Characterization of Ionic Liquid-Based Biocatalytic Two-Phase Reaction System for Production of Biodiesel

B. L. A. Prabhavathi Devi
National Food Institute, Technical University of Denmark, 2800 Lyngby, Denmark

Lipid Science and Technology Div., Indian Institute of Chemical Technology, Hyderabad 500607, India

Zheng Guo
National Food Institute, Technical University of Denmark, 2800 Lyngby, Denmark

Dept. of Molecular Biology, Aarhus University, 8000 Aarhus C, Denmark

Xuebing Xu
Dept. of Molecular Biology, Aarhus University, 8000 Aarhus C, Denmark

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The property of a variety of ionic liquids (ILs) as reaction media was evaluated for the production of biodiesel by enzymatic methanolysis of rapeseed oil. The IL Ammoeng 102, containing tetraaminum cation with C_{18} acyl and oligoethyleneglycol units, was found to be capable of forming oil/IL biphasic reaction system by mixing with substrates, which is highly effective for the production of biodiesel with more than 98% biodiesel yield and nearly 100% conversion of oil. Conductor-like screening model for real solvent (COSMO-RS) in silico prediction of substrate solubility and simulation of partition coefficient change vs. reaction evolution indicated that the amphiphilic property of Ammoeng 102 might be responsible for creating efficient interaction of immiscible substrates; while big difference of partition coefficients of generated biodiesel and glycerol between the two phases suggests a large chemical potential to move reaction equilibrium for maximum oil conversion and yield of target biodiesel. The reaction behavior and specificity of oil/IL biphasic system for enzymatic production of biodiesel were theoretically delineated through COSMO-RS computation with experimental validation. © 2010 American Institute of Chemical Engineers *AIChE J,* 57: 1628–1637, 2011

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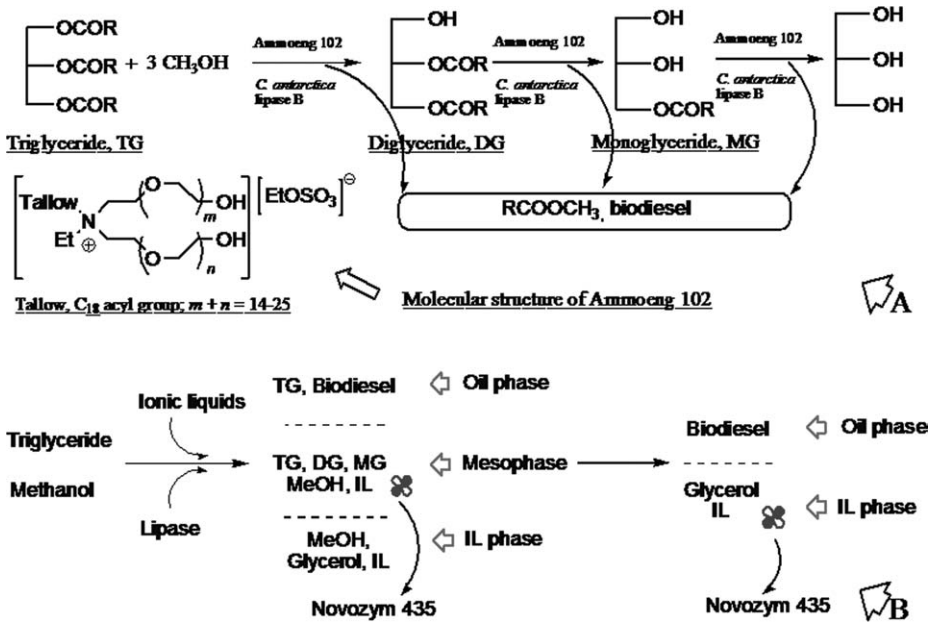
Introduction

The use of biodiesel (normally referred to as monoalkyl esters of long-chain fatty acids derived from oils and fats) has grown considerably in last decades because of increasing environmental concern and unsustainable availability of petroleum oil. Biodiesel does have significant advantages for

Additional Supporting Information may be found in the online version of this article.

Correspondence concerning this article should be addressed to X. Xu at xu@mb.au.dk.



Scheme 1. Schematic representation of enzymatic production of biodiesel in Ammoeng 102 (A) and phasic behaviors during the reaction evolution (B).

this situation: renewability (emitted CO_2 is recycled from the atmosphere to produce biomass), biodegradability, and improved exhaust emissions.[1,2] Although biodiesel has been successfully produced from oil and fat feedstock in large scale using chemical catalysts,[3–5] there are several associated problems such as high-energy requirements, difficulties in the recovery of catalyst and glycerol, and environmental impact from the postprocessing of generated wastes. Nowadays, biocatalysts are gaining more attention and have the potential to outperform chemical catalysts for biodiesel production in the future. Lipase-catalyzed esterification or/and transesterification with methanol represents promising alternates that might avoid those technical problems existing in chemically catalyzed reactions.[6–8] To learn related reaction behaviors and develop practical technology, extensive studies, with respect to development of enzyme preparations, kinetic delineation, and processing technology, have been conducted by a few groups.[9–11] Although there exist technical problems, such as enzyme inactivation by methanol, immiscibility of methanol and oil leading to lower reaction rate and incomplete conversion to biodiesel remained to be explored for enzymatic approaches. To this end, some approaches like successive addition of methanol and use of alternative alcohol donors have been proposed.[12,13] However, the attempts for a better understanding of the reaction behaviors and a more efficient approach are still on going, among which application of ionic liquids (ILs) represents one important respect of those efforts.[14,15]

Recently, ILs as a nonconventional solvent are becoming increasingly attractive because of their specific physicochemical characteristics, their ability to dissolve wide range of substrates, and the ease in fine tune as designer solvents.[16,17] The attempts that use ILs for biodiesel production include the synthesis of novel IL-based Brønsted acids used as catalyst for esterification[18] and transesterification[19] and as a carrier (support) of metal catalysts to create homogeneous catalysis and facilitate catalyst recycling.[20] Few reports also involved the use of ILs as reaction media[21,22] and also as lipase support[15] for lipase-catalyzed biodiesel production, which demonstrated the possibility and some specificities of IL-mediated reaction for biodiesel production. The enzyme/IL combinations were shown to exhibit somehow synergistic effect that enhances the activity and durability of the catalytic system; moreover, ILs as enzyme support proved to generate more stable and active catalysts for various enzymes.[15] However, to explore the enormous possibilities and fulfill the tremendous potentials of ILs, especially in theoretical understanding and description of phasic behavior as well as the interaction of substrates, enzyme, and media in IL-based biodiesel production system, more investigations are imperative.

In this work, we investigated the potential of a special class of Ammoeng series ILs, representing acyclic ammonium salts that contain cations with alkyl or acyl substituents and oligoethyleneglycol units of different chain length, for biocatalytic production of biodiesel. For comparison, the enzymatic reactions in other 19 types of ILs with differing cations and anions were preliminarily evaluated. The focus of this work is centered on a systematic investigation and characterization of two promising IL [Ammoeng 102 (Scheme 1) and Ammoeng 120]-mediated enzymatic production of biodiesel. The reaction characteristics, phasic behaviors, and possible mechanism accounting for excellent oil conversion and biodiesel yield were delineated with the assistance of conductor-like screening model for real solvent (COSMO-RS) in silico prediction.

Experimental

Materials

Lipozyme TL IM (*Thermomyces lanuginosa*), Lipozyme RM IM (*Rhizomucor miehei*), and Novozym 435 (*Candida antarctica* lipase B) lipases were kindly provided by Novozymes A/S (Bagsvaerd, Denmark). Rapeseed oil was obtained from Aarhus United (Aarhus, Denmark), and

triolein with 90% purity was purchased from Dr. Frischer GmbH (Bremen, Germany). Methanol (>99% purity) and other solvents were purchased from Sigma-Aldrich (St. Louis, MO) and were used without further purification. Fatty acid methyl esters used as standards were procured from Sigma Chemical (St. Louis, MO). The ILs used are BMIM·BF_4, BMIM·PF_6, OMIM·BF_4, OMIM·PF_6, BMIM·CF_3SO_3, MeEtPy·$C_4F_9SO_3$, MeOcPy·BF_4, *t*OMA·Tf_2N, MeBuPyo·$N(CN)_2$, HMIM·Cl, DMIM·DMP, EMIM·$MDEGSO_4$, EMIM·$OctSO_4$, MeBuPy·$N(CN)_2$, ETDAA·$EtSO_4$, BMPyo·Tf_2N, EMIM·OTs, and EMIM·Et_2SO_4, and Ammoeng 100, 111, 102, 112, and 120 were procured from Solvent Innovation (Cologne, Germany). *t*OMA·TFA was obtained from Merck (Darmstadt, Germany). The full name and molecular structure of the ILs are available in the Supporting Information. All other chemicals were of analytical reagent grade.

General procedure for biodiesel production

The enzymatic transesterification was conducted in a 10-mL screw-capped bottle and thermostated by water bath at 50°C. A mixture of rapeseed oil (0.882 g, 1 mmol), IL or *t*-butyl alcohol (0.50 g), and methanol (0.160 mL, 4 mmol) were kept under agitation by magnetic stirring at 300 rpm under heating in water bath at 50°C for 10 min for equilibration. The reaction was then initiated by the addition of Novozym 435 (0.05 g, 5 wt % of oil) and continued for desired time. For solvent-free system, rapeseed oil (0.882 g, 1 mmol) and Novozym 435 (0.05 g, 5 wt % based on oil weight) were placed in a 15-mL screw-capped vial, followed by adding methanol. To decouple the effects of water contents in different ILs, 24 kinds of commercial ILs to be screened were dried by applying vacuum of 1 mm Hg at 50°C for 8 h and its water content was measured with Karl Fischer titration. Then all ILs were adjusted to the same water content 1.5% (wt %) by adding a certain amount of water based on the calculation of mass balance, respectively. (If no statement elsewhere, the water content adjustment was only applied in IL screening reactions. In all other reactions, the ILs was used as received). The conditioned ILs was used for conducting enzymatic methanolysis as above described for a typical reaction. For the comparison of fatty acid methyl ester (FAME) production in 24 kinds of ILs, a control transesterification reaction in *tert*-butyl alcohol adjusted to the same water content 1.5% was carried out under the identical conditions. From this screening study, two promising ILs, i.e., Ammoeng 102 and Ammoeng 120, were selected and used for further optimization of methanol, IL, and enzyme concentration on the production of biodiesel. The reactions were monitored by thin layer chromatography (TLC), and 20 μL of samples were withdrawn while stirring at desired intervals and dissolved in 1 mL of chloroform/methanol (1:1, v/v) mixture and analyzed by TLC with flame ionization detector (TLC-FID).[23]

All reactions are conducted in duplicates, and the means of the two determinations are used for evaluation.

TLC-FID analysis

Twenty microliter of the reaction mixture was taken at time intervals and was used for analysis of the composition of triacylglycerols (TAGs), diacylglycerols (DAGs), free fatty acids (FFAs), and FAMEs by TLC-FID (Iatroscan MK-5, Iatron Laboratories, Tokyo, Japan).[24] The sample was dissolved in chloroform/methanol (1:1, v/v) at the concentration of 1% (vol %). The solution (5 μL) was spotted on a silica gel rod, and the components were developed with a mixture of hexane/diethyl ether/acetic acid (60.2:6.7:0.134, v/v/v) for 35 min and then with a mixture of *n*-hexane/diethyl ether/acetic acid (45:25:1, v/v) for 5 min. Standard mixture of FAME/TAG/FA/DAG/MAG was used as the standard for identifying the peaks. Quantification of all the components was calculated based on the peak area percentage. The formation of FAME was also confirmed by TLC using 10% EtOAc in hexane as developing solvent. Then the spots on the TLC plate were visualized under UV lamp after spraying with 2,7-dichlorofluoresene in ethanol (2 g/L) as spray reagent.

Model processing and calculation of partition coefficients with COSMO-RS

COSMO-RS integrates concepts from quantum chemistry, dielectric continuum models, electrostatic interactions, and statistical thermodynamics and extends to real solution system.[25] As a physically sound in silico approach, COSMO-RS is capable to correctly describe complex solvation behaviors in a real solvent system qualitatively and quantitatively or at least semiquantitatively.[26] With theoretical documentation and evaluation of many COSMO-RS practical performance,[27] COSMO-RS represents one of the most efficient models to delineate complicated and simultaneously occurred multiple interactions of substrates, products, and solvent in ILs, and therefore, it is used here to predict and simulate partition behaviors of products between oil and IL phases and to uncover the thermodynamic reasons behind the unique reaction behaviors of enzymatic production of biodiesel in ILs.

The molecular structures of cations of Ammoeng series of ILs and glycerides were sketched as two-dimensional structures and subsequently converted to three-dimensional geometries using Chemdraw Ultra 8.0. The energy of molecular conformations was minimized by Molecular Orbital PACkage 2000. The COSMO calculations were performed with the TURBOMOLE 5.7 program package on the density functional theory level by using the BP (B88-VWN-P86) functional with a triple-ζ valence polarized basis set (TZVP). The partition coefficients of solute i between oil and IL phase at infinite dilution ($P_i^{\mathrm{IL-Oil}}$) are direct results of COSMOtherm calculations[25,26]:

$$\log_{10}(P_i^{\mathrm{IL-Oil}}) = \log_{10}\left[\exp((\mu_i^{\mathrm{Oil}} - \mu_i^{\mathrm{IL}})/RT) \cdot (V_{\mathrm{Oil}}/V_{\mathrm{IL}})\right], \quad (1)$$

where μ_i^{Oil} and μ_i^{IL} are the chemical potential of solute i in oil and IL phase, respectively; R is the gas constant and T is the temperature input for computation. The volumes of oil and IL are estimated as the volume sum of components in each phase, respectively. All COSMO-RS calculations of activity coefficients and solubilities are performed using COSMOthermX program (COSMO/logic GmbH & Co KG, Leverkusen, Germany). The latest parameterization BP_TZVP_C21_0108 was used to achieve the best prediction quality.

Table 1. Dependency of Biodiesel Production on the Properties of Media*

Entry†	Medium‡	MG (wt %)	DG (wt %)	TG (wt %)	Conversion of TG (mol %)§	Yield of Biodiesel (wt %)
1	BMIM·BF_4	0.47 ± 0.10	0.07 ± 0.01	97.04 ± 0.63	3.89 ± 0.76	2.32 ± 0.21
2	*t*OMA·Tf_2N	1.59 ± 0.07	0.18 ± 0.02	61.28 ± 3.11	38.50 ± 2.24	36.81 ± 2.13
3	Ammoeng 100	0.86 ± 0.24	0.21 ± 0.01	78.81 ± 3.56	21.25 ± 2.86	19.67 ± 2.16
4	Ammoeng 102	0.79 ± 0.17	0.21 ± 0.02	0.96 ± 0.21	99.04 ± 1.35	97.66 ± 1.17
5	Ammoeng 111	0.15 ± 0.04	0.11 ± 0.02	96.83 ± 0.57	2.69 ± 0.14	2.28 ± 0.14
6	Ammoeng 112	3.54 ± 0.31	3.42 ± 0.34	85.69 ± 2.60	14.38 ± 1.06	6.33 ± 0.85
7	Ammoeng 120	2.14 ± 0.14	0.38 ± 0.03	2.25 ± 0.35	98.51 ± 2.14	93.39 ± 1.77
8	DMIM.DMP	2.54 ± 0.22	1.82 ± 0.13	77.86 ± 1.47	21.78 ± 1.19	16.47 ± 0.42
9	*t*OMA·TFA	21.62 ± 1.21	13.89 ± 0.64	17.89 ± 1.47	82.97 ± 3.27	44.79 ± 2.28
10	*t*-Butyl alcohol	11.83 ± 1.54	3.23 ± 0.22	0.55 ± 0.02	99.17 ± 0.37	80.85 ± 2.51

*Typical reaction conditions: 1 mmol rapeseed oil (0.882 g) is mixed with 0.5 g ionic liquid (or *t*-butyl alcohol) and 4 mmol methanol (0.128 g) in a 15-mL-capped vial thermostated at 50°C. All media were adjusted to the same water content of 1.5% (wt %) before being applied for reaction. The reaction is initiated by the addition of 50 mg Novozym 435 and kept on running for 24 h with magnetic agitation at 300 rpm. The reaction is monitored by periodical sampling and TLC-FID analysis. Area percentage used as weight of analyte is used for result evaluation. The data reported are means ± standard deviations of duplicate determinations.

†In BMIM·PF_6-, OMIM·BF_4-, OMIM·PF_6-, BMIM·CF_3SO_3-, MEPy·$C_4F_9SO_3$-, MeOcPy·BF_4-, MBPyo·$N(CN)_2$-, HMIM·Cl-, EMIM·MDEGSO_4-, EMIM·OctSO_4-, MBPy·$N(CN)_2$-, ETDAA·EtSO_4-, BMPyo·Tf_2N-, EMIM·OTs-, and EMIM·EtSO_4-mediated reactions, the conversion of oil was lower than 2% or no reaction was observed. Hence, the data are not shown.

‡The molecular structures of the ionic liquids used here are available in the Supporting Information.

§Molar conversion of triglyceride (TG) is defined as the percentage of deformed TG of the initial TG concentration. The calculation is based on the mass balance of TG and converted acyl groups. Biodiesel, MG, FFA, and DG are thus treated as 1/3, 1/3, 1/3, and 2/3 of TG unit in the calculation, respectively.

Measurement of the distribution of glycerol and biodiesel in oil and IL phase

To validate the COSMO-RS-predicted partition coefficients of biodiesel and glycerol between oil/IL phases, 1 mmol triolein and 3 mmol methanol were mixed with 1 mmol Ammoeng 102 and 120, respectively. The reaction was initiated with the addition of 50 mg Novozym 435 and monitored with periodical sampling. The sample of reaction mixture was centrifuged at 14,000 rpm for 30 min to allow the components to reach distribution equilibrium between oil/IL phases. Then, the oil and IL phase were sampled and dissolved in methanol/chloroform (2/1, v/v), respectively. Relative concentration of glycerol and biodiesel in oil or IL phase was analyzed with Hitachi-Merck HPLC Series 7000 (Hitachi-Merck, Japan), conjugated with a PL-ELS 2100 evaporative light-scattering detector (ELSD; Polymer Laboratories, Shropshire, UK), as previously described.[28] The reverse phase column used was Supelcosil LC-18 (250 mm × 4.6 mm; Supelcosil, Bellefonte, PA). The ELSD was operated at an evaporating temperature of 30°C and a nebulizing temperature of 30°C with air as the nebulizing gas. The peaks were quantitated by reference to response curves generated with standards.

The reactions were conducted in triplicates. Means and standard deviations of three determinations were used for result evaluation. A better accuracy of HPLC analyses was obtained with <3% standard deviations of triplicate measurements.

Results and Discussion

Dependency of biodiesel production on the properties of media

As illustrated in Scheme 1, enzymatic synthesis of biodiesel is a multistep reaction, starting from triglyceride with stepwise release of fatty acid as the corresponding ester form and ending up with glycerol. This fact indicates that the reaction could simultaneously stay in every stages and biodiesel only represents one of the possible products. Because of the same reason, reaction medium may play more important role, at least theoretically, in the determination of the yield of a product by shifting reaction equilibrium through their specific interaction with reactants, intermediate, and end products. Table 1 reveals part of the results of Novozym 435 (*Candida antarctica* lipase B)-catalyzed transesterification of rapeseed oil with methanol in different solvents. Initially, a total of 24 kinds of ILs were tested for biodiesel production and compared with the reaction in *tert*-butyl alcohol under an identical condition. However, as stated in Table 1, the attempts in 15 types of ILs with variable cation and anion combination end up with <2% of triglyceride conversion. The possible reasons differ from case to case. Some ILs such as HMIM·Cl and EMIM·OTs inactivate enzyme[29]; while in other ILs such as BMIM·PF_6 and OMIM·BF_4, the IL itself might not allow efficient interaction of property-differing reactants (hydrophilic methanol and hydrophobic triglyceride). Surprisingly, the results from Ammoeng 102 (97.66%) and 120 (93.39%) are very encouraging in terms of the yield of biodiesel, which are much higher than in *tert*-butyl alcohol (80.85%; one of the most efficient conventional systems for biodiesel production[19,21]). Another point worthy to note is that for Ammoeng 102-mediated reaction, not only high conversion of triglyceride (TG) but also remarkably low partial glycerides (monoglycerides, diglycerides, and free fatty acid) allow the biodiesel preparation to directly meet the specifications of European biodiesel standards (in terms of ester content), without any further processing.[30]

A close inspection of the data in Table 1 reveals that some reactions achieve similar conversion of TG but different product profiles. For example, for *t*OMA·TFA-based system with 82.97% converted TG produces only 44.79% biodiesel and the left is converted into diglyceride (DG) (13.89%) and monoglyceride (MG) (21.62%; entry 9); while

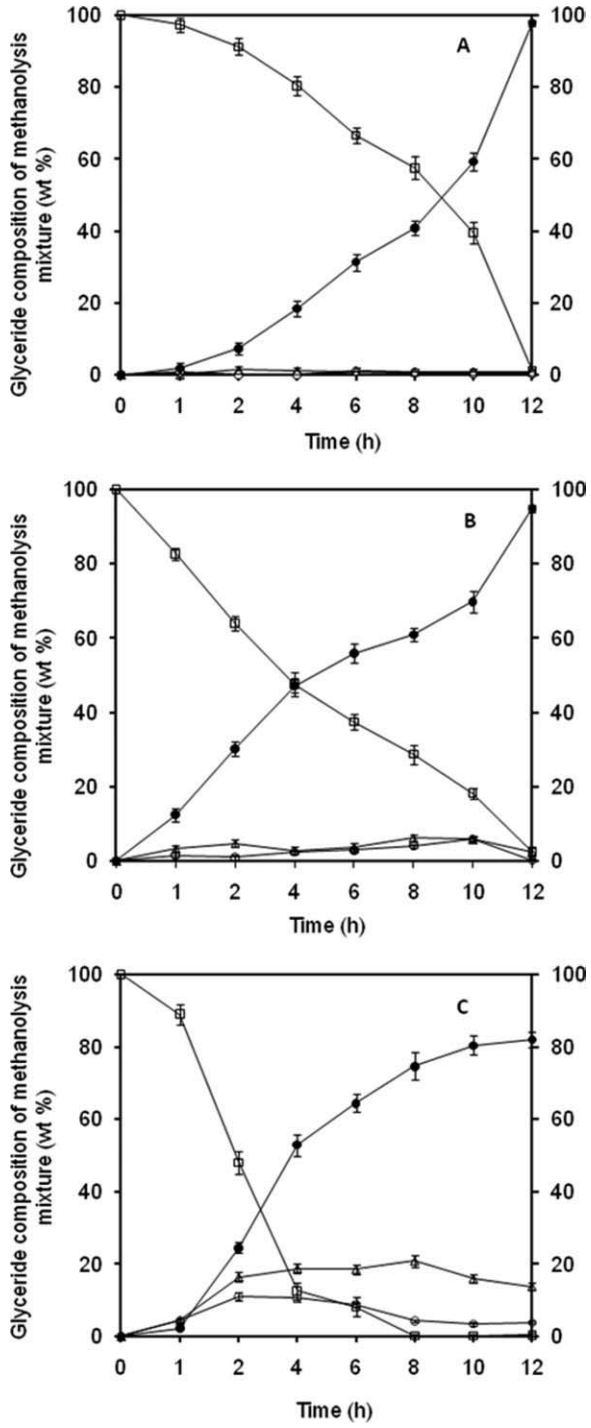


Figure 1. Time course of enzymatic production of biodiesel from methanolysis of rapeseed oil in Ammoeng 102 (A), Ammoeng 120 (B), and *tert*-butyl alcohol (C).

Typical reaction conditions are the same as the description in Table 1. The legends for glyceride components in the reaction mixture are triglyceride (□), diglyceride (○), monoglyceride (△), and biodiesel (●). The plotted data are means ± standard deviations (error bars) of two determinations.

for *t*OMA·tf_2N 95.6% of converted TG (36.81/38.50) becomes biodiesel (entry 2). Similar observation appears between Ammoeng 100 and 112, the former deforms 92.56% of converted TG into biodiesel (entry 3) and the latter produce only 6.33% biodiesel of 14.38% converted TG (entry 6). The results suggested that the reaction profiles (conversion and selectivity) are strongly dependent on the structure of ILs. The ILs with the same cation but differing anions may yield different selectivity (herein referred as selectivity toward biodiesel formation; entries 2 and 9). The results in Table 1 also suggested that the reaction behaviors of enzymatic alcoholysis in the ILs with differing cations and anions cannot be simply ascribed to one single factor. Because ILs represent most complex solvation system, among which multisolvations (electrostatic, H-bonding, and van der Waals interactions) occur simultaneously.[31] These multiple but different solvations among different ILs might interact with the reactants, intermediate, and end products, leading to distinct reaction profile pertaining to respective ILs. Unusually, different operation conditions sometimes also yield different and even contrary results. For instance, almost no reaction was observed in BMIM·PF_6-mediated reaction in our test (Table 1). However, Sunitha et al.[22] used higher IL dosage and methanol concentration and obtained more than 95% biodiesel yield.

As well documented theoretically and experimentally by Hailing,[32] Zaks and Klibanov,[33] and Afflect et al.,[34] for nonaqueous biocatalysis system, the water activity plays an important role in determining enzyme activity and reaction rate. However, to control the water activity of an IL-mediated system is a complicated issue and thus an intensive study was not carried out to avoid missing its focus on investigation of the effects of structural variations of ILs on enzymatic production of biodiesel.

Characteristic reaction profiles in Ammoeng 102 and 120

Figure 1 shows the time courses of enzymatic production of biodiesel in Ammoeng 102 (A), 120 (B), and *t*-butyl alcohol (C). The reactions in Ammoeng 102 and 120 achieve significantly high biodiesel yield than in *t*-butyl alcohol at 12 h, even though the reaction in Ammoeng 102 undergoes a lower initial rate.[35] Another hallmark to differentiate these three systems is their reaction profiles. Apparently, throughout the reaction course, there are considerable amount of monoglycerides and diglycerides in *t*-butyl alcohol system, even when TG is almost consumed after 8 h. In Ammoeng 120 system, partial glycerides exist following reaction evolution but with a comparably low amount, leading to high final yield of biodiesel. However, the sum of DG and MG content in Ammoeng 102 system is always less than 2% throughout the whole reaction course. This characteristic profile is unique and preferable for biodiesel production, which suggests that the system does not favor the accumulation of DG and MG. This also indicates that there is an intrinsic impetus to promote further degradation of the partial glycerides derived from triglyceride, which needs further investigation.

To further understand the systems of Ammoeng 102 and 120, the effects of variable parameters have been examined (Table 2). First, we evaluated the effects of the ratio of methanol/triolein (entries 1–6) to see whether higher methanol concentration results in improved yield of biodiesel or leads to inactivation of lipase as observed in conventional

Table 2. Effects of Important Parameters on the Production of Biodiesel in Ammoeng 102 and 120*

Entry	Ionic Liquid*	T (°C)	Time (h)	Methanol/TG (mol/mol)	IL Dosage (wt % of Oil)	Enzyme/ Amount (g)	Biodiesel (wt %)†	Conversion of TG (mol %)†
1	102	50	24	4:1	56.69	CAL B/0.05	98.62 ± 1.21	99.24 ± 0.34
2	102	50	24	6:1	56.69	CAL B/0.05	97.65 ± 1.41	98.48 ± 0.55
3	102	50	24	8:1	56.69	CAL B/0.05	98.87 ± 0.79	99.53 ± 0.28
4	120	50	24	3:1	56.69	CAL B/0.05	95.21 ± 2.03	99.35 ± 0.51
5	120	50	24	4:1	56.69	CAL B/0.05	94.93 ± 1.78	97.50 ± 0.97
6	120	50	24	6:1	56.69	CAL B/0.05	51.15 ± 3.92	54.49 ± 1.22
7	102	50	10	4:1	22.68	CAL B/0.05	69.04 ± 4.15	69.69 ± 1.75
8	102	50	10	4:1	45.35	CAL B/0.05	96.05 ± 1.59	98.67 ± 0.77
9	102	50	10	4:1	90.70	CAL B/0.05	99.05 ± 0.88	99.34 ± 0.53
10	120	50	10	4:1	22.68	CAL B/0.05	40.61 ± 3.53	43.45 ± 1.76
11	120	50	10	4:1	45.35	CAL B/0.05	82.59 ± 2.78	83.82 ± 0.91
12	120	50	10	4:1	90.70	CAL B/0.05	88.07 ± 1.83	98.37 ± 1.15
13	102	50	10	4:1	56.69	CAL B/0.10	99.65 ± 0.41	99.97 ± 0.02
14	120	50	10	4:1	56.69	CAL B/0.10	99.52 ± 0.39	99.97 ± 0.03
15	102	50	8	4:1	56.69	CAL B/0.05	40.85 ± 2.95	42.13 ± 1.99
16	102	60	8	4:1	56.69	CAL B/0.05	68.92 ± 3.69	72.74 ± 1.65
17	120	50	8	4:1	56.69	CAL B/0.05	60.95 ± 4.07	70.53 ± 2.06
18	120	60	8	4:1	56.69	CAL B/0.05	29.47 ± 2.79	32.70 ± 1.18
19	102	50	24	4:1	56.69	CAL B/0.10	99.65 ± 0.19	99.97 ± 0.04
20	102	50	24	4:1	56.69	RM IM/0.10	22.32 ± 1.68	62.41 ± 1.23
21	102	50	24	4:1	56.69	TL IM/0.10	18.67 ± 1.26	24.85 ± 1.04
22	120	50	24	4:1	56.69	CAL B/0.10	99.52 ± 0.36	99.97 ± 0.09
23	120	50	24	4:1	56.69	RM IM/0.10	10.82 ± 1.02	14.98 ± 0.72
24	120	50	24	4:1	56.69	TL IM/0.10	2.93 ± 0.57	6.53 ± 1.11

*The other reaction conditions, unless stated in this table, are the same as in Table 1.
†Molar conversion of triglyceride (TG) is defined as the percentage of deformed TG of the initial TG concentration. The calculation is based on the mass balance of TG and converted acyl groups. Biodiesel, MG, FFA, and DG are thus treated as 1/3, 1/3, 1/3, and 2/3 of TG unit in the calculation, respectively. The data reported are means ± standard deviations of duplicate determinations.

solvents.[12,13] The results demonstrated that in Ammoeng 120 increasing concentration of methanol does lead to the decrease in either TG conversion or biodiesel yield (entries 1–3). However, for Ammoeng 102 at methano/TG ratio up to 8, Novozym 435 still remains robust to produce excellent TG conversion as well as biodiesel yield (entries 4–6). This seems to indicate that the molecular structure of Ammoeng 102 might have some protective effect on enzyme activity, which was also observed in other ILs with similar cationic structure.[36] For the case of methanol existence, the real reason accounting for the protective effect from ILs has not been clarified. It is suggested here that the end free OH group in cation of Ammoeng 102 (Scheme 1) might participate in the ''immobilization'' of excessive methanol through H-binding so as to alleviate the direct interaction of methanol with the active site of lipase. Furthermore, the above suggestion does not rule out but reclaim that the PEG structures in Ammoeng series ILs may provide the protective effect on enzyme activity because they also contribute to the formation of H-bonding with methanol as an acceptor.[37–39]

Lower dosage of ILs functioning as higher amount of conventional solvents has been claimed as one of the advantages of expensive ILs as a reaction medium.[37] To clarify this issue for biodiesel production, the effects of Ammoeng 102 and 120 dosages were examined at three levels (Table 2, entries 7–12). The effects of dosage reduction for Ammoeng 120 are much significant, for example, TG conversion at 10 h is reduced from 83.82% to 43.45% when IL dosage is decreased from 45.35% to 22.68% by oil weight. Contrarily, Ammoeng 102 shows better ''buffering capacity,'' e.g., half-reduced IL dosage only results in slight decrease of TG conversion and biodiesel yield (entries 8 and 9), and further half-reduction results in 69% biodiesel yield after 10 h but still being able to obtain more than 90% yield of biodiesel after 24 h (data not shown). This result suggests that Ammoeng 102 possesses this excellent property that many other ILs and conventional solvents do not possess, which is of great interest to reduce the total cost of IL-based systems.

Higher enzyme loading generally results in a faster total reaction rate. The effect is also significant for the reactions in Ammoeng 102 and 120 (Table 2, entries 13 and 14). The yield of biodiesel in Ammoeng 102 after 10 h is increased from 59.30% (Figure 1A) to 99.65% with Novozym 435 increasing from 50 to 100 mg (Table 2, entry 13); similarly in Ammoeng 120 from 69.88% (Figure 1A) to 99.52% (Table 2, entry 14). Elevated temperature, in general, leads to improved reaction rate; however, it gives difference for the system with the presence of methanol that may speed inactivation of enzyme. For example, a temperature increase from 50 to 60°C for the reaction in Ammoeng 120 leads to TG conversion decrease instead of an increase in TG conversion (Table 2, entries 17 and 18). Differently, in Ammoeng 102, both TG conversion and biodiesel yield increase significantly (Table 2, entries 15 and 16), which might be principally ascribed to the amelioration of kinetics and mass transfer due to the decrease of viscosity of the IL at enhanced temperature.[37] This result also suggests that Ammoeng 102, different from Ammoeng 120, might provide some protection to retain enzyme activity against methanol inactivation as observed above (Table 2, entries 1–3). Finally, the biocatalysis performance of three commercial immobilized lipases is evaluated in both ILs (Table 2, entries 19–24). Agreed with

many previous observations,[10,12,19,37] Novozym 435 gives the best results in terms of TG conversion and biodiesel yield, followed by Lipozyme RM IM and Lipozyme TL IM. The ionic nature of ILs to strip of the essential water for catalysis action of enzyme has been suggested to be responsible for the lower activity of lipases in IL environment; however, *Candida antarctica* lipase B is structurally unique that could bind the essential water for catalysis and consequently works well at low water activities.[35]

Partition equilibrium and reaction specificity

If one does not consider the solid state of biocatalyst (here, immobilized lipase), the enzymatic alcoholysis of triglycerides in ILs (most of them evaluated in this work) can be seen as proceeded in a typical biphasic system (Scheme 1B). The specificity of the system is that one of the substrates itself (triglyceride) constitutes starting "organic" phase, and IL resembles mostly "aqueous" phase where another substrate methanol is dissolved. The advantages of a biphasic reaction system have been well documented,[26] including timely delivery of dissolved substrates at high concentration and immediate removal of products by redistribution between the two phases, which facilitates removal of product inhibition for an enzymatic reaction. Therefore, a biphasic reaction system may allow an easy separation of products or greatly shift reaction equilibrium towards the maximum obtainable conversion or yield of desired product if the solvent is properly selected. For enzymatic reaction, biphasic system may bring other accompanying benefits such as improved activity, stability, or selectivity because it has been certain that the enzyme specificity is profoundly influenced by solvation state of substrate in solvents.[40] To explore tremendous opportunities for creating an efficient biphasic reaction system for lipase-catalyzed production of biodiesel, COSMO-RS might be able to play a role to integrate or understand the reaction behaviors by simulating and evaluating the partition behavior of substrates and products between oil and IL phases,[26,41] which just represents the focus of the following discussion.

The concentration-dependent partition coefficient of a solute ($P_i^{\mathrm{IL-Oil}}$) is calculated based on the thermodynamic equilibrium conditions as follows:

$$P_i^{\mathrm{IL-Oil}} = \frac{\gamma_i^{\mathrm{Oil}}}{\gamma_i^{\mathrm{IL}}} = \frac{x_i^{\mathrm{IL}}}{x_i^{\mathrm{Oil}}} \tag{2}$$

where x_i^{Oil} and x_i^{IL} are the molar fractions of solute i in oil and IL phase, respectively, and γ_i^{Oil} and γ_i^{IL} correspond to their activity coefficients. Equation 2 indicates that the exchanges of components (substrates, ILs, and products) between oil/IL phases are governed by the difference of the multiplying product of molar fraction and activity coefficients ($x_i \cdot \gamma_i$) between the two phases (namely, chemical potential) until reaching equilibrium.[41] Thus, we could understand the reaction behavior by evaluating partition coefficients or vice versa by using partition coefficients to predict equilibrium conversion or yield. The specific reaction profiles in particular ILs and their difference from conventional solvent have been observed (Figure 1), of which the reason most likely comes from the differing properties of solvents as other conditions are the same. For a multistep reaction, examination of the changes of activity coefficients of intermediate and end products in different biphasic systems with reaction evolution might be a good start to probe into the intrinsic reasons accounting for respective reaction specificity.[35] To simplify the simulation of the reaction progress, the following assumptions are established based on the observed characteristics of representative IL reaction system for enzymatic production of biodiesel (typically as in Ammoeng 102; Figure 1A): (1) this prediction is based on 1 mol triolein reacted with 3 mol methanol using 1 mol IL as a medium; (2) this work focuses on the partition coefficients of the end products (glycerol and biodiesel), all other components treated as part (composition) of two phases; (3) the formation of intermediate products (MG and DG) are treated as a steady state that the accumulation of MG and DG is neglected; (4) regarding the mutual solubilities of solvents, oil (TG and/or biodiesel, changing with reaction evolution) concentrations in ILs are estimated by COSMO-RS calculation at an infinite mode; and the solubilities of methanol, glycerol, and ILs in oil phase are omitted; (5) the volumes of oil phase (TG and biodiesel) and IL phase (IL, TG, biodiesel, methanol, and glycerol) are the sum of volume contributions from the compositions. Partition coefficients of biodiesel and glycerol in oil and IL phases (Ammoeng 102 and 120) are thus calculated on the basis of the above assumptions (Figure 2A). To examine the prediction, a validation experiment was conducted as described in the Experimental section (Figure 2B). It should be pointed out that the evaluation of partition coefficients for the reaction in *t*-butyl alcohol is impossible because no phase separation occurs.

Figure 2A shows evident difference of partition coefficient of glycerol and biodiesel between oil/IL phases for Ammoeng 102 and for 120 systems. The partition coefficients [$\log_{10}(P)$] of glycerol in oil/Ammoeng 102 system increase from 1.09 to 1.53 following the reaction evolution, whereas the corresponding values of biodiesel vary, in parallel, from -1.24 to -1.07. Similarly, the $\log_{10}(P)$ values of glycerol in oil/Ammoeng 120 system increase from 0.85 to 1.22, and the $\log_{10}(P)$ values of biodiesel change from -0.97 to -0.85. These changing trends indicate that oil/Ammoeng 102 is a better biphasic system than oil/Ammoeng 120, in terms of a larger distribution difference of biodiesel and glycerol between oil/IL phases. For example, $\log_{10}(P)$ value (1.53) of glycerol between oil/Ammoeng 102 means that the mole concentration distribution of glycerol in oil phase and Ammoeng 102 (equal mole mixed) is 1:34, that is, 97.12% glycerol is enriched in IL phase (calculated by Eq. 2). For biodiesel at the same examination condition, the distribution ratio is around 11.8:1, which means that there is 7.8% of biodiesel dissolved in IL phase. However, the discrimination efficiency of oil/Ammoeng 120 is comparatively lower, with 16.5:1 for glycerol and 7.1:1 for biodiesel (Figure 2A). A larger partition difference of a product facilitates its immediate removal from reaction vicinity and shifts the equilibrium accordingly, because many biphasic reactions are observed to occur in the interface of two phases.[26,37] Thus, this could explain why Ammoeng 102, other than Ammoeng 120, is capable to greatly drive alcoholysis to completion (Figure 1). This prediction was verified by experimental measurement of the actual distribution change of biodiesel and glycerol in oil/IL phase with reaction progress (Figure 2B). As shown in Figure 2B, the measured data of

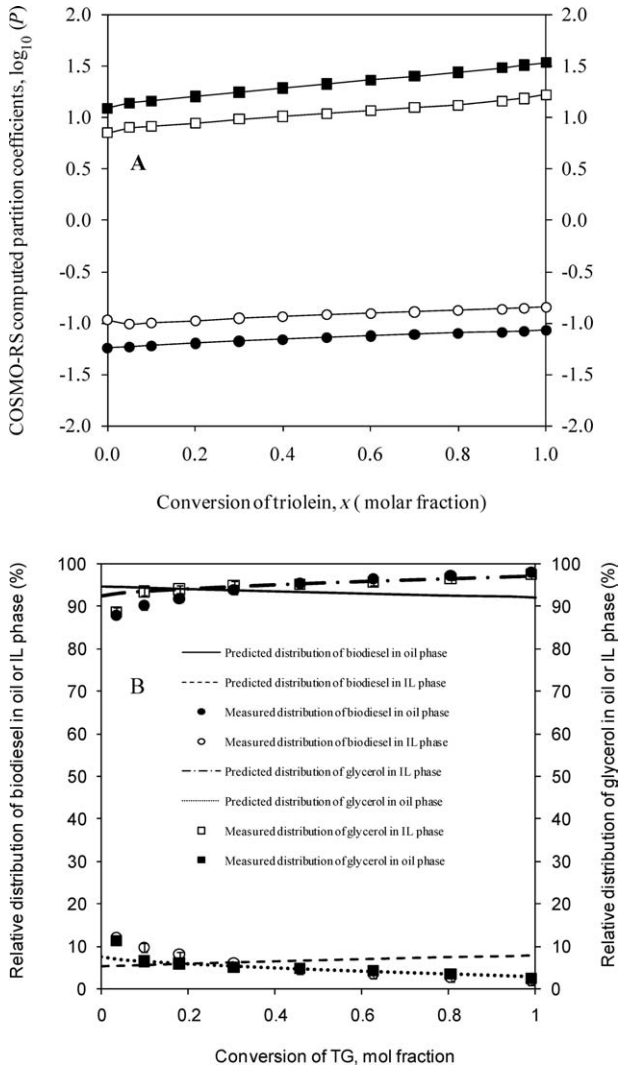


Figure 2. (A) COSMO-RS-predicted partition coefficients [$\log_{10}(P)$] of biodiesel (●, ○) and glycerol (■, □) in oil/ionic liquids [Ammoeng 102 (●, ■) or Ammoeng 120 (○, □)] systems against reaction evolutions at 323.15 K; (B) measured and predicted relative distribution of biodiesel (or glycerol) in oil and IL phase of Ammoeng 102-based system.

The plotted measured data in (B) are means ± standard deviations (error bars) of three determinations. The equilibrium partitioning at finite concentrations, however, was determined iteratively using the activity coefficients in both phases calculated by COSMOtherm.

glycerol distribution, in general, agree well with the predicted values. However, a larger deviation was observed for the distribution of biodiesel in oil/IL phase at the two ends of time course. For example, at 99% of TG conversion, the measured relative content of biodiesel in oil/IL is 98.1%/1.9%, which is higher than predicted 92.2%/7.8%. For Ammoeng 120-based system, the measured distribution of biodiesel in oil/IL phases at TG conversion 98.5% is 90.2%/9.8%, which is in agreement with predicted 87.6%/12.4%. Both prediction and experimental data demonstrated that Ammoeng 102 could achieve larger distribution difference for biodiesel and glycerol than Ammoeng 120. The reason for the prediction deviations from measured data may need further investigation, but the prediction direction is correct, that is, the COSMO-RS prediction could be used to explain the reaction behavior in IL-based system at least qualitatively.

For the reaction in oil/IL system, Novozym 435 is initially observed to suspend in between of the two phases due to its density with a value in between of that of oil and IL. Interestingly, for both IL systems, a cloudy interphase is always observed, and here we name it as pseudo-mesophase (Scheme 1B). This pseudo-mesophase, with enzyme resided in, is relatively stable, which may exist more than half-hour until a clear separation after agitation is stopped.[37] This phenomenon most likely can be ascribed to partial miscibility of oil and ILs because of amphiphilic property of Ammoeng 102 and 120.[35] The existence of pseudo-mesophase provides a microenvironment where triglyceride and methanol (dissolved in ILs) interact effectively, which facilitates progressing of the reaction.

Another characteristic of enzymatic production of biodiesel in these oil/IL systems, as aforementioned, is much less MG and DG detected in the reaction mixture, which indicates that both oil/IL systems (Figs. 1A,B) do not favor the accumulation of intermediate products. To clarify this issue, we also computed the partition coefficients of TG (triolein), DG (1,3-diolein), and MG (1-monoolein) in both systems throughout reaction evolution course. For the reaction in oil/Ammoeng 102 system, $\log_{10}(P)$ of TG varies from −2.16 to −2.03, DG from −0.96 to −0.71, and MG 0.28 to 0.61 with reaction evolution. This suggests that DGs have a larger portion than TG dissolved in the IL phase available for interaction with methanol in the IL; at the same time, the formed MG potentially prefer staying in the IL phase where it allows a further reaction with methanol. Therefore, from chemical potential point of view, both cases facilitate further in situ degradation of generated intermediate products, which might also contribute more to the formation of mesophase (Scheme 1B). Furthermore, in the last step of the reaction (MG + methanol to biodiesel + glycerol; Scheme 1A), the generated biodiesel is thermodynamically preferable to be redistributed into oil phase, driven by chemical potential difference, which greatly moves the reaction to completion. With this model prediction, we may partly explain why the content of intermediate products is kept at a lower concentration throughout the reaction course. Overall, the above discussion clearly shows that the reaction specificities of enzymatic alcoholysis in oil/IL systems are heavily associated with the partition behaviors of reactants, intermediate, and end products in the two phases.

Properties of ionic liquids that govern oil conversion and yield of biodiesel

The solubility of triglycerides in ILs has been suggested being a crucial factor to determine the reaction rate of enzymatic glycerolysis in IL-based systems[35]; such that, the solubilities of triolein in the evaluated ILs have been predicted by COSMO-RS (Table 3). Clearly, the reaction degrees show a certain correspondence with the oil solubilities in the ILs

Table 3. COSMO-RS-Predicted Solubility of Triolein in ILs and Partition Coefficients of Biodiesel and Glycerol Between Oil (Represented by Triolein) and IL Phases in the Presence of Methanol at 298.15 K

Ionic Liquid	Solubility of Triolein (x)*	$\log_{10}(P)$ of Biodiesel†	$\log_{10}(P)$ of Glycerol†
BMIM·BF_4	3.532 E −9	−1.9412	2.7524
*t*OMA·Tf_2N	0.5962	0.0863	1.9108
Ammoeng 100	0.0132	−0.8029	2.3237
Ammoeng 102	0.0432	−0.6630	2.0876
Ammoeng 111	1.4877 E −3	−0.5883	3.7311
Ammoeng 112	2.0058 E −4	−0.8873	3.2047
Ammoeng 120	0.1316	−0.3824	1.8776
DMIM·DMP	4.6347 E −5	−1.9233	3.8824
*t*OMA·TFA	0.3408	0.0083	2.4751

*COSMO-RS-computed solubility at 298.15 K is a zero-order approximation, which is based on a noniterative mode.
†COSMO-RS calculation of partition coefficients between oil (triolein) and IL (with dissolved methanol) is based on similar assumption as in Figure 2. The partition coefficients of biodiesel and glycerol at infinite dilution are direct results of COSMOtherm calculations. The volume quotient was model-estimated value.

(Tables 1 and 3). For instance, except DMIM·DMP, all IL systems that produce >10% TG conversion have triolein solubility >0.01 order of magnitude. DMIM·DMP is an exception, and a similar phenomenon has also been observed by Ha et al.[21] In EMIM·TfO (ethylmethylimidazolium bis(trifluoromethylsulfonyl) methane) with similar simple cation (the decisive moiety of ILs for the solubility of hydrophobic oil), biodiesel is also produced efficiently.[21] Both cases are difficult to find out a reasonable explanation from their remarkably low oil solubilities in the ILs according to COSMO-RS estimation.

The partition coefficients of biodiesel and glycerol in other IL systems evaluated in Table 1 are also predicted (Table 3). *t*OMA·Tf_2N- and *t*OMA·TFA-based systems represent two exceptions with positive $\log_{10}(P)$ values of biodiesel, which means that biodiesel have larger partition fraction in IL phase, and thus the equilibrium shifting will be strongly hindered by accumulation of biodiesel in IL phase. Probably, this is why the reactions could be easily initiated but do not move to completion (Table 1, entries 2 and 9). This prediction agrees well with experimental observation that no clear phase separation was achieved in these two systems. However, a larger partition difference of biodiesel (BMIM·BF_4 and DMIM·DMP) or glycerol (Ammoeng 111 and 112) between oil/IL phases (Table 3) did not result in a good conversion of oil (Table 1, entries 1, 5, 6, and 8). The most likely explanation is that this larger difference also indicates larger property difference of the related IL from oil, which corresponds to a lower solubility of oil in the IL available for reaction (Table 3). This may partly explain why the conversions of oil in these biphasic systems (Table 1, entries 1, 5, 6, and 8) are very low. It should be mentioned that exotic additives or excessive substrate could result in significant property change of IL system, and as a result can give considerable improvement of oil conversion.[22] Similar phenomenon has been observed for Ammoeng 100 in this work, which resulted in up to 95% yield of biodiesel while excessive methanol was used (data not shown).

From the above discussion, it is clear that oil/IL reaction system evaluated in this work behaves somehow like a typical biphasic system; however, being a highly concentrated system (solvent free for oil phase and methanol/IL (3:1, mol/mol) for IL phase), the property of the system can be greatly altered with substantial components added, which sometimes give quite different results.[26] Thus, it is not practical to accurately characterize oil/IL reaction system in a quantitative way, as COSMOtherm calculation of solubility and partition coefficient are based on an infinite mode as used in this work. However, the results from this work still show that in silico COSMO-RS calculation and simulation could predict the conversion and reaction specificity correctly, namely, the conversion of oil is pronouncedly associated with the solubility of oil in IL and the yield of biodiesel is largely controlled by the partition coefficient difference of end products between oil and IL phases.

Ammoeng series of ILs have all toxic evaluation data, which have been listed in the Toxic Substances Control Act and the European Inventory of Existing Commercial Chemical Substances. This group of products is available in technical quantities for comparably lower prices, which represents an interesting alternative to "conventional" ILs.[42] This work disclosed some interesting and distinct characteristics of Ammoeng IL-based system that other systems have not disclosed, for example, small amount of ILs engender an efficient reaction, protective effect for enzyme from inactivation by high concentration of methanol, and automatic phase separation. These properties will definitely contribute to the competence of this group of ILs as potential solvents for enzymatic production of biodiesel.

In the attempts of evaluation of reusability of enzyme and IL, positive results have been obtained. Novozym 435 could be reused for a number of times without significant activity lost, and Ammoeng 102 could also be recovered and reused more than 10 times without significant mass loss; however, these processes are still by means of organic solvents with many step extraction and separation. Therefore, for practical applications, developing more advanced technology for reusability of ILs and recovery of glycerol will be the next focus of the related research.

Conclusion

This work examined enzymatic production of biodiesel with oil/IL biphasic system by applying a variety of ILs with different cations and anions. It turned out that the reaction specificities are strongly dependent on the molecular structures of the ILs. Among the ILs evaluated, Ammoeng 102 and 120 display excellent reaction profiles in terms of oil conversion and biodiesel yield. The oil/Ammoeng 102 system also shows some unique properties, such as very less MG and DG in final product, only small amount IL needed to host an efficient reaction, protective effect for enzyme against inactivation by methanol and automatic phase separation for biodiesel recovery, etc. These characteristics are of paramount importance to explore the industrial interests of the system. This work also demonstrated that reaction specificity and oil/IL biphasic behaviors could be described by an experiment-independent model COSMO-RS, in which the initiation of reaction and conversion of oil are highly relevant to mutual solubility of oil and ILs and the completion of reaction and yield of biodiesel are largely governed by partition coefficients of solutes between oil/ILs. The results presented in this work suggested that the prediction quality is acceptable and instructive when in silico calculation of COSMO-RS based on an infinite mode to delineate nonideal biphasic behavior of a highly concentrated system was used,

which is believed to be useful to better understand oil/IL system and design more efficient reaction approaches.

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Literature Cited

1. Kalscheuer R, Stölting T, Kalscheuer R, Stölting T. Microdiesel: *Escherichia coli* engineered for fuel production. *Microbiology*. 2006;152:2529–2536.
2. Schubert C. Can biofuels finally take center stage? *Nat Biotechnol*. 2006;24:777–784.
3. Ma F, Hanna MA. Biodiesel production: a review. *Bioresour Technol*. 1999;70:1–15.
4. Liu YJ, Lotero E, Goodwin JG, Lu CQ. A novel sulfonated carbon composite solid acid catalyst for biodiesel synthesis. *J Catal*. 2007;246:428–433.
5. Chai F, Cao F, Zhai F, Chen Y, Wang X, Sua Z. Transesterification of vegetable oil to biodiesel using a heteropolyacid solid catalyst. *Adv Synth Catal*. 2007;349:1057–1065.
6. Akoh CC, Chang SW, Lee GC, Shaw F. Enzymatic approach to biodiesel production. *J Agric Food Chem*. 2007;55:8995–9005.
7. de Oliveira D, Di Luccio M, Faccio C, Rosa CD, Bender JP, Lipke N, Menoncin S, Amroginski C, de Oliveira JV. Optimization of enzymatic production of biodiesel from castor oil in organic solvent medium. *Appl Biochem Biotechnol*. 2004;113:771–780.
8. Chang HM, Liao HF, Lee CC, Shieh CJ. Optimized synthesis of lipase-catalyzed biodiesel by Novozym 435. *J Chem Technol Biotechnol*. 2005;80:307–312.
9. Al-Zuhair S. Production of biodiesel by lipase-catalyzed transesterification of vegetable oils: a kinetics study. *Biotechnol Prog*. 2005;21:1442–1448.
10. Watanabe Y, Shimada Y, Sugihara A, Noda H, Fukuda H, Tominaga Y. Continuous production of biodiesel fuel from vegetable oil using immobilized *Candida antarctica* lipase. *J Am Oil Chem Soc*. 2000;77:355–360.
11. Paula AV, Urioste D, Santos JC, de Castro HF. Porcine pancreatic lipase immobilized on polysiloxane-polyvinyl alcohol hybrid matrix: catalytic properties and feasibility to mediate synthesis of surfactants and biodiesel. *J Chem Technol Biotechnol*. 2007;82:281–288.
12. Shimada Y, Watanabe Y, Sugihara A, Tominaga Y. Enzymatic alcoholysis for biodiesel fuel production and application of the reaction to oil processing. *J Mol Catal B*. 2002;17:133–142.
13. Du W, Xu YY, Liu DH, Zeng J. Comparative study on lipase-catalyzed transformation of soybean oil for biodiesel production with different acyl acceptors. *J Mol Catal B*. 2004;30:125–129.
14. Zhou W, Boocock DGB. Phase distributions of alcohol, glycerol, and catalyst in the transesterification of soybean oil. *J Am Oil Chem Soc*. 2006;83:1047–1052.
15. Gamba M, Lapis AAM, Dupont J. Supported ionic liquid enzymatic catalysis for the production of biodiesel. *Adv Synth Catal*. 2008;350:160–164.
16. Brennecke F, Maginn EJ. Ionic liquids: innovative fluids for chemical processing. *AIChE J*. 2001;47:2384–2389.
17. Cull SG, Holbrey JD, Vargas-Mora V, Seddon KR, Lye GJ. Room-temperature ionic liquids as replacements for organic solvents in multiphase bioprocess operations. *Biotechnol Bioeng*. 2000;69:227–233.
18. Cole AC, Jensen JL, Ntai L, Tran KLT, Weaver KJ, Forbes DC, Davis JH. Novel Brønsted acidic ionic liquids and their use as dual solvent-catalysts. *J Am Chem Soc*. 2002;124:5962–5963.
19. Wu Q, Chen H, Han M, Wang D, Wang J. Transesterification of Cottonseed oil catalyzed by Brønsted acidic ionic liquids. *Ind Eng Chem Res*. 2007;46:7955–7960.
20. Neto BAD, Alves MB, Lapis AAM, Nachtigall FM, Eberlin MN, Dupont J, Suarez PAZ. 1-*n*-Butyl-3-methylimidazolium tetrachloroindate (BMI·InCl4) as a media for the synthesis of biodiesel from vegetable oils. *J Catal*. 2007;249:154–161.
21. Ha SH, Lan MN, Lee SH, Hwang SM, Koo YM. Lipase-catalyzed biodiesel production from soybean oil in ionic liquids. *Enzyme Microb Technol*. 2007;41:480–483.
22. Sunitha S, Kanjilal S, Reddy PS, Prasad RBN. Ionic liquids as a reaction medium for lipase-catalyzed methanolysis of sunflower oil. *Biotechnol Lett*. 2007;29:1881–1885.
23. Knothe G. Analyzing biodiesel: standards and other methods. *J Am Oil Chem Soc*. 2006;83:823–833.
24. Freedman B, Pryde EH, Kwolek WF. Thin-layer chromatography flame ionization analysis of transesterified vegetable oils. *J Am Oil Chem Soc*. 1984;61:1215–1220.
25. Eckert F, Klamt A. Fast solvent screening via quantum chemistry: COSMO-RS approach. *AIChE J*. 2002;48:369–385.
26. Spieß AC, Eberhard W, Peters M, Eckstein MF, Greiner L, Büchs J. Prediction of partition coefficients using COSMO-RS: solvent screening for maximum conversion in biocatalytic two-phase reaction systems. *Chem Eng Proc*. 2008;47:1034–1041.
27. Franke R, Krissmann J, Janowsky R. What can the process engineer expect of COSMO-RS? *Chem-Ing-Tech* 2002;74:85–89.
28. Marcato B, Cecchin G. Analysis of mixtures containing free fatty acids and mono-, di- and triglycerides by high-performance liquid chromatography coupled with evaporative light-scattering detection. *J Chromatogr A*. 1996;730:83–90.
29. Chen B, Guo Z, Let MB, Lue B-M, Xu X. Preparation of CLA ascorbyl ester with improved volumetric productivity by an ionic liquid-based reaction system. *Org Biomol Chem*. 2008;6:3196–3201.
30. Mittelbach M. Diesel fuel derived from vegetable oils. VI. Specifications and quality control of biodiesel. *Bioresour Technol*. 1996;56:7–11.
31. Anderson L, Ding J, Welton T, Armstrong DW. Characterizing ionic liquids on the basis of multiple solvation interactions. *J Am Chem Soc*. 2002;124:14247–14254.
32. Hailing PJ. Salt hydrates for water activity control with biocatalysts in organic media. *Biotechnol Tech*. 1992;6:271–276.
33. Zaks A, Klibanov AM. The effect of water on enzyme action in organic media. *J Biol Chem*. 1988;263:8017–8021.
34. Affleck R, Xu Z-F, Suzawa V, Focht K, Clark DS, Dordick JS. Enzymatic catalysis and dynamics in low-water environments. *Proc Natl Acad Sci USA*. 1992;89:1100–1104.
35. Chen B, Guo Z, Tan T, Xu X. Structures of ionic liquids dictate the conversion and selectivity of enzymatic glycerolysis: theoretical characterization by COSMO-RS. *Biotechnol Bioeng*. 2008;99:18–29.
36. Dreyer S, Kragl U. Ionic liquids for aqueous two-phase extraction and stabilization of enzymes. *Biotechnol Bioeng*. 2008;99:1416–1424.
37. Guo Z, Xu X. Lipase-catalyzed glycerolysis of fats and oils in ionic liquids: a further study on the reaction system. *Green Chem*. 2006;8:54–62.
38. Laszlo JA, Compton DL. α-Chymotrypsin catalysis in imidazolium based ionic liquids. *Biotechnol Bioeng*. 2001;75:181–186.
39. Murakamia Y, Hoshi R, Hirata A. Characterization of polymer–enzyme complex as a novel biocatalyst for nonaqueous enzymology. *J Mol Catal B*. 2003;22:79–88.
40. Janssen AEM, Halling PJ. Specificities of enzymes corrected for solvation depend on the choice of the standard state. *J Am Chem Soc*. 1994;116:9827–9830.
41. Mokrushina L, Buggert M, Smirnova I, Arlt W, Schomäcker R. COSMO-RS and UNIFAC in prediction of micelle/water partition coefficients. *Ind Eng Chem Res*. 2007;46:6501–6509.
42. Weyershausen B, Lehmann K. Industrial application of ionic liquids as performance additives. *Green Chem*. 2005;7:15–19.

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