Supporting Information

Hydrophobic deep eutectic solvents as an emerging green reaction medium for biocatalytic processes: Impacts of solvent properties and compositions

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1. **Table S1. The yields obtained in 24 h from the acylation reaction to produce tyrosol acetate in hydrophilic and hydrophobic DESs**

Hydrophilic DES	Hydrophobic DES	Yield (%)
Pro/G (1:2)		4.0%
Bet/G (1:2)		0
ChCl/LA (1:1)		0
ChCl/U (1:2)		5.4%
ChCl/G (1:2)		0
ChCl/EG (1:2)		0
ChAc/U (1:2)		0
ChAc/G (1:2)		0
ChAc/EG (1:2)		0
ChAc/A (1:2)		0
	Men/Ner (1:2)	61.8
	Men/t-BuOH (1:2)	45.8
	Men/2M2B (1:2)	56.1
	Men/cHex (1:2)	1.4
	Men/Cin (1:2)	58.7
	Men/Ibu (1:2)	24.5
	Men/Lid (1:2)	42.4
	Men/Dec (1:2)	0.3
	Men/Thy (1:2)	69.5
	DecA/Hex (1:2)	0
	DecA/Oct (1:2)	0
	DecA/Dec (1:2)	0
	DecA/Ger (1:2)	14.1
	DecA/Ner (1:2)	20.5
	DecA/Lin (1:2)	16.2
	DecA/Thy (1:2)	14.2
	DecA/Men (1:2)	2.4
	DecA/N44Br (2:1)	21.6
	DecA/N88Br (2:1)	10.3
	DecA/*t*-BuOH (1:2)	11.1
	DecA/2M2B (1:2)	13.6

Reactions were carried out by adding 20 mg Novozym 435 to 2 mL of a DES containing 5 mM tyrosol and 25 mM vinyl acetate, with agitation of 250 rpm at 40 ^{o}C for 24 h.

Abbreviations: Pro = proline, Bet = betaine, G = glycerol, U = urea, A = acetamide, EG = ethylene glycol, ChCl = choline chloride, ChAc = choline acetate, LA = lactic acid, Cin = cinnamaldehyde, Ibu = ibuprofen, Lid = lidocaine, Thy = thymol, Men = menthol, Ner = nerolidol, Lin = linalool, Ger = geraniol, *t*-BuOH = *tert*-butanol, 2M2B = 2-methyl-2-butanol, DecA = 1-decanoic acid, Hex = 1-hexanol, Oct = 1-octanol, Dec = 1-decanol, cHex = cyclohexanol, N44Br = tetrabutylammonium bromide, N88Br = tetraoctylammonium bromide.

2. Effect of water addition on the enzymatic synthesis of tyrosol decanoate in DecA-based HDES

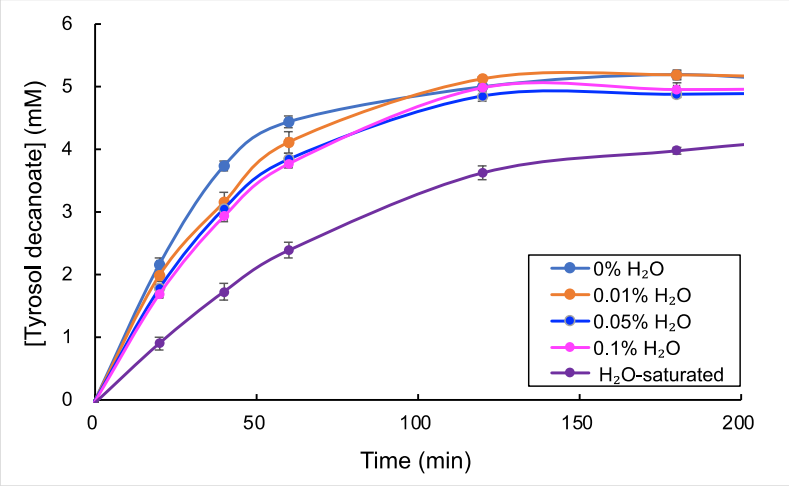


Figure S1. Impact of water content on the acylation reaction in HDES

Five tyrosol solutions (5.0 mM) were prepared in water-saturated DecA/Ner (1:2), and in the molecular sieve-dried HDES with addition of 0, 0.01%, 0.05% and 0.1% (v/v) water. Novozym 435 (20 mg) was added to 2.0 mL of each of these tyrosol solutions, and the reactions were carried out at 40 °C with agitation of 250 rpm.

3. Measurement of the solvent properties

The densities and viscosities of the DESs were detected by using a multifunctional electronic densimeter (MH-300G) and a digital display rotary viscometer (NDJ-9S), respectively, from the Shanghai Lichen Bangxi Instrument Technology Co. Ltd., China.

The procedure of detecting the solubility of the substrate, tyrosol, in a DES was as follows: Excess amount of tyrosol was first added to a glass bottle followed by addition of 5 mL of a DES, the mixture was then mixed thoroughly with a vortex mixer for 5 min before being incubated at 25 °C for 24 h. The supernatant was then subjected to HPLC analysis.[1]

The polarity of a DES was estimated by measuring the maximum absorption wavelength (λ_{max}) of the solvent containing a solvachromic dye, Nile Red (NR), in a concentration of ~15 μM, using a Shimadzu UV-1800 UV-Vis spectrophotometer.[1] The λ_{max} is positively related to the polarity of the solvent.[2] As the molar electronic transition energy of the dye (Nile Red in our case) in the solvent, E_T (NR) is employed to express the polarity of the solvent based on the equation E_T (NR) = 28591/λ_{max}.[3] Lower E_T (NR) values imply higher polarity of the solvent.

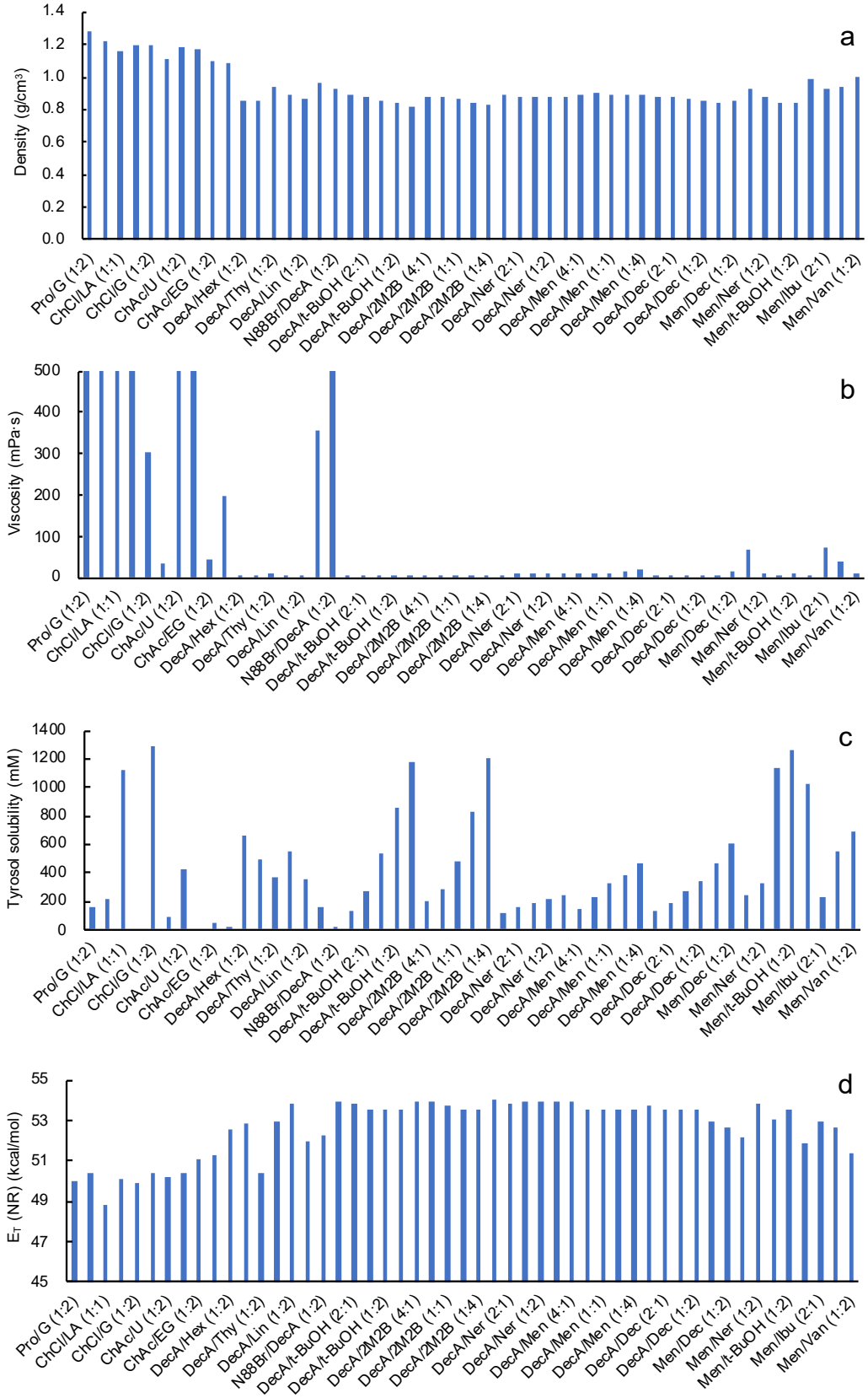


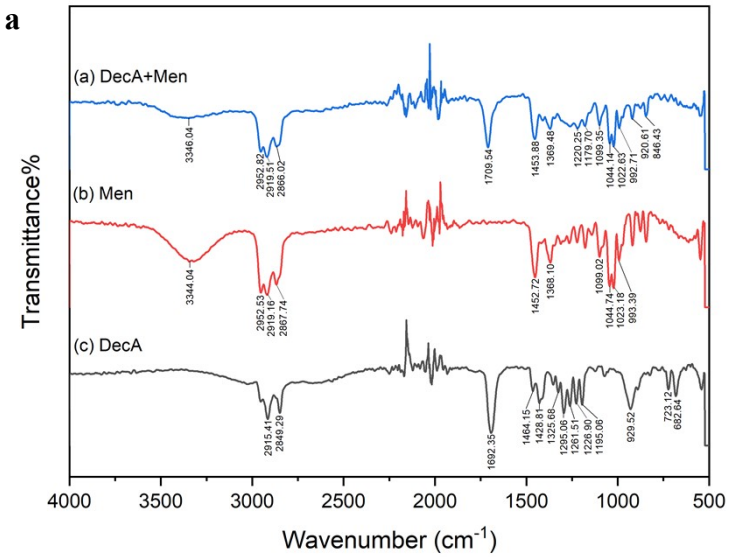
Figure S2. Physicochemical properties of DESs. a. density; b. viscosity; c. tyrosol solubility; d. polarity

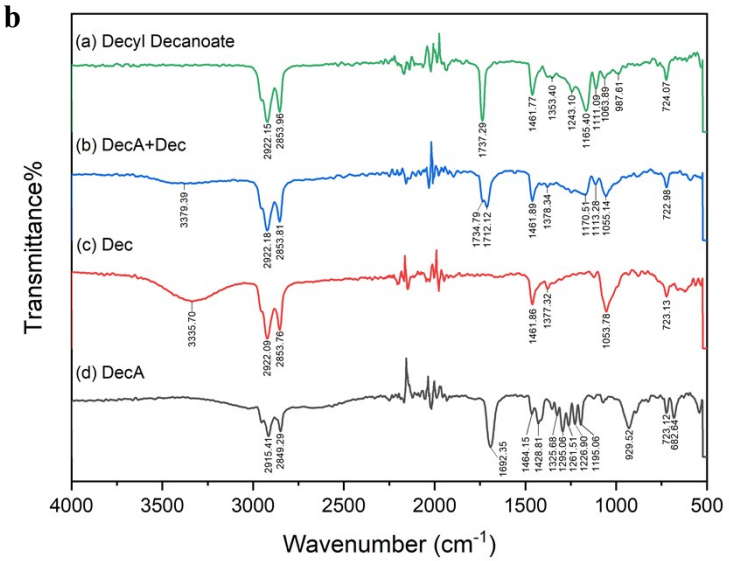
4. Structural analysis of HDES and discussion about the H-bonding interactions in the HDES

4.1 Structural analysis with FT-IR and ^{1}H-NMR

Fourier transform infrared (FTIR) spectroscopy (Nicolet iS50, Thermo Scientific) and ^{1}H-nuclear magnetic resonance (NMR) spectroscopy (Avance III 500 MHz, Bruker) were used to study the interactions involved in the HDES.

4.2 FT-IR and ^{1}H-NMR spectra





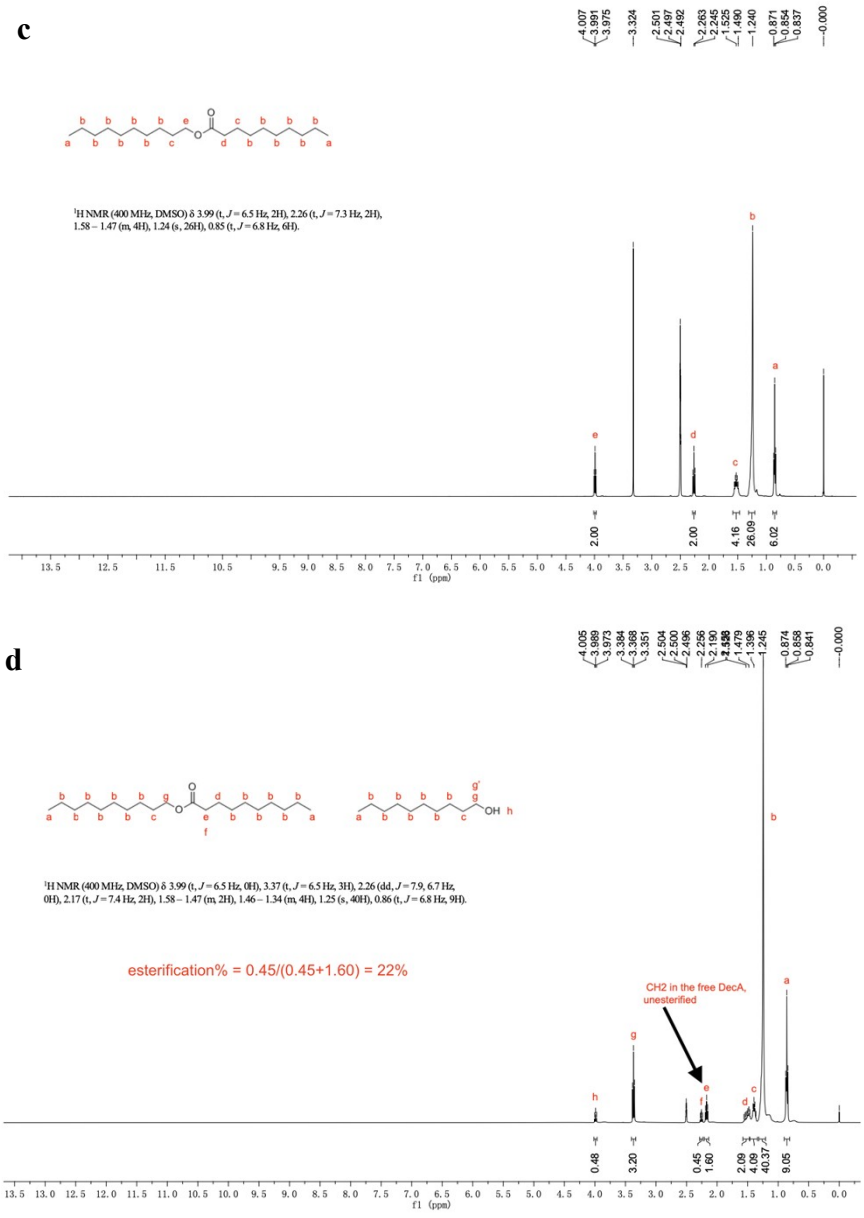


Figure S3. Structural analyses of HDESs

a. FT-IR spectra of the HDES DecA/Men (1:2) and its two components, decanoic acid (DecA) and DL-menthol (Men);

b. FT-IR spectra of the standard sample of decyl decanoate, the HDES DecA/Dec (1:2) and its two components, DecA and 1-decanol (Dec);

c. ^{1}H-NMR spectra of the standard sample of decyl decanoate;

d. ^{1}H-NMR spectra of DecA/Dec (1:2).

4.3 A discussion

The FT-IR spectra of all the DecA/alcohol HDESs, except DecA/Dec (1:2), were similar as the one presented in Fig. S3a for DecA/Men (1:2), showing a sharp peak at ~1710 cm^{-1}. The carbonyl stretching vibration peak for the free decanoic acid is originally located at a lower wavelength (1692 cm^{-1}). A blue shift from 1692 cm^{-1} to 1710 cm^{-1} indicates strongly a new hydrogen bond formation,[4,5] thus confirming the H-bond interactions in the HDES.

For DecA/Dec (1:2), however, beside the sharp peak at 1712 cm^{-1} there is a shoulder peak appearing at 1735 cm^{-1}(Fig. S3b), which is the stretching vibration peak characteristic for the C=O double bond in an ester linkage. A strong absorption peak at 1737 cm^{-1} is indeed observed for the standard sample of decyl decanoate. This suggests that while most of decanoic acid present in the HDES is involved in the H-bonding interactions with decanol, another component of the HDES, a small portion of decanoic acid is already esterified with decanol. The ^{1}H-NMR spectrum of the HDES (Fig. S3d) further reveals that 22% of decanoic acid is present as an ester, calculated from the ratio of the integral areas.

References

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