



# Design and application of a novel ionic liquid with the property of strengthening coenzyme regeneration for whole-cell bioreduction in an ionic liquid-distilled water medium



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## HIGHLIGHTS

- [N1,1,1,1][Cys]-containing system was successfully applied in the reduction of BTAP.
- [N1,1,1,1][Cys] exhibits novel property of strengthening coenzyme regeneration.
- [N1,1,1,1][Cys]-water medium is the best for *T. asperellum*-catalyzed bioreduction.
- Fungus/yeast-catalyzed reductions are improved in [N1,1,1,1][Cys]-containing system.

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## ABSTRACT

Focusing on the task-specific properties of ionic liquids (ILs), a novel IL was designed by combining the quaternary ammonium cationic surfactant, [N1,1,1,1]<sup>+</sup>, with benign amino acid anion ([Cys]<sup>-</sup>), and was successfully employed in whole-cell-catalyzed bioreduction by *Trichoderma asperellum* ZJPH0810 using an ionic liquid-distilled water medium. As expected, based on better understanding about the effects of ILs' characteristics of cations and anions on *T. asperellum* ZJPH0810-catalyzed bioreduction and the optimization of reaction parameters, the developed tetramethylammonium cysteine ([N1,1,1,1][Cys])-containing system is more efficient for the asymmetric reduction of 3,5-bis(trifluoromethyl) acetophenone to (*R*)-[3,5-bis(trifluoromethyl)phenyl] ethanol by whole-cell catalysis in contrast to that in monophasic aqueous system. Herein, a new biotechnological application for designed type of ILs is proposed due to its novel property of strengthening coenzyme regeneration during the bioreduction process. The designed IL [N1,1,1,1][Cys] was also evaluated in *Candida tropicalis* 104-catalyzed bioreduction to board its application, with remarkable success.

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## 1. Introduction

The human health and environmental impact of chemical processes have led to a re-evaluation of conventional chemical methods, and provide impetus for a move towards Green Chemistry. Catalyst and solvent replacement have been identified as complementary approaches that may help mitigate the impact of chemical synthesis (Anastas and Eghbali, 2010). Enantiomerically pure chiral alcohols are among the valuable key intermediates for the manufacture of pharmaceuticals, pesticides, pheromones, flavors and advanced materials (Gamenara and de María, 2009). Recently, in the quest for a mild and environmentally friendly process for the production of chiral alcohols, an alternative biocatalysis approach

has been developed and gains increasing attention. (*R*)-[3,5-bis(trifluoromethyl) phenyl] ethanol ((*R*)-BTPE) is an important chiral building block for the synthesis of antiemetic drug of tumor chemotherapy Aprepitant (Emend®) and Fosaprepitant (Ivemend®) (Jin et al., 2012). A biocatalytic methodology has been established for asymmetrically reducing 3,5-bis(trifluoromethyl) acetophenone (3,5-BTAP) to (*R*)-BTPE by employing microbial cells as biocatalyst to replace conventional chiral chemical catalysts (complexes of Ru and ligands) (Li et al., 2009). In our previous study, whole cells of *Trichoderma asperellum* ZJPH0810 were employed to catalyze the bioreduction of 3,5-BTAP to (*R*)-BTPE with excellent enantioselectivity in aqueous reaction medium (Li et al., 2013). It was found however, like most whole-cell biocatalytic processes, poor water-solubility of 3,5-BTAP and its toxicity to microbial cells themselves are adverse to this biocatalytic process, thus limiting the productivity. Therefore, the development

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of more efficient reaction medium calls for novel alternatively solvents to improve the catalytic efficiency. In recent years, ionic liquids (ILs), as alternative environmentally sustainable solvents, have been subjected to in-depth evaluation as potential media for performing biocatalytic reactions, thus providing an effective way to overcome above limitations. Xiao et al. reported the use of a [C<sub>2</sub>OHMIM][NO<sub>3</sub>]-containing system in asymmetric reduction of 4-(trimethylsilyl)-3-butyn-2-one to (R)-4-(trimethylsilyl)-3-butyn-2-ol. The substrate concentration (12 mM versus 6 mM), initial reaction rate (14.0 μmol min<sup>-1</sup> g<sub>cell</sub><sup>-1</sup> versus 4.3 μmol min<sup>-1</sup> g<sub>cell</sub><sup>-1</sup>) and maximum yield (91% versus 71%) were greatly improved in comparison with the neat aqueous buffer system (Xiao et al., 2012). Up to date, however, the reported reaction systems suitable for biocatalysis in ILs by whole cells are still limited, and mainly focus on conventional imidazolium or pyridinium-based ILs (Huang et al., 2012; Zhang et al., 2013).

Currently, ILs as tunable solvents, have been investigated to match the specific requirements of particular process by the combination of different kinds of cations and anions with inexpensive greener starting materials and renewable sources (Samorì, 2011; Naushad et al., 2012). Much of this interest has been focused on the design of a 'green' IL, with ideally addressed issues such as better biocompatibility (Pan et al., 2010) and lower toxicity (Nancharaiyah and Francisa, 2011). In addition to above mentioned, of equal importance is the functional performance of the environmentally benign ILs. Surfactant ILs as new type of functional ILs, have been investigated in the biocatalytic system, with sometimes remarkable results. For example, dialkylimidazolium-based ILs, which are similar in structure to cationic surfactants, might increase the permeability of microbial cell membrane, thus not only lowering the product concentration in cells, but also reducing the inhibition and toxic effects of the product (Ranke et al., 2007). Although it was reported that quaternary ammonium-based ILs show low toxicity to some filamentous fungi, e.g. *Penicillium brevicompactum*, *Penicillium glandicola*, and so on (Petkovic et al., 2009), only a few studies have examined the performances of the quaternary ammonium-based cationic surfactant ILs in biocatalytic reactions. Cornmell et al. described the use of quaternary ammonium-based IL ([NMeOct<sub>3</sub>][NTF<sub>2</sub>]) in the biocatalytic dihydroxylation of toluene to toluene *cis*-glycol (Cornmell et al., 2008). Nevertheless, IL "greenness" is often challenged. Amino acids, as one of the abundant renewable sources and chiral pools in nature, are excellent feedstock for the preparation of ILs (Tao et al., 2006). Notably, cysteine (Cys) displays a ability to improve the yield and enantioselectivity in the bioreduction of (4R)-(-)-carvone, which may act as a hydride source to regenerate coenzyme NAD(P)H due to its mercapto group (Silva et al., 2012).

For the consideration of above mentioned, firstly, we focused on the performance evaluation for a variety of quaternary ammonium salt as the cations of ILs in the bioreduction of 3,5-BTAP to (R)-BTPE with the property of cationic surfactant. In order to obtain green and task-specific ILs used in the bioreduction of prochiral aromatic ketone, we subsequently centered our examination on various amino acids as anions to form ILs with the additional property of promoting coenzyme regeneration. In the bioreduction processes, coenzyme (e.g. NADH or NADPH) regeneration is a concern. Enzymes which catalyze reduction of carbonyl groups require a coenzyme from which a hydride is transferred to the carbonyl carbon. Many alcohols and sugars have been reported as co-substrate for recycling of coenzyme (Matsuda et al., 2009; Li et al., 2013; Ouyang et al., 2013). However, to date, cofactor regeneration via ILs remains unexplored largely and rarely reported.

We now report a new rational designed IL tetramethylammonium cysteine ([N1,1,1,1][Cys]) with a novel property of strengthening coenzyme regeneration and its superior performance in *T. asperellum* ZJPH0810-catalyzed bioreduction of 3,5-BTAP. To the

best of our knowledge, this is the first reported case on the bioreduction of (R)-BTPE in an IL-containing system. Furthermore, *Candida tropicalis* 104-catalyzed bioreduction was also evaluated to expand [N1,1,1,1][Cys]-containing system application in whole-cell bioreduction.

## 2. Methods

### 2.1. Materials

Chemically synthesized 3,5-BTAP, used as substrate for model reaction in this study, was supplied by Beijing Golden Olive Company, China. The model reaction product of (R)-BTPE and (S)-BTPE were purchased from Capot Chemical Co. Ltd., China. The ILs used throughout the investigation were synthesized and supplied by Shanghai Chengjie Chemical Co. Ltd., China. The chemical structures of cations and anions of ILs were shown in Table S1. Other reagents and chemicals were from commercial sources and were of analytical grade.

### 2.2. Synthesis of ILs

[N1,1,1,1][Cys] was synthesized by a similar method described previously (Abbott et al., 2003, 2006). Briefly, 0.1 M tetramethylammonium hydroxide solution was mixed with 0.1 M cysteine solution at a molar ratio of 1:1 at 60 °C under vigorous agitation for 24 h. The resulted clear liquid was dried with P<sub>2</sub>O<sub>5</sub> at 45 °C for at least 2 weeks. <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) δ/ppm: 3.64–3.61 (q, J = 3.6 Hz, 1H, CH), 3.09 (s, 12H, CH<sub>3</sub> × 4), 2.84–2.80 (q, J = 5.6 Hz, 2H, CH<sub>2</sub>). <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O) δ/ppm: 179.8 (COOH), 57.9 (CH), 55.7 (CH<sub>3</sub> × 4), 27.2 (CH<sub>2</sub>).

The following three kinds of ILs were synthesized separately by the method similar to [N1,1,1,1][Cys].

Tetraethylammonium cysteine [N2,2,2,2][Cys], <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) δ/ppm: 3.47–3.44 (q, J = 3.2 Hz, 1H, CH), 3.18–3.13 (q, J = 6.0 Hz, 8H, CH<sub>2</sub> × 4), 2.90–2.86 (m, 2H, CH<sub>2</sub>), 1.16 (t, J = 5.2 Hz, 12H, CH<sub>3</sub> × 4). <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O) δ/ppm: 176.8 (COOH), 64.6 (CH), 52.5 (CH<sub>3</sub> × 4), 28.5 (CH<sub>2</sub>), 7.1 (CH<sub>2</sub> × 4).

Tetramethylammonium glutamate [N1,1,1,1][Glu], <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) δ/ppm: 3.05 (s, 12H, CH<sub>3</sub> × 4), 2.10–2.06 (t, J = 8.0 Hz, 1H, CH), 1.79–1.72 (m, 2H, CH<sub>2</sub>), 1.67–1.62 (m, 2H, CH<sub>2</sub>). <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O) δ/ppm: 182.5 (COOH), 182.2 (COOH), 57.8 (CH), 56.0 (CH<sub>3</sub> × 4), 35.1 (CH<sub>2</sub>), 32.7 (CH<sub>2</sub>).

Tetraethylammonium glutamate [N2,2,2,2][Glu], <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) δ/ppm: 3.18–3.14 (q, J = 5.6 Hz, 8H, CH<sub>2</sub> × 4), 2.15–2.07 (m, 1H, CH), 1.85–1.76 (m, 2H, CH<sub>2</sub>), 1.72–1.64 (m, 2H, CH<sub>2</sub>), 1.16 (t, J = 6.0 Hz, 12H, CH<sub>3</sub> × 4). <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O) δ/ppm: 182.7 (COOH), 182.5 (COOH), 56.6 (CH), 52.6 (CH<sub>3</sub> × 4), 35.0 (CH<sub>2</sub>), 32.6 (CH<sub>2</sub>), 7.4 (CH<sub>2</sub> × 4).

### 2.3. Measurements of physicochemical properties of IL

Density and viscosity of IL were measured at the temperature T = 298.15 ± 0.02 K by a rotational automated viscometer Anton Paar Stabinger AMA 5000 M and viscometer ND58S, Shanghai Inspection & Measurement Co. Ltd., respectively. Each data point of the density and viscosity is the average value of three measurements. The uncertainty of the density and viscosity measurements is 0.0005 g cm<sup>-3</sup> and ±3% respectively according to the manufacturer.

Experimental surface tension result is determined at the temperature 298.15 ± 0.02 K at atmospheric pressure (101 Kpa). A digital tensiometer (DropMeter Standed A-100, Maist Vision Inspection & Measurement Co. Ltd.) calibrated by pure water, was used to determine the surface tension by means of the

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