Impacts of ionic liquids on enzymatic synthesis of glucose laurate and optimization with superior productivity by response surface methodology

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ABSTRACT

As biosurfactants derived from naturally renewable resources, sugar fatty acid esters have broad applications in food, cosmetic and pharmaceutical industries. Glucose laurate was synthesized by a lipase-catalyzed acylation of glucose with vinyl laurate in ionic liquid (IL) systems. 16 ILs were screened for investigating the impact of the IL's compositions on both the solvent properties of the IL and the synthetic process. The conversions obtained in ILs showed a bell-shaped relationship with their log P values. ILs that carry hydrophobic cations and hydrophilic anions seemed to favor the sugar ester synthesis. 1-Hexyl-3-methylimidazolium trifluoromethylsulfonate ([HMIm][TfO]) was selected both as a pure solvent and as a co-solvent with 2-methyl-2-butanol (2M2B) for this application, and the influence of the affecting factors (such as reaction temperature, enzyme amount, molar ratio of the two substrates, and reaction time) has been studied. Response surface methodology (RSM) was applied to optimize the [HMIm][TfO]/2M2B bisolvent system, and an optimal productivity of 14.2 mmol/L/h was achieved, which is superior to other literature results.

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1. Introduction

Sugar fatty acid esters (SFAEs) are nonionic biosurfactants produced from naturally renewable resources (i.e., carbohydrates and fatty acids), thus being nontoxic, biodegradable and biocompatible. SFAEs are also tasteless, odorless and nonirritant, and have been widely used in food, cosmetic and pharmaceutical industries [1,2].

Lipase-mediated enzymatic synthesis of SFAEs has proven to be superior to the currently dominating chemical synthesis, and Novozym 435 (*Candida antarctica* lipase B (CALB) immobilized on acrylic resins from Novozyme), or CALB in general, is the most studied enzyme used for this application [1,2]. But the requirement of using volatile organic solvents (VOSs) as the reaction medium is problematic, mainly due to their inability to solubilize the two substrates of opposite polarity as well as concerns regarding their environmental health risks and enzyme compatibility [3]. Ionic

liquids (ILs) have recently emerged as an attractive "green" alternative to VOSs for carbohydrate biotransformation [3,4], particularly because of their superior solubilizing power [5] and their excellent compatibility with enzymes in support of the enzyme activity, stability and selectivity [6,7]. A search for appropriate ILs for this application requires a good understanding of the impacts of ILs on the enzymatic performance. However, So far only a few popular ILs (such as [BMIm][BF$_4$] and [BMIm][PF$_6$]) have been tested on sugar ester synthesis [8–10]. Therefore, it is important to set up an investigation on how the structure of an IL determines its properties and in turn the enzymatic performance in SFAE synthesis. Kim and Koo [11] have reported a preliminary study about the effects of physicochemical properties of 4 TfO$^-$-based imidazolium ILs on enzymatic synthesis of butyl acetate. A comprehensive survey of IL-enzyme/protein interactions has been given by Yang [12].

On the other hand, optimization of the synthetic process by response surface methodology (RSM) is worth an investigation. RSM has been recognized as a successful tool for experimental design and optimization because by running only a small number of experimental trials, it can enable the building of models and the evaluation of the significance of the different factors considered as
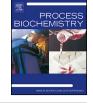
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well as their interactions [13]. Up till now only a few investigations have been reported dealing with applying RSM to optimization of lipase-catalyzed sugar ester synthesis in ILs [14–16].

In this context, the production of glucose laurate by lipase-catalyzed transesterification of glucose with vinyl laurate was taken as a model reaction, and a systematic investigation was carried out by screening 16 different ILs as the reaction medium. The use of vinyl ester is superior to that of a free fatty acid in improving the production yield by driving the reaction through the tautomerization of the enol product. The major goals of this study were: (1) to evaluate the structure-property relationship of ILs; (2) to explore the impact of ILs on the SFAE synthesis; and (3) to investigate the SFAE synthesis in both a pure IL system and an IL/VOS bisolvent system and to optimize the process by utilizing RSM. It is anticipated that the results obtained from this study can provide some insights not only to the enzymatic synthesis of SFAEs but, more generally, to all biocatalytic processes in ILs.

2. Materials and methods

2.1. Materials

Novozym 435 (*C. antarctica* lipase B immobilized on acrylic resins) was purchased from Novozymes (China) Investment Co., Ltd. Ionic liquids (99%) were obtained from ShangHai Cheng Jie Chemical Co., Ltd. Vinyl laurate (VL) and the Reichardt's dye (2,6-diphenyl-4-(2,4,6-triphenyl-1-pyridinio)phenolate) were from Sigma–Aldrich China Inc. α-D-Glucose (Glc), lauric acid (LA), molecular sieves (4 Å), and all other reagents used were of analytical grade from local manufacturers.

2.2. Dissolution and solubility of glucose in ILs

Glucose (100 mg) was added to a test tube containing 2.0 mL of an IL, followed by vortex mixing for 5 min and then shaking in an incubator/shaker at 30 °C and 220 rpm for 12 h. After centrifugation, the supernatant was obtained for determining the glucose solubility by using the dinitrosalicylic acid (DNS) method [17].

2.3. Physical properties of ILs

Viscosity was measured at 40 °C by using an AR1000 rheometer (TA Instruments, USA). Electron pair acceptor strength was determined spectrophotometrically by means of the Reichardt's dye [18], which was dissolved in an IL at 0.5 mM. After centrifugation, the visible spectrum of the solution was scanned at 25 °C with a PerkinElmer Lambda 25 UV–vis spectrophotometer. The wavelength of the absorption peak (λ_{max}, in nm) was recorded for determination of the IL's acceptor strength (E_T^N) following the equation: $E_T^N = [28591/\lambda_{max} - 30.7]/32.4$, where E_T^N is set on a dimensionless normalized scale between 0.0 for Si(CH$_3$)$_4$ and 1.0 for H$_2$O. Log P (P is the partition coefficient of a solvent in an octanol/H$_2$O biphasic system) [19], a commonly used parameter to represent the solvent hydrophobicity, was estimated with the aid of a software, KowWin (http://www.epa.gov/opptintr/exposure/pubs/episuite.htm). It has been shown to give the best result in log P determination among the four software packages (HyperChem, Pallas, KowWin and TOPKAT) [20], and has been used for predicting log P values of ILs [11,21].

2.4. Enzymatic synthesis of glucose laurate in pure IL or IL/VOS systems

A typical reaction was carried out by adding 0.054 g glucose (corresponding to 0.3 mol/L of the reaction system, only partially

dissolved) to a 5-mL capped test tube containing 0.3 M vinyl laurate (totally dissolved) and 100 mg 4 Å molecular sieves in 1-mL solvent (pure IL or IL/VOS mixture, dried over molecular sieves for over a week prior to use). After vortex mixing for 5 min to maximize the glucose dissolution in the solvent, 100 mg Novozym 435 was added, and the tube was placed in an incubator/shaker with agitation of 300 rpm at 40 °C to start the reaction. Periodically, a 10 μL sample was taken and 4 times diluted with DMSO for HPLC analysis as indicated below. The conversion was calculated based on the total amount of glucose added to the reaction system. The product is 6-*O*-lauroyl-D-glucopyranose, which has been verified by using HPLC and structural analyses with NMR, IR and MS (Lin et al., unpublished results).

2.5. HPLC analysis

This was performed on a Shimadzu LC-20AT HPLC system equipped with a refractive index detector (Shimadzu RID-10A) and a 150 × 4.6 mm, 5 μm inertsil ODS-SP column (GL Sciences Inc. Japan). A 10-μL sample was injected, and a solvent mixture of methanol/water (85:15 v/v, pH adjusted to 2.3 with acetic acid prior to addition of methanol) was employed as the mobile phase with a flow rate of 1.0 mL/min, operated at 40 °C. The retention times for glucose laurate (GL), lauric acid (LA) and vinyl laurate (VL) were 3.31, 5.30 and 10.90 min, respectively.

2.6. Experimental design for optimizing the IL/VOS bisolvent system by RSM

Four factors (i.e., reaction temperature, enzyme amount, VL/Glc molar ratio and reaction time) were selected for optimization. Single-factor experiments were carried out first, i.e., to obtain the optimum for one parameter at a time while keeping others constant. Then based on the above results, a 4-factor-5-level central composite rotatable design (CCRD) of response surface methodology (RSM) was undertaken using Design-Expert 8.0.6, a DOE software developed by Stat-Ease, Inc. The model was evaluated by regression analysis of the experimental data by means of ANOVA (analysis of variance) technique implemented in the Design-Expert software.

3. Results and discussion

3.1. Correlation between properties and structures of the ILs

Table 1 summarizes the physical properties of the 16 ILs (all are liquid under ambient temperature) that were tested in our study, all varying according to a change in the IL's cation or anion. For instance, the viscosity of the ILs increased as a result of an increase in the alkyl chain length of the cation (e.g., [EMIm][TfO] < [BMIm][TfO] < [HMIm][TfO]) or a decrease in the size of the anion (e.g., [BMIm][Tf$_2$N] < [BMIm][TfO] < [BMIm][MeSO$_4$] < [BMIm][PF$_6$]).

The E_T^N of common immidazolium and ammonium ILs normally fall in the range of 0.50–0.75 and 0.37–1.0, respectively, corresponding to that of dipolar solvents (protic or non-protic) [18]. Our measurements agree well with these ranges, suggesting that all these tested ILs are highly polar. In agreement with Carmichael and Seddon [22], our data have also revealed that the IL polarity increased slightly with a decrease in either the chain length of the alkyl substituents on the cation (e.g., [EMIm][TfO] > [BMIm][TfO] > [HMIm][TfO]) or the size of the anion (e.g., [BMIm][PF$_6$] > [BMIm][TfO] > [BMIm][Tf$_2$N]).

Log P has been generally considered as a major descriptor for the hydrophobicity of an organic solvent [19], and was used here to assess the hydrophobicity/hydrophilicity of the ILs and their