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Cholinium-based deep eutectic solvents and ionic liquids for lipase-catalyzed synthesis of butyl acetate

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ABSTRACT

The aim of this study was to analyze the advantages and limitations of cholinium-based ionic liquids (ILs) and deep eutectic solvents (DESs) used as green solvents for immobilized *Candida antarctica* lipase B-catalyzed synthesis. The reaction of acetic anhydride with 1-butanol to give short chain ester butyl acetate was chosen as a model reaction. Results showed that selected ILs (choline glycinate, choline alaninate, choline asparaginate, choline malate) and DESs (choline chloride mixtures with glycerol Gly, ethylene glycol EG, and urea U as hydrogen bond donors in molar ratio 1:2) are poor media for tested reaction if applied as pure solvents (yield <5%). This happens probably due to the substrate "entrapment" within DES structure through H-bonding. The addition of water (as a protic solvent) to the DESs strongly enhances both enzyme activity and reaction yield. Among different DES:water mixtures tested, the one with EG as hydrogen bond donor at water content of 5% (w/w) proved to be the most effective, resulting in esterification yield 80% (higher than yield obtained in a reference solvent *n*-heptane). Kinetic parameters of Ping-Pong Bi–Bi reaction mechanism were estimated for reactions in ChCl:EG:water mixtures and the chosen model seems to fit reaction mechanism description. Furthermore, in all ChCl:EG:water tertiary mixtures about 50–70% lipase activity decrease was observed after 3 days incubation. The enzyme activity stayed unchanged even after extending the incubation time to 15 days. Finally, the used DESs were assayed for cytotoxicity in fish (CCO) and human (MCF-7) cell lines, where they exhibited low cytotoxicity (EC_{50} >5 mM) for both cell lines. Obtained results suggest that DESs are promising candidates for green biocatalysis.

1. Introduction

Within a green chemistry concept a growing area of research is devoted to the design of new, more environment friendly solvents, indicatively called green solvents. Various tunable and smart solvents have been evaluated for this purpose, and so far ionic liquids (ILs) and deep eutectic solvents (DESs) stand out as the most promising candidates in the field of solvent innovation for food technology, biotechnology and chemical technology [1]. Properties that attribute ILs the title "environment friendly" are non-volatility, non-flammability and excellent stability. Conventional imidazolium- and pyridinium-based ILs so far showed excellent technological properties, however, they are expensive, based on non-sustainable feedstock and possess relatively high environmental impact [2]. This led to development of ILs from cheap and carefully selected naturally-derived materials (*e.g.* cholinium salts, sugars, organic acids and amino acids) that are suitable for CO_2 separation processes [3] and pretreatment of lignocellulosic biomass [4]. As for their environmental impact, choline-amino acid ILs for example displayed low toxicity toward the bacteria [5], aquatic crustacean *Artemia salina* and *HeLa* cell culture [6], and were also classified as 'readily biodegradable' [5]. However, further ILs structure-toxicity studies have to be conducted before claiming them as truly eco-friendly [2]. The DESs represent a new generation of liquid salts and are generally based on mixtures of cheap and readily available components: nontoxic quaternary ammonium salts (*e.g.* choline chloride) and a naturally-derived uncharged hydrogen-bond donor (HBD) (*e.g.* sugars, alcohols, amides and carboxylic acids) [7]. They are liquid supramolecules usually composed of common metabolites at specific molar ratios, including water in some cases, and are characterized by extensive intermolecular interactions. Since their emergence in broad use, these solvents have attracted attention in synthesis, electrochemistry, nanomaterials, biochemistry, sep-

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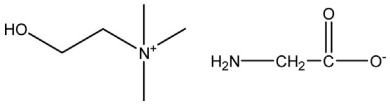
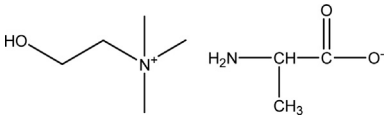
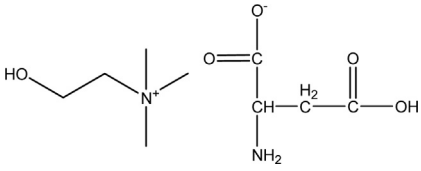
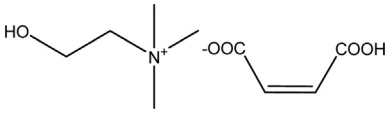
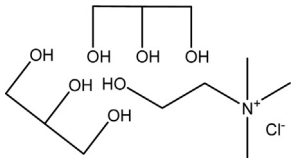
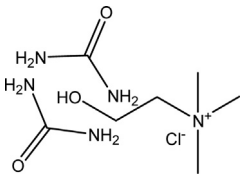
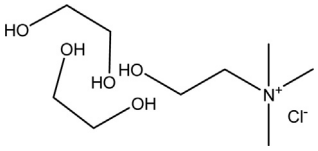
Table 1
List of ILs and DESs used for lipase-catalyzed esterification.

	Name	Abbreviation	Structure
ILs	Choline glycinate	[Ch][Gly]	
	Choline alaninate	[Ch][Ala]	
	Choline asparaginate	[Ch][Asp]	
	Choline malate	[Ch][MA]	
DESs	Choline chloride:glycerol (1:2)	ChCl:Gly	
	Choline chloride:urea (1:2)	ChCl:U	
	Choline chloride:ethylene glycol (1:2)	ChCl:EG	

aration, and analysis [8]. The assumption that these solvents are environmentally friendly is based on the benign property of their starting materials, however, recent studies indicate that these assumptions have to be carefully examined [2,8].

Lipases, as catalysts in synthetic (ester synthesis, interesterification and transesterification reactions) or hydrolytic reactions, are the most industrially applied enzymes in food, cosmetic, and pharmaceutical industry, as well as in, surfactant synthesis and preparation of biodiesel [9]. These enzymes tolerate non-natural environments surprisingly well, and have been established as promising catalysts for biotransformations in organic solvents, certain ILs and DESs [10–12]. So far, hydrophobic imidazolium- and pyridinium-based ILs with long alkyl chains attached to cation core and anions, such as bis(trifluoromethylsulfonyl) imide and hexafluorophosphate, proved to be excellent media for various lipase-catalyzed synthetic and hydrolytic reactions, as well as for regio- and stereoselective transformations [13,14], whereas the potential of ILs from natural sources (*e.g.* cholinium-based ILs) have been reported only by Deive et al. [15]. The DESs were at first considered as a poor choice for biocatalysis due to denaturing properties of HBD like urea. However, Gorke et al. [16] were the first to find that many hydrolytic enzymes retain excellent activity in such environment and associated this to the fact that strong H-bonds between DES components lowered reactivity of individual components. To date, several publications have pointed out the use of DESs based on choline chloride or ethylammonium chloride with a HBD such as alcohols, acids or amides as media for lipase-catalyzed esterification [17,18], transesterification [16,19], alcoholysis [12,20], lipophilization of phenolic acids [21], epoxidation of fatty acids and phenolic compounds [22] and acylation reactions [23].

Esterification reactions are commercially interesting for lipase-catalyzed preparation of short chain flavour esters by a reaction between short chain acid and alcohol. These esters are commonly used as flavour additives in food-, pharma- and cosmetic industry [14,25,26]. A number of papers regarding their enzymatic synthesis in nonconventional media, such as *n*-heptane [24], supercritical CO_2 [25] and imidazolium ILs [26,14] have been published so far. Herein the potential of chlolinium-based solvents as media for lipase-catalyzed preparation of short chain esters have been investigated. The synthesis of butyl acetate, as a bench reaction for proposed estrification, was conducted in a series of cholinium-based ILs and DESs using immobilized lipase. Additionally, to evaluate environmental properties of DESs, cytotoxicity in fish (CCO) and human (MCF-7) cell lines have been investigated.