

Lipase-catalyzed Transesterification in Several Reaction Systems: An Application of Room Temperature Ionic Liquids for Bi-phasic Production of *n*-Butyl Acetate

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Abstract Organic solvents are widely used in biotransformation systems. There are many efforts to reduce the consumption of organic solvents because of their toxicity to the environment and human health. In recent years, several groups have started to explore novel organic solvents called room temperature ionic liquids in order to substitute conventional organic solvents. In this work, lipase-catalyzed transesterification in several uni- and bi-phasic systems was studied. Two representative hydrophobic ionic liquids based on 1-butyl-3-methylimidazolium coupled with hexafluorophosphate ([BMIM][PF₆]) and bis(trifluoromethylsulfonyl) imide ([BMIM][Tf₂N]) were employed as reaction media for the transesterification of *n*-butanol. The commercial lipase, Novozym 435, was used for the transesterification reaction with vinyl acetate as an acyl donor. The conversion yield was increased around 10% in a water/[BMIM][Tf₂N] bi-phasic system compared with that in a water/hexane system. A higher distribution of substrates into the water phase is believed to enhance the conversion yield in a water/[BMIM][Tf₂N] system. Partition coefficients of the substrates in the water/[BMIM][Tf₂N] bi-phasic system were higher than three times that found in the water/hexane system, while *n*-butyl acetate showed a similar distribution in both systems. Thus, RTILs appear to be a promising substitute of organic solvents in some biotransformation systems.

Keywords: ionic liquid, lipase, transesterification, bi-phasic system, butyl acetate

INTRODUCTION

The development of cost effective production processes and efficient purification techniques is important for industrial scale production systems [1]. Simultaneous production and recovery using various bi-phasic reaction system is an important technique [2]. Among various enzymes, lipases are used as catalysts in organic solvents for many reactions, such as esterification, transesterification (alcoholysis), perhydrolysis, and ammoniolysis (amide synthesis) [3]. However, the original function of a lipase is hydrolysis of oils [4]. Recently, novel applications of lipases have been developed for selective acylation and deacylation of natural and synthetic compounds and for resolution of racemic alcohols and acids [5]. However, environmentally harmful organic solvents are frequently used in lipase-catalyzed production systems because of the low solubility of hydrophobic substrates in water. Residuals of the toxic organic solvents in a product are another problem in the fine chemical and pharmaceutical industries.

Room-temperature ionic liquids (RTILs) are compounds composed only of ions and are liquid at room temperature. RTILs can substitute organic solvents for many chemical and biological reactions as environmentally-friendly media due to their low vapor pressure, high thermal stability and high solubility of various hydrophilic and hydrophobic molecules [6]. Thus, RTILs have increasing relevance as green and effective reaction media for the future. Vast tunable physical and chemical properties are other important advantages of RTILs through appropriate modification of the cation and anion, such as polarity, hydrophobicity and miscibility behaviors [7]. Moreover, RTILs can be recycled and reused by simple processes because of their low vapor pressure and heat stability [8,9]. Several groups recently reported enzyme-catalyzed reactions in RTILs and suggested RTILs as a substitute of conventional organic solvents [10].

The current study attempted a production of *n*-butyl acetate in uni- and bi-phasic systems using RTILs. Two representative hydrophobic ionic liquids based on 1-butyl-3-methylimidazolium coupled with hexafluorophosphate and bis(trifluoromethylsulfonyl) imide were employed as reaction media. A lipase-catalyzed conversion of butanol was selected as a model biocatalysis system with vinyl acetate as the acyl donor. The production yield

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and partition of the substances were compared with the conventional organic solvent system, hexane.

MATERIALS AND METHODS

Materials

Novozym 435 (lipase B from *Candida Antarctica*; immobilized on macro-porous polyacrylic resin beads, Novo Nordisk Co., Denmark) was used for the transesterification reaction. Two substrates, *n*-butanol (Sigma-Aldrich Co., USA) and vinyl acetate (Junsei Chem. Co., Japan) were used as an acyl acceptor and donor, respectively. *n*-Butyl acetate was purchased from Sigma-Aldrich Co. RTILs, 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF₆]) and bis[(trifluoromethyl sulfonyl)imide] ([BMIM][Tf₂N]) were gifted from C-Tri Co. (Korea). Hexane and acetonitrile were purchased from Acros Organics Co. (USA) and Fisher Scientific Co. (USA), respectively. A reciprocally shaking water bath was used for mixing and temperature control during the reaction. The reciprocal mixing speed was 150 rpm.

Analytical Methods

Concentrations of the substrates and product were analyzed by a gas chromatograph (M-600D, Young-Lin Instrument Co., Korea) equipped with an HP-INNOWAX column (30 m × 0.250 mm) and an FID detector. The split ratio of the sample was 1:25 and nitrogen was used as a carrier gas. The temperature of the injector and detector was 160°C. The temperature of the oven was initially kept at 100°C for 1 min and then ascended stepwise to 160°C at the rate of 10°C/min. In each sample, 10% (v/v) of 1-propanol was added as an internal standard prior to the analysis.

Lipase-catalyzed Transesterification in Uni-phasic System

RTILs ([BMIM][PF₆] or [BMIM][Tf₂N]) or organic solvents (acetonitrile or hexane) were used for the uni-phasic reaction medium. *n*-Butanol (4.0 mM, 370 μL) and vinyl acetate (1.1 mM, 100 μL) were added in 2 mL of each reaction medium. The reaction was started by the addition of 3 mg of Novozym 435. The conversion yields were compared at different temperatures: 40, 50, 60, and 70°C, respectively. The reaction was stopped after 24 h by the addition of the mixture of acetone and hydrochloric acid (volume ratio, 99:1, 200 μL) on 20 μL of the reaction solution.

Lipase-catalyzed Transesterification in Bi-phasic System

The bi-phasic lipase-catalyzed transesterification medium was formed with 1 mL of deionized water and the same volume of hydrophobic solvents in a glass vial. RTILs ([BMIM][PF₆], [BMIM][Tf₂N]) or hexane was

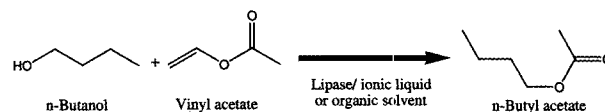


Fig. 1. Schematic drawing of the transesterification reaction of *n*-butanol catalyzed by lipase using vinyl acetate as an acyl donor.

used as a water-immiscible solvent. *n*-Butanol (4.0 mM, 370 μL) and vinyl acetate (1.1 mM, 100 μL) were added in the bi-phasic system. The reaction was started by the addition of 3 mg of Novozym 435 at 50°C. The operation temperature range recommended by manufacturer is 40~60°C, because of thermal inactivation. The middle point of suggested temperature range was chosen. The bi-phasic system was composed of an aqueous top phase and a solvent bottom phase. The concentration of the substrates and product in each phase was measured by gas chromatograph as described earlier. A partition coefficient is the concentration ratio of the molecules in both phases ($C_{\text{aqueous phase}}/C_{\text{solvent phase}}$). Partition coefficients of *n*-butyl acetate, vinyl acetate and *n*-butanol in each bi-phasic system were determined after the reaction ended.

RESULTS AND DISCUSSION

Lipase-catalyzed Transesterification in Uni-phasic System

n-Butyl acetate was produced from *n*-butanol and vinyl acetate by Novozym 435 at various temperatures. The reaction scheme of the lipase-catalyzed transesterification of *n*-butanol is shown in Fig. 1. Normally, the conversion yield is decreased by a reverse reaction of lipase. However, there is no reverse reaction in this production system because the by-product of this reaction is aldehyde. The enzyme activity could be influenced by aldehyde, but further investigation on kinetic properties of lipase and the inhibition effect in ionic liquid are necessary.

Two hydrophobic RTILs, [BMIM][PF₆] or [BMIM][Tf₂N] were used as reaction media. [BMIM][PF₆] contains more anions than [BMIM][Tf₂N]. Generally, more anions leads to a decrease of polarity [11]. Hexane was used as a conventional reaction medium for the comparison of conversion yield with RTILs. The conversion yield in each reaction medium at different temperatures is displayed in Fig. 2. The optimum temperature for the production was 60°C except in water. The higher solubility of hydrophobic substrates in water at higher temperatures is supposed to give a larger conversion yield at 70°C than 60°C. A solvent-free reaction system using water often requires a higher operation temperature to reduce the viscosity of the reaction medium and to improve the solubility of the substrates. The conversion yield in water, [BMIM][PF₆], [BMIM][Tf₂N], acetonitrile and hexane at 60°C was 42.7, 78.1, 68.7, 51.8, and 71.2%, respectively. The conversion yields in [BMIM][PF₆] were highest at all reaction temperatures. The activity and/or sta-

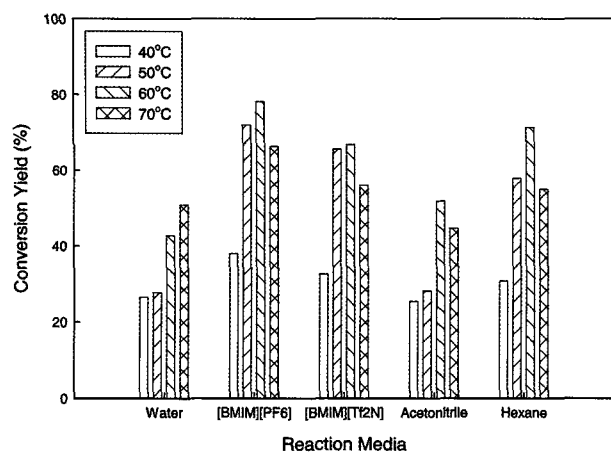


Fig. 2. Conversion yield of *n*-butylacetate in several uni-phasic reaction media at various temperatures.

bility of lipase were expected to be better in RTILs than in organic solvents. The increased stability of lipase in RTILs has been reported in a few research papers [12, 13]. It has been shown that Novozym 435 is stable up to 60°C [14], however 50°C was selected for further experiments in the present study because of small differences of conversion yields in RTILs and better heat stability of the lipase.

Lipase-catalyzed Transesterification in Bi-phasic System

The enzymatic production in an organic solvent is an important issue when hydrophobic substrates are involved in the reaction. Although many enzymes are active in various organic solvents, a major problem is the much lower activity and stability of enzymes in anhydrous media than in water. Thus, it is very important to develop appropriate techniques to enhance the productivity in organic solvents. The addition of a small amount of water in an organic solvent is a possible solution for a few reactions. However it is restricted to a few systems because of the limit of available reactions and water/organic solvent systems. Furthermore, the addition of water in a lipase mediated reaction system is improper considering only a small amount of water can induce the hydrolysis of the product by lipase. Incorporation of an additional process for the continuous removal of water gives a higher conversion yield by lipase [15].

A higher conversion can be obtained by simultaneous production and recovery of the product in a bi-phasic system. The larger difference of the partition coefficients of substrates and product gives higher conversion and supports easier recovery of the product. Hydrophobic RTILs comprising the bi-phasic system were [BMIM][PF₆] or [BMIM][Tf₂N]. The volumetric content of the aqueous phase against the total volume of the reaction medium was 50%. The immobilized Novozym 435 was retained on the interface of the organic and water phases because the density of the lipase immobilized polyacrylic

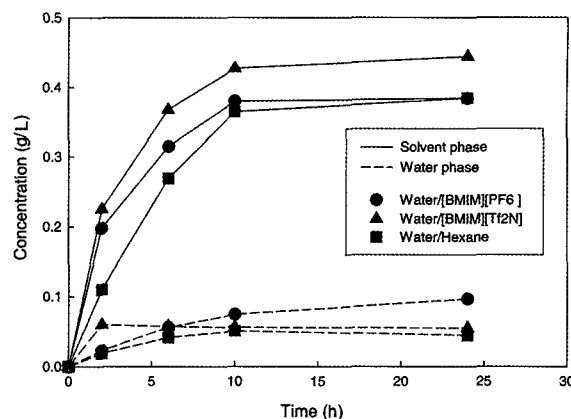


Fig. 3. Production of *n*-butylacetate in several bi-phasic reaction systems.

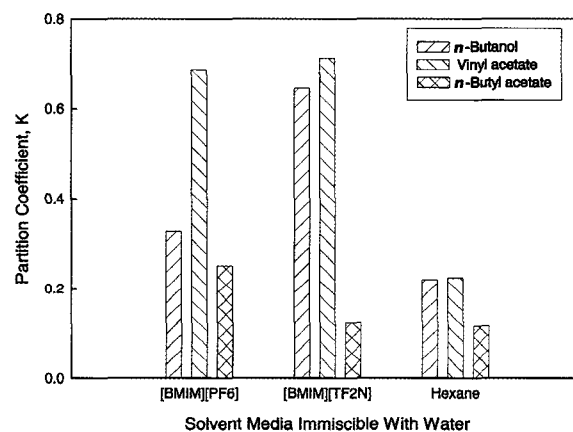


Fig. 4. Partition coefficients of the components in bi-phasic reaction systems.

resin is in between that of water and the ionic liquid. Lipase immobilized beads can be positioned in the ionic liquid phase by changing the density of the bead to be similar or higher than that of the ionic liquid. The applications using immobilized lipase has been reported [16, 17].

The concentrations of *n*-butyl acetate produced in both phases are shown in Fig. 3. The concentration of produced *n*-butyl acetate was higher in solvent phases than in the water phase. The water/[BMIM][Tf₂N] bi-phasic system showed the highest production yield of *n*-butyl acetate among the tested bi-phasic systems. The final conversion yields after 24 h were 75, 78, and 67% for the bi-phasic system composed of water and [BMIM][PF₆], [BMIM][Tf₂N], and hexane, respectively (data not shown). The conversion yield was enhanced up to 10% in the water/RTIL bi-phasic system when compared with that in the water/hexane system.

The partition coefficients of the substrates and product of the reactions were measured after the reaction ended (Fig. 4). The partition coefficients of *n*-butyl acetate were

0.25, 0.59 and 0.12 for the bi-phasic system composed of water and [BMIM][PF₆], [BMIM][Tf₂N], and hexane, respectively. All the components of the reaction preferred the organic phase than water because of the hydrophobic nature of the components. The partition coefficient of vinyl acetate was more than three times higher in RTILs than in the water/hexane bi-phasic system. Conversion yields in all of the bi-phasic systems were higher than those of uni-phasic systems. A relatively smaller amount of dissolved substrates in an RTIL phase due to lower solubility than in hexane is supposed to enhance the conversion in water/RTIL bi-phasic systems than in a water/hexane system. Further investigation to obtain an understanding and prediction of the effect of RTILs on various reactions is essential. A thorough understanding of the effect of the components and structure of RTILs on an enzymatic reaction system would increase the applicability of RTILs as green solvents.

CONCLUSION

In this study, enzymatic transesterification of *n*-butanol in RTILs was compared with the conventional organic solvent system, hexane. As representative hydrophobic RTILs, [BMIM][PF₆] and [BMIM][Tf₂N] were employed. There was no remarkable inhibition effect by RTILs on the activity of lipase. The bi-phasic production of *n*-butyl acetate was tried using the immiscible characteristic of RTILs with water. The conversion yield of *n*-butanol was increased around 10% in the water/[BMIM][Tf₂N] bi-phasic system compared with that in the water/hexane system. Consequently, RTILs appeared as a good substitute of organic solvents as clean media in bio-transformation systems because of favorable physico-chemical properties such as negligible vapor pressure, high chemical activity, thermal stability and high solubility.

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