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Assessing the toxicity and biodegradability of deep eutectic solvents

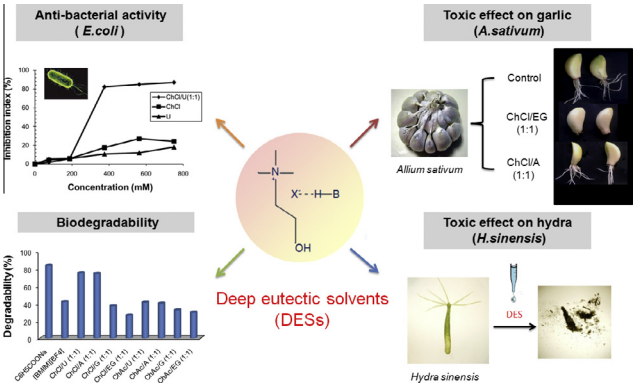
Qing Wen [a], Jing-Xin Chen [a], Yu-Lin Tang [b], Juan Wang [b], Zhen Yang [b,*]

[a] College of Life Sciences, Shenzhen Key Laboratory of Marine Bioresources and Ecology, Shenzhen University, Shenzhen 518060, Guangdong, China
[b] College of Life Sciences, Shenzhen Key Laboratory of Microbial Genetic Engineering, Shenzhen University, Shenzhen 518060, Guangdong, China

HIGHLIGHTS

- A systematic assessment on both toxicity and biodegradability of DESs.
- DESs cannot be simply regarded as nontoxic or readily biodegradable.
- Strong relationship between toxicity/biodegradability of DESs and their structures.
- Toxicity of DES components can be drastically lowered by incorporation into DES.
- Toxicity mechanism may be related to interactions of DESs with cellular membranes.

GRAPHICAL ABSTRACT



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ABSTRACT

Deep eutectic solvents (DESs) have emerged as a new type of promising ionic solvents with a broad range of potential applications. Although their ecotoxicological profile is still poorly known, DESs are generally regarded as "green" because they are composed of ammonium salts and H-bond donors (HBDs) which are considered to be eco-friendly. In this work, cholinium-based DESs comprised of choline chloride (ChCl) and choline acetate (ChAc) as the salt and urea (U), acetamide (A), glycerol (G) and ethylene glycol (EG) as the HBD were evaluated for their toxic effects on different living organisms such as *Escherichia coli* (a bacterium), *Allium sativum* (garlic, a plant) and hydra (an invertebrate), and their biodegradabilities were assessed by means of closed bottle tests. These DESs possessed an anti-bacterial property and exhibited inhibitory effects on the test organisms adopted, depending on the composition and concentration of the DES. The mechanism for the impact of DESs and their components on different living organisms can be associated to their interactions with the cellular membranes. Not all DESs can be considered readily biodegradable. By extending the limited knowledge about the toxicity and biodegradation of this particular solvent family, this investigation on DESs provides insight into our structure-based understanding of their ecotoxicological behavior.

1. Introduction

As a promising "green" alternative to conventional ionic liquids (ILs), deep eutectic solvents (DESs) have currently attracted widespread academic and industrial interests with a broad range of applications (Abbott et al., 2003; Zhang et al., 2012; Paiva et al., 2014). A DES can be easily prepared by thermal mixing of an ammonium salt (such as choline chloride) with a hydrogen-bond donor (HBD, such as urea and glycerol) at a specified stoichiometric ratio or by freeze drying of this mixture. The melting point of the resulting DES is lower than that of either of its components,

* Corresponding author. Tel.: +86 755 2653 4152; fax: +86 755 2653 4277.
E-mail address: zyang@szu.edu.cn (Z. Yang).

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which can be ascribed to the hydrogen bonding between the HBD and the salt anion (Abbott et al., 2003). From both environmental and technological perspectives, DESs are superior to conventional ILs with the following advantages: (1) lower price; (2) easier preparation with higher purity; (3) higher biodegradability and lower toxicity; and (4) greater designability with a broad selection of cations, anions, HBDs, and salt/HBD molar ratios.

Because the two components of DESs are environmentally-friendly ingredients that are abundantly found in nature, DESs are usually considered "green". For instance, choline, the so-called vitamin B4, is an essential nutrient widely distributed in biosphere (USDA; Zeisel and da Costa, 2009). However, extensive assessment is required before we can judge whether DESs are truly "green". So far there are only 3 reports dealing with the study in this regard: Hayyan et al. have tried to assess the toxic effects of both cholinium- (Hayyan et al., 2013a) and phosphonium-based (Hayyan et al., 2013b) DESs on four bacteria and on brine shrimps, while Radošević et al. (2015) have evaluated 3 choline chloride-based DESs for their cytotoxicity towards fish and human cell lines, phytotoxicity on wheat, and biodegradability using closed bottle tests.

The goal of this study was to take up a systematic assessment on DESs about their likely toxicity towards different living organisms and to obtain a structure-based understanding of their toxicity and biodegradability. It is anticipated that these results can extend our very limited knowledge about the ecotoxicological profile of DESs, thus providing useful information for the design of more biocompatible, less toxic and readily biodegradable DESs. To this end, 24 DESs were prepared by combining two cholinium salts (choline chloride (ChCl) and choline acetate (ChAc)) with four HBDs (i.e. urea (U), acetamide (A), glycerol (G) and ethylene glycol (EG)) at three molar ratios (1:2, 1:1, 2:1), their toxicity towards a bacterium (*Escherichia coli*), an invertebrate (*Hydra sinensis*) and a plant (garlic, *Allium sativum*) were evaluated, and their biodegradability assessed.

2. Materials and methods

2.1. Reagents and test organisms

Choline acetate (ChAc, 99%) was purchased from ShangHai Cheng Jie Chemical Co. Ltd. Choline chloride (ChCl), urea (U), glycerol (G), acetamide (A), ethylene glycol (EG), and all other reagents used were of analytical grade from Shenzhen Xinlixiang Technology Co. Ltd. *E. Coli* DH5α was obtained from the China Center for Type Culture Collection (CCTCC). *H. sinensis* was collected from Dongjiang River of Huizhou, China. Garlic bulbs (*A. sativum*) were purchased from a local supermarket in Shenzhen, China. DESs were prepared by following the procedures described in Wu et al. (2014); Huang et al. (2014). The DES concentration was defined based on the molar concentration of the cholinium salt. DESs with all three salt/HBD molar ratios (1:1, 1:2 and 2:1) were used in the hydra tests (Section 2.3) while those with a salt/HBD molar ratio of 1:1 were selected for use in the other tests (Section 2.2, 2.4, 2.5).

2.2. Toxicity test on E. coli

The impact of DESs on the growth of microorganisms was estimated by inoculating the *E. coli* strain into a DES-containing Mueller–Hinton broth, as compared to the same bacterium grown in the DES-free medium. A series of tubes (25 × 200 mm) were prepared which contained 3 mL of sterilized Mueller–Hinton broth with or without addition of a DES or its components (0–750 mM, pH 7.0). Each tube was inoculated with 1 mL of a suspension of the *E. coli* cells (in a concentration of 1.7×10^9 cfu mL^{-1} (cfu, colony forming units)) and incubated for 24 h at 37 °C in a slanted position. The growth of the bacterium was followed by measuring the absorbance at 550 nm, and the inhibition effect of the DES was estimated by using the inhibition index (I) defined as: $I = (A_0 - A)/A_0 * 100\%$ (Kommanee et al., 2012), where A_0 and A are the absorbances at 550 nm for the inoculated solutions in the absence and presence of the DES, respectively. Experiments were duplicated (with %CV for each absorbance reading lower than 5%).

2.3. Toxicity test on hydras

A hydra medium containing 1.0 mM $NaHCO_3$, 0.1 mM KCl, 0.1 mM $MgCl_2$ and 1.0 mM $CaCl_2$ in deionized water (pH determined to be 7.79) was prepared, where polyps were maintained for at least 24 h prior to microscopic observations. As a control, a few drops of this hydra specimen (containing 1–2 healthy hydras) were placed on a clean glass slide for microscopic observations with an Olympus BX51 fluorescence microscope (40×), normally at 1–2 h intervals. The morphological changes of the hydras were followed and their survival times were recorded. For testing the toxicity of DESs and their components, a few drops of the above hydra specimen were placed on the glass slide, the water of which was gently absorbed with a soft absorbent paper before 2–3 drops of a solution were added immediately; the solution contained in the hydra medium either 10 mM of a salt (ChCl or ChAc), an HBD (A, U, EG, or G), a mixture of the two at a molar ratio of 1:1, 1:2 or 2:1, or a DES prepared by combining the two at a molar ratio of 1:1, 1:2 or 2:1 (pH re-adjusted to pH 7.79). Microscopic observations were done as for the control, at 1–2 h intervals.

2.4. Toxicity test on garlics

Healthy and equal-sized garlic cloves were selected from the bulbs that had not started the root growth nor the formation of green leaves. Fifty one such garlic cloves were soaked in deionized water overnight at 26 °C before proceeding as follows (3 cloves per treatment): Three of them were treated with 1% H_2O_2 for 4 h; three were placed in boiling water for 20 min; and the rest were further treated for germination in the presence of 3 types of test solutions (final pH all adjusted to 7.0): (1) deionized water (as a blank control); (2) aqueous solution containing a DES component (a cholinium salt or an HBD, 0.01 M); and (3) aqueous solution containing a DES (0.01 M). The procedures of treatment with the above solutions for germination were as follows: A piece of gauze was placed in each 90 × 15 mm Petri dish containing 5 mL of a test medium as mentioned above, and the garlic cloves were transferred onto the gauze, with 3 cloves per dish (~2 cm apart from each other, sitting perpendicularly to the water level). These Petri dishes were then placed in an incubator for 7 d with the incubation conditions controlled as 26 °C, 16 h light/8 h dark per day, and 60% relative humidity. The test medium solutions in the Petri dishes were renewed regularly every 24 h. Seven days later, the germination was halted, the root lengths were measured, and the root tips were subjected to staining with carbol fuchsin for observation of the changes in the nucleus with an Olympus BX51 fluorescence microscope (1000×). For the first two groups, staining and microscopic observation were performed immediately after the treatment as mentioned above. The root length data were analyzed by using the IBM SPSS Statistics 19 software for one-way analysis of variance (ANOVA).

2.5. Closed bottle tests

The biodegradability of DESs was assessed by adopting the closed bottle tests following the OECD guideline 301 D (OECD, 1992). A mineral medium was prepared that contained