ionic liquids (ILs) containing trifluoromethanesulfonate, $[TfO]^-$ using *Candida antarctica* lipase B was demonstrated. Among the ILs tested, the highest butyl acetate production and its initial rate was achieved in [Bmim][TfO]. Compared with *tert*-butanol, which has been the conventional solvent for transesterification reactions, the initial reaction and conversion rate were approximately 1.4 and 1.1 times, respectively, higher. Some physicochemical parameters, such as viscosity, hydrophobicity, and partial charge of ILs were examined to find the relations which influence reaction rates. Although only weak correlations between each parameter and enzyme activity were found, it shed a light on understanding the generalization complexity of ILs for enzyme reaction.

Key words: Ionic Liquids, Butyl Acetate, CALB, Viscosity, Hydrophobicity

INTRODUCTION

Butyl acetate is a pivotal solvent not only in the chemical industry but also in the food industry [1]. It has been commonly used for manufacturing paint, varnish and coating materials [2]. Also, butyl acetate is able to be used as a natural flavor ester, which is often expensive material for commercial usage, due to lower impact on the environment [3]. Conventionally, butyl acetate has been synthesized by chemical methods adding liquid acids as a catalyst [4]; however, those methods require post-treatment. Several researchers have reported that the usage of bio-catalysts is suitable in various esterification reactions under milder conditions [5-8]. Lipase B from *Candida antarctica* (CALB) is an efficient bio-catalyst for esterification [9]. Bio-catalysis has been widely used because of high activity, enantioselectivity, thermal stability, and the choice of a wide range of materials as substrates [10]. So far, organic solvents have been widely used in ester synthesis processes using CALB; however, its toxicity, and flammability yields severe problems on the environment. Room temperature ionic liquids (ILs) are organic salts that are not crystallized at room temperature [11]. ILs are being spotlighted as "green solvent," and have attracted significant attention in recent years because, unlike organic solvents, ILs have very low vapor pressure, thus being advantageous to the environment [12-17]. Our group has investigated that the conversion rate and activity of esterification reaction using CALB was increased when imidazolium ILs containing trifluoromethanesulfonate anion ($[TfO]^-$) was used as a reaction medium compared to organic solvents or other ILs, which have been known as a good solvent for transesterification [18,19]. In this study, butyl acetate synthesis reactions were performed using CALB in four kinds of [TfO] containing ILs to measure the activity and productivity. Also, the parameters which can affect this reaction were measured to obtain the correlation between reaction activity and these parameters.

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MATERIALS AND METHODS

1. Materials

Lyophilized CALB was obtained from C-LEcta (Leipzig, Germany). The following chemicals, vinyl acetate, *n*-butanol, *tert*-butanol, butyl acetate, were purchased from Sigma Aldrich (St. Louis, USA). 1-ethyl-3-methylimidazolium trifluoromethanesulfonate ([Emim][TfO]), 1-butyl-3-methylimidazolium trifluoromethanesulfonate, ([Bmim][TfO]), 1-hexyl-3-methylimidazolium trifluoromethanesulfonate ([Hmim][TfO]), and 1-metyl-3-octylimidazolium trifluoromethanesulfonate ([Omim][TfO]) were synthesized and purified by C-TRI (Suwon, Korea) (Fig. 1). The solvents used in this study were of analytical grade and used without additional purification. All solvents contained less than 0.1% (w/w) of water.

2. Butyl Acetate Synthesis Using CALB in Ionic Liquids

For elimination of water, which is one of the critical impurities in reaction in ILs, all ILs were stored in vacuum oven at 100 °C for more than 24 hrs. The water content of all ILs and *tert*-butanol was estimated by Karl-Fischer Titration (831-KF Coulometer, Metrohm, Switzerland) using HYDRANAL-Coulomat AK reagent. All the reactions were carried out in 5 ml reaction vials with magnetic bar. *n*-butanol (0.05 mol/*l*) and CALB (50 mg) were added into 1 ml each of [Emim][TfO], [Bmim][TfO], [Hmim][TfO], [Omim][TfO], and *tert*-butanol. These mixtures were placed in reaction block for 1 hr at 300 rpm, 27 °C for preheating. By adding 0.1 M vinyl acetate into each mixture, the reactions were started. To measure butyl acetate in each reaction, gas chromatography (CP 9001, Chrompack, Netherlands) equipped with DB-23 capillary column (30 m*0.25 mm, film thickness 0.25 mm; J&W Scientific, USA) was used. 20 μl aliquots were periodically taken from solutions and diluted with 80 μl of methanol. Further treatments to remove ILs in the sample were not needed, since glass wool, which is located in the injector of the GC, prevented ILs from passing into the column. The oven temperature was kept at 35 °C for 4 min, followed by the temperature increase of 30 °C/min up to 120 °C, which was then maintained at same temperature for 2 min. The injector and detector tempera-

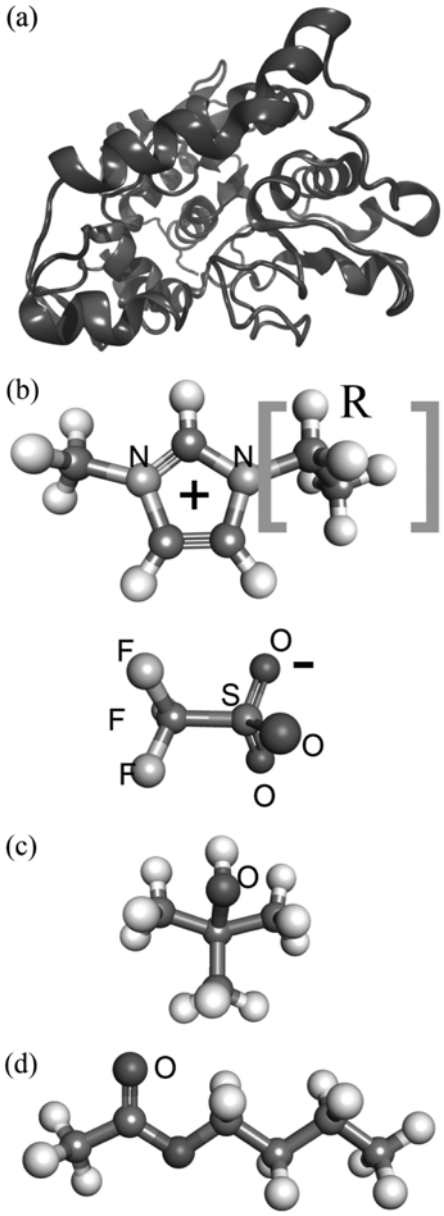

Fig. 1. Chemical structure of materials used in this study. (a) Cartoon model of CALB obtained by PDB (code: 1TCA), (b) 1-Alkyl-3-methylimidazolium trifluoromethanesulfonate ([Rmim][TfO], R=ethyl, butyl, hexyl, octyl), (c) *tert*-butanol, (d) butyl acetate. All atoms not otherwise labeled are carbon (Dark), and hydrogen (Light).

ture was set at 220 and 275 °C, respectively. The pure nitrogen gas was used as a carrier gas. All experiments were performed in triplicate in same condition and average values were presented.

3. Viscosity Measurement of Ionic Liquids

The viscosity of each solvent was measured by Ubbelohde calibrated viscometer (CANNON, USA) at 27 °C. Since enzyme reactions were generally performed in ILs where water contents are less than 1,000 ppm (w/w) [20], and no significant difference of viscosity of ILs was observed when water content ranged from 10 ppm to 1,000 ppm [21], all ILs were dried in vacuum oven at high temperature (150 °C) until water content was under 1,000 ppm. The water content of solvents was carefully measured by Karl-Fischer Titration using HYDRANAL-Coulomat AK reagent. The temperature was strictly controlled by water-bath. All experiments were performed in triplicate at same condition and average values were presented.

4. Hydrophobicity Prediction for Ionic Liquids

To predict hydrophobicity of each solvent, KOWWIN and ALOGPS 2.1 software were used [22,23]. These tools have been used for the prediction of log *P* values of ILs [24]. The chemical structures which are input for measuring hydrophobicity using computer software were drawn by Discovery Studio Visualization 2.5 and JME.

5. Partial Charge Prediction for Ionic Liquids

The partial charges of ILs were taken from the force field developed by Sambasivarao et al. [25]; those of *tert*-butanol were referred from Lee et al. [26]; 3D modeling related with van der Waal's surface was illustrated by Discovery Studio Visualization 3.1.

RESULTS AND DISCUSSION

1. Production of Butyl Acetate

We performed butyl acetate synthesis reaction for 12 hr to measure the conversion and initial reaction rates of synthesized butyl

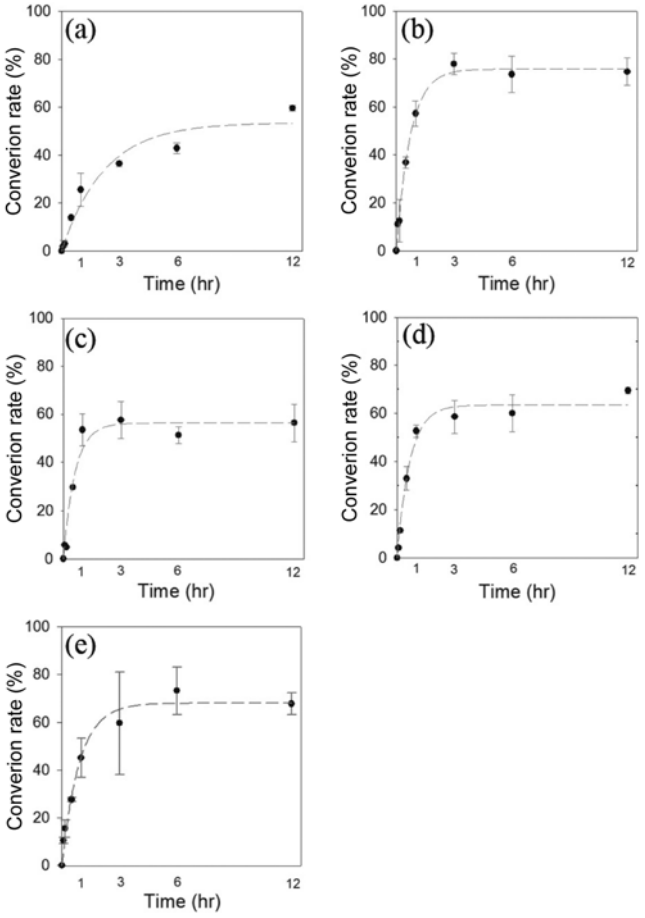

Fig. 2. Conversion rate of production of butyl acetate using CALB at 27 °C during 12hrs reaction. (a) [Emim][TfO], (b) [Bmim][TfO], (c) [Hmim][TfO], (d) [Omim][TfO], and (e) *tert*-butanol. Dotted line indicates the regression of experimental data.

Table 1. Initial reaction rate of butyl acetate synthesis in solvents used in this study

Solvents	Initial reaction rate (mmol/L·hr)
[Emim][TfO]	0.20
[Bmim][TfO]	0.85
[Hmim][TfO]	0.77
[Omim][TfO]	0.74
tert-Butanol	0.62

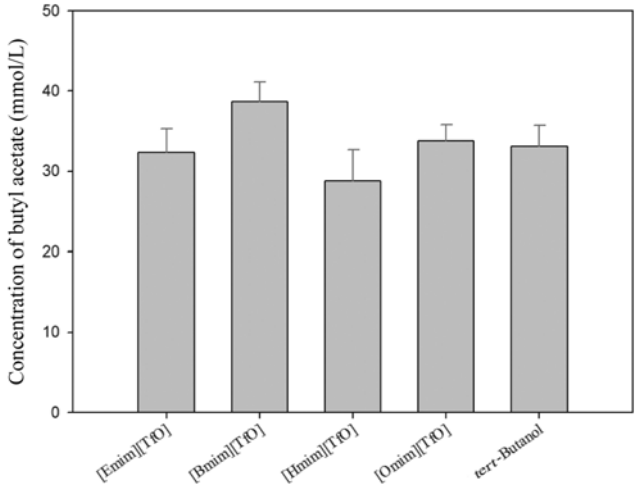

Fig. 3. Comparison of butyl acetate production in ILs and *tert*-butanol after 12 hrs reaction.

acetate using CALB as an enzyme in various solvents. All reactions reached equilibrium state after 3 hrs, except in [Emim][TfO]. The highest conversion rate was obtained in [Bmim][TfO], while the lowest conversion rate was acquired in [Emim][TfO] (Fig .2). In case of *tert*-butanol, which has been commonly used as a conventional reaction medium for transesterification reaction [27,28], the conversion rate was lower than [Bmim][TfO], but it showed higher conversion rate than other ILs. Table 1 represents initial reaction rate of butyl acetate synthesis, which was calculated through differentiation of the fit curve which ranges from 0 to 1 hr plotted as a dotted line in Fig. 2. The highest initial reaction rate was obtained in [Bmim][TfO], and the rate decreased in the order of [Hmim][TfO], [Omim][TfO], *tert*-butanol, and [Emim][TfO]. Fig. 3 shows the production of butyl acetate after 12 hr reaction in four kinds of ILs compared with *tert*-butanol. The maximum productivity of butyl acetate was obtained in [Bmim][TfO], which was approximately 1.1 times higher than in *tert*-butanol. Unlike the initial reaction rate of butyl acetate synthesis explained above, the production of butyl acetate in [Emim][TfO] was higher than that in [Hmim][TfO]. Previous researchers have reported that alkyl chain length highly affects enzyme reactions, and the alkyl chain of imidazolium could change various properties which lead to enzyme activity and stability [29, 30]. Furthermore, several parameters, such as hydrophobicity, viscosity, and ion coordinating ability, are known to influence enzyme activity. To clarify the relationship between enzyme activity and these parameters, additional studies were performed.

2. Viscosity

Table 2 shows the viscosity of ILs used in this study. As the alkyl chain length of imidazolium ring in ILs increased, viscosity was also increased. Even though the absolute value of viscosity differed from that referred from previous researches, the viscosity showed upward tendency as alkyl chain length was increased. Since viscosity is generally independent of temperature in liquid, and tends to decrease as temperature increases, the main reason for the difference of viscosity between experimental and reference values might be caused by temperature.

In general, viscosity is one of the key factors which affect the enzymatic reactions, because it plays a significant role in mass-transfer of substrates and enzyme. A lower reaction rate would be expected in solvents with higher viscosity [36]. In this study, the result agreed with the correlation between the enzyme activity and viscosity, except the case of [Emim][TfO]. There is a general tendency that viscosity has a negative effect on the enzyme activity for butyl acetate synthesis in ILs. Nevertheless, other parameters need to be considered to explain the exception of [Emim][TfO], which had the lowest viscosity among the media conducted in this study, but with low initial reaction rate. It is notable that [Bmim][TfO] showed the highest reaction rate, while its viscosity was approximately 25 times higher than that of *tert*-butanol.

3. Hydrophobicity and Partial Charges of ILs

Hydrophobicity has been considered as one of the key parameters of enzyme activity in organic solvents [29,38]. The partition coefficient, also known as log *P* of a solvent in an octanol/water mixture, has been used as a standard of hydrophobicity [22]. We predicted hydrophobicity of ILs and *tert*-butanol by using log *P* calculation tools named ALOGPS, and KOWWIN [23]. As shown in Table 3, two simulation results showed that all ILs except [Omim][TfO] are predicted to be hydrophilic. Especially, hydrophobicity of [Emim][TfO] and [Bmim][TfO] was even lower than that of ethanol (−0.24) [39], which has been known as a typical hydrophilic

Table 2. Viscosity of solvents used in this study at 27 °C

Solvents	Water contents (ppm)	Kinematic viscosity (mm^2/s)	Density (g/ml)	Viscosity (cP)	Ref.[a] (cP)
[Emim][TfO]	941.90	28.24	1.39 [31]	39.25	45 [31]
[Bmim][TfO]	229.20	59.86	1.30 [32]	77.90	90 [33]
[Hmim][TfO]	460.00	103.34	1.20 [31]	124.01	160 [31]
[Omim][TfO]	352.40	159.51	1.19 [34]	189.81	240[b]
tert-Butanol	-	-	0.78 [35]	-	4.31 [19]

[a]All reference data related with viscosity were determined at 20-25 °C

[b]Data referred to ionic liquid chart issued by Sigma-Aldrich and Fluka

Table 3. Partition coefficient of ILs and *tert*-butanol calculated by log *P* prediction programs

Solvents	Hydrophobicity		
	ALOGPS	KOWWIN	Ref.
[Emim][TfO]	−1.28	−2.82	-
[Bmim][TfO]	−0.75	−1.84	−1.63 [20]
[Hmim][TfO]	−0.06	−0.86	-
[Omim][TfO]	0.65	0.13	-
tert-Butanol	0.70	0.73	0.60 [37]

solvent. Previous study indicated that the solvents with a high log *P* are more hydrophobic and thus more favorable for enzyme reaction than those with a low log *P* [39]. It is because the solvents with higher hydrophilic properties have more tendencies to attract essential waters around enzymes which counterpoise the stability [40]. However, though most of the ILs used in this study had hydrophilic characteristics, the reaction rate of butyl acetate synthesis in ILs was similar to that in hydrophobic organic solvent, *tert*-butanol, or even better in case of [Bmim][TfO]. We observed that hydrophobicity had no direct influence on enzyme activity for butyl acetate synthesis. The consideration that the loss of essential water is a major cause of deactivating enzyme seems unlikely in case of CALB, which stays active under strictly anhydrous conditions [9,41]. Many exceptions to this rule have been reported [29]. However, though ILs containing imidazolium ring as a cation have been spotlighted as suitable solvents for enzymatic reaction [42], the extremely low experimental log P value of [Emim][TfO] (−2.82) may explain the low enzyme activity in this study.

It has been demonstrated that ILs containing a strongly coordinating anion, such as nitrate or chloride, are able to dissolve the enzyme. However, these anion groups can change secondary structure of enzyme by intra-molecular hydrogen bonding interruption, yielding deactivation [12,43-45]. As shown in Fig. 4, the oxygen region of $[TfO]^-$ has maximum partial charge of −0.70. It indicates that $[TfO]^-$ had stronger coordination compared with $[NO_3]^-$ (−0.60)

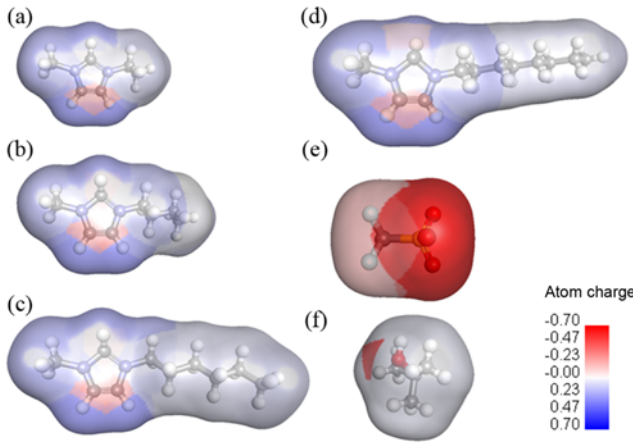

Fig. 4. Partial charge of (a) [Emim], (b) [Bmim], (c) [Hmim], (d) [Omim], (e) [TfO], and (f) *tert*-butanol. The blue color represents the tendency for positive atom charges, and the red color shows the tendency for negative atom charges.

[25], which can lead to enzyme deactivation. A possible explanation for the low enzyme activity in [Emim][TfO] may be drawn from the correlation between enzyme reaction and partial charge. The relatively small size of weakly coordinating cation, $[Emim]^+$, might be insufficient to offset the strongly coordinating anion in case of [Emim][TfO], thereby yielding enzyme deactivation. In contrast, it is plausible that other cations with bigger size than $[Emim]^+$ efficiently countervail the strong coordinating anion, $[TfO]^-$. In the sense of enzyme activity, [Hmim][TfO] and [Omim][TfO] are assumed to be able to offset the negative effect of anion, of strong coordination, but high viscosity (124 cP and 190 cP, respectively) might decrease the reaction rate by limiting mass-transfer rate between substrates and enzyme. In the same way, it would be explained that $[Bmim]^+$ effectively counterbalances the strongly coordinating anion, with not much mass transfer limitation with proper viscosity of 90 cP as well.

CONCLUSION

Our experimental results showed that using ILs with imidazolium as cation is a potential for enzymatic synthesis of butyl acetate. [Bmim][TfO]; especially, it showed the highest reaction rate, when compared with *tert*-butanol which has been the conventional medium for butyl acetate synthesis. The viscosity of ILs is found to influence the reaction rate by mass transfer limitation. The exceptional case of [Emim][TfO] was tried to be explained by hydrophobicity and partial charges of ILs. The extremely low log *P* value of [Emim][TfO] is considered to cause low enzyme activity, along with the fact that relatively small size of $[Emim]^+$ is insufficient to offset the strongly coordinating $[TfO]^-$. It is concluded that various parameters and complexity of their interaction must be comprehensively considered to understand enzymatic reactions in ILs.

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NOMENCLATURE

[Emim][TfO] : 1-ethyl-3methylimidazolium trifluoromethansulfonate
[Bmim][TfO] : 1-butyl-3methylimidazolium trifluoromethansulfonate
[Hmim][TfO] : 1-hexyl-3methylimidazolium trifluoromethansulfonate
[Omim][TfO] : 1-metyl-3octylimidazolium trifluoromethansulfonate
CALB : *Candida antarctica* Lipase B

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