



Current Status and Future Prospects of Biocatalysis in Deep Eutectic Solvents.

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BIOCATALYSIS AND SOLVENTS

Water

Natural resource, easy to obtain, low cost, safe.
Restricted solvation properties, high reactivity.

SCF ~10

Hybrid of gas/liquid obtain under specific conditions of temperature and pressure (most popular is carbon dioxide).
Tuning properties, strong and unusual solvation properties.
Restricted range of molecule solubility, high cost.

Molecular solvents number ~ 100

Biomass-derived solvents: such as EtOH, limonene, ethyl lactate, glycerol, or 2-methyltetrahydrofuran.
Biorenewable, innocuous, and not very expensive.
Low choice, restricted versatility and solvation properties.

Organic solvents: such as acetone and ethyl acetate, or alkyl alcohols such as methanol (MeOH), ethanol (EtOH), and propanol.
Large choice with several and different solubilization and extraction capacities.
Accumulation in the atmosphere (low boiling points), flammability, high toxicity, limited-biodegradability, synthetic from fossil source, cost.

Multi-molecular based solvents number > 10⁶

Mixing of at least two molecules in such proportions that they can lead to a liquidus temperature significantly lower than pure equivalents.

Ionic liquids (IL)

Expensive, difficult to purify, toxicity

1914 (P. Walden)

Deep Eutectic Solvents (DES)

2003 (AP. Abbott)

Natural Deep Eutectic Solvents (NaDES)

2011 (R. Verpoorte)

SOLVENTS: Multi-molecular based solvents:

Deep Eutectic Solvents (DES)

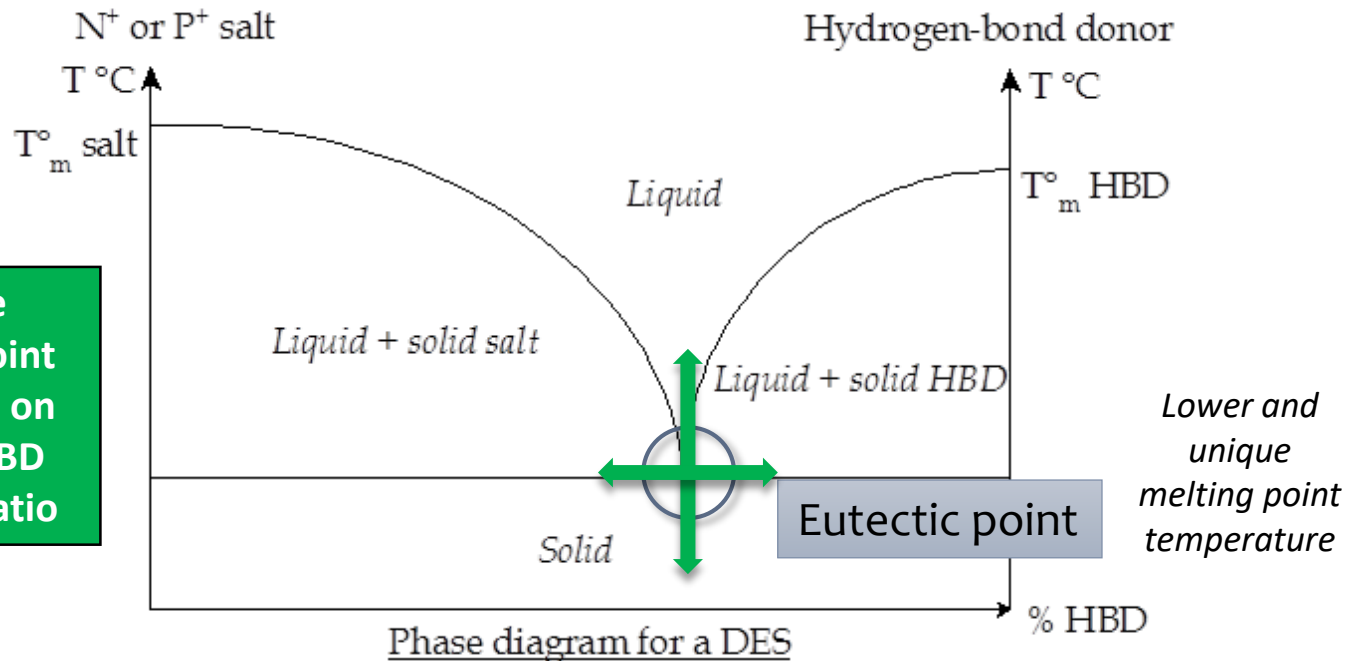
Solvents obtained by mixing ammonium/phosphonium salts with hydrogen-bond donors (HBD) in proportions such that, at the “eutectic point”, the mixture behaves like a pure compound with a melting point lower than that of each pure component.

Ammonium (N⁺) or phosphonium (P⁺) salt

Choline chloride, Betaine
Methyltriphenylphosphonium bromide

Hydrogen-Bond Donor (HBD)

Alcohols, Acids, Amines,
Amides, Thiols...



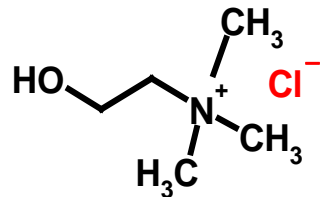
Variable eutectic point depending on Salt and HBD type and ratio

SOLVENTS: Multi-molecular based solvents: Deep Eutectic Solvents (DES)

Choline Chloride (ChCl)



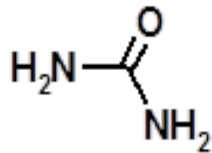
$T_f = 302^\circ\text{C}$



Urea (U)



$T_f = 135^\circ\text{C}$



1 mole

2 moles

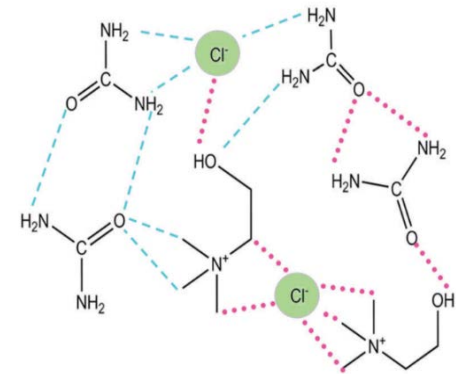
60°C

1h

ChCl:U



$T_f = 12^\circ\text{C}$



SOLVENTS: Multi-molecular based solvents: Natural Deep Eutectic Solvents (NaDES)

Natural Deep Eutectic Solvents (NADES): DES made from molecules widely present in living organisms

Storage and biosynthesis of water-insoluble molecules

e.g.: Alkaloids, Flavonoids, Macromolecules



Protect plants from harsh environmental conditions



NaDES involved in flowers and plants for nectar, sap, exudate production

Prevent pathogenic microorganisms, attract or catch insects.



NaDES: THIRD LIQUID PHASE IN LIVING ORGANISMS?

Natural Deep Eutectic Solvents (NaDES)

About 150 described NaDES so far.

NaDES composition	Mole Ratio
malic acid:glucose	1:1
malic acid:fructose	1:1
maleic acid:sucrose	1:1
citric acid:sucrose	1:1
citric acid:glucose	2:1
glucose:fructose	1:1
glucose:sucrose	1:1
alanine:malic acid:water	1:1:3
proline:malic acid:water	1:1:3

NaDES composition	Mole Ratio
ChCl:fructose:water	5:2:5
ChCl:xylose:water	2:1:2
ChCl:sucrose:water	4:1:4
Betaine:sucrose:proline	1:1:1
glycerol:ChCl:water	2:1:1
sorbitol:ChCl:water	2:5:6
xylitol:ChCl:water	1:2:3
lactic acid:glucose:water	5:1:3
fructose:glucose:sucrose:water	1:1:1:11

Organic acids

Amino acids

Sugars

Polyols

Ammonium

Water

Properties and Applications of DES and NaDES

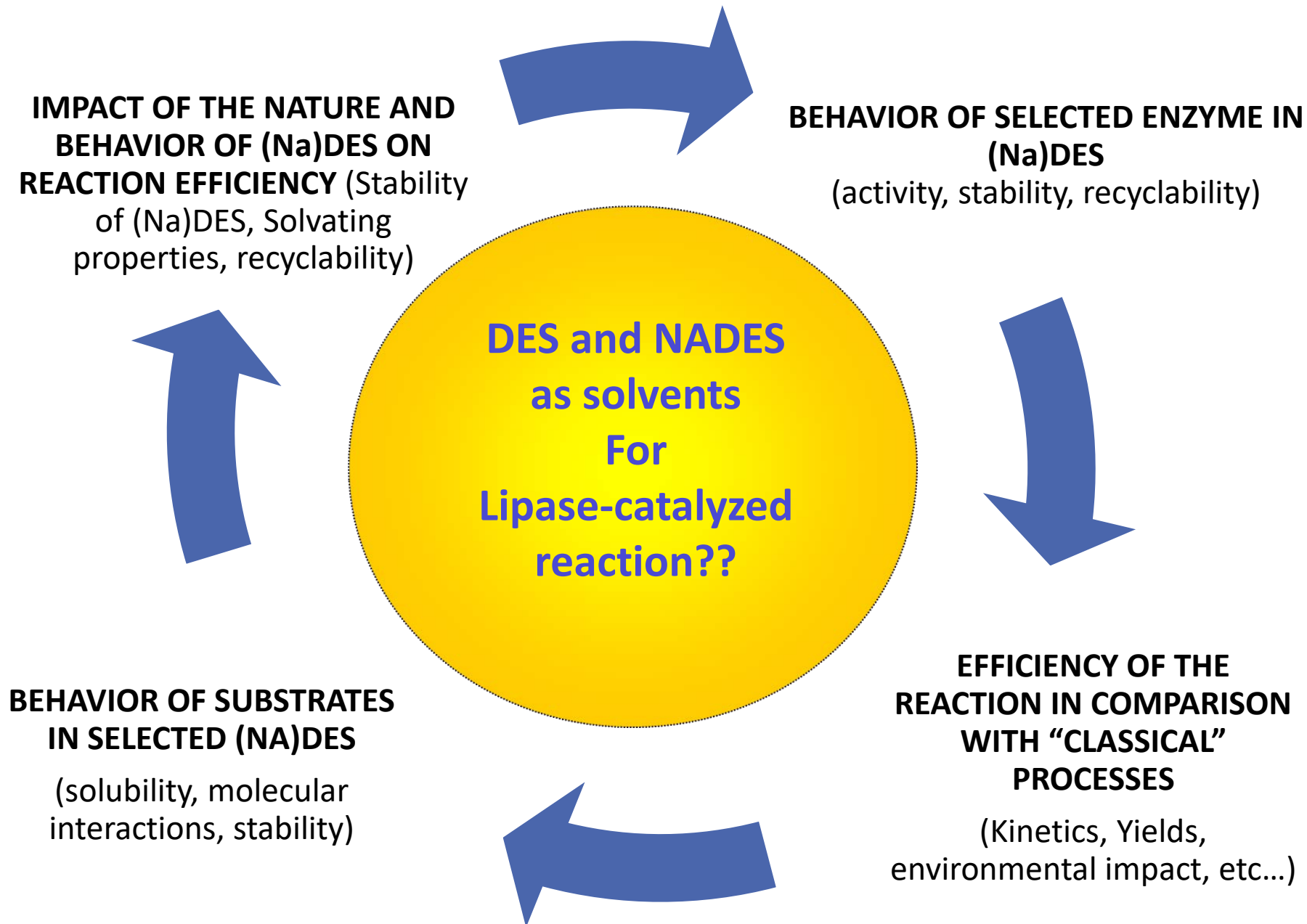
Properties

- ✓ Thermally stable, non-flammable, very low vapour pressure
- ✓ Easy and fast to prepare, relatively inexpensive
- ✓ Wide range of polarity, viscosity, pH, melting point...
- ✓ Most are expected to be biodegradable, safe and even renewable
- ✓ Strong solvation capacity of molecules poorly or non water soluble

Some current or potential applications

- ✓ metal processing, (bio)Polymer and nanomaterials sciences
- ✓ Carrier for bioactive molecules
- ✓ Functionnal additives, monomers for MIP synthesis
- ✓ Solvent for solubilisation or extraction of natural compounds
- ✓ Solvents for chemical/enzymatic synthesis or catalysis

DES and NaDES as media for Biocatalysis?



DES and NaDES as media for Biocatalysis?

First publication on Lipases in DES: Kazlauskas' Group, 2008

COMMUNICATION

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Hydrolase-catalyzed biotransformations in deep eutectic solvents†

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Table 1 Percentage conversion of ethyl valerate to butyl valerate at 60 °C

	<i>i</i> CALB	CALB	CALA	PCL	No enzyme
ChCl:Acet	23 ^a	96	0.5	0.0	0.0
ChCl:EG	11 (99) ^b	32 (93) ^b	3.0	0.2	0.0
ChCl:Gly	96	96	70	22	0.0
ChCl:MA	30	58	0.7	0.0	0.7
ChCl:U	93	99	1.6	0.8	0.0
EAC:Acet	63	92	2.7	0.0	0.0
EAC:EG	23 (54) ^b	33 (79) ^b	20	0.0	0.0
EAC:Gly	93	91	2.1	0.5	0.0
Toluene	92	92	76	5.0	0.0

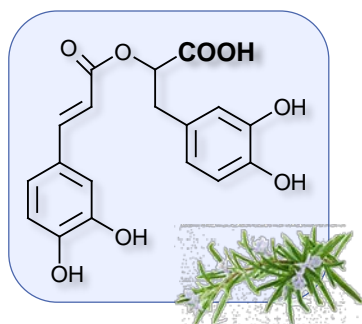
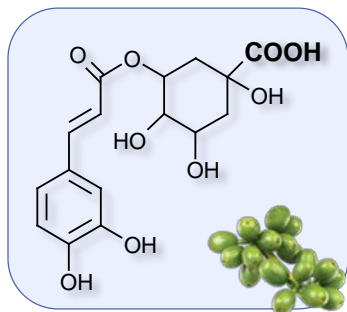
^a 40 mM ethyl valerate, 400 mM 1-butanol, 10 mg ml⁻¹ enzyme, 24 h. ^b Number in parentheses is the percentage conversion including the side reaction with the ethylene glycol component of the deep eutectic solvent. No side reaction was detected in the other reactions.

Table 2 Initial specific activity of *i*CALB after 15 min of reaction

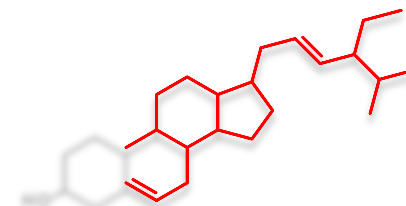
	Transesterification activity ^a	Aminolysis activity ^a
Toluene	37 (100%)	46 (100%)
ChCl:Gly	33 (89%)	52 (113%)
ChCl:U	20 (54%)	22 (48%)
EAC:Gly	50 (135%)	Not determined ^b
BMIM[Tf ₂ N]	24 (65%)	11 (24%)
BMIM[BF ₄]	7 (19%)	9 (19%)

^a μmol ethyl valerate h⁻¹ mg solid⁻¹, activity relative to toluene in parentheses. *i*CALB contains ~10 wt% protein. ^b Aminolysis with ethylamine from the DES was the dominant reaction.

(Na)DES AS NEW MEDIUMS FOR LIPASE-CATALYZED REACTIONS: SYNTHESIS OF PHENOLIPIDS



ANTIOXIDANTS
(Phenolic acids,
polyphenols)



LIPIDS (hydrophobic chain)

(Na)DES AS NEW MEDIA FOR LIPASE-CATALYZED REACTIONS: SYNTHESIS OF PHENOLIPIDS

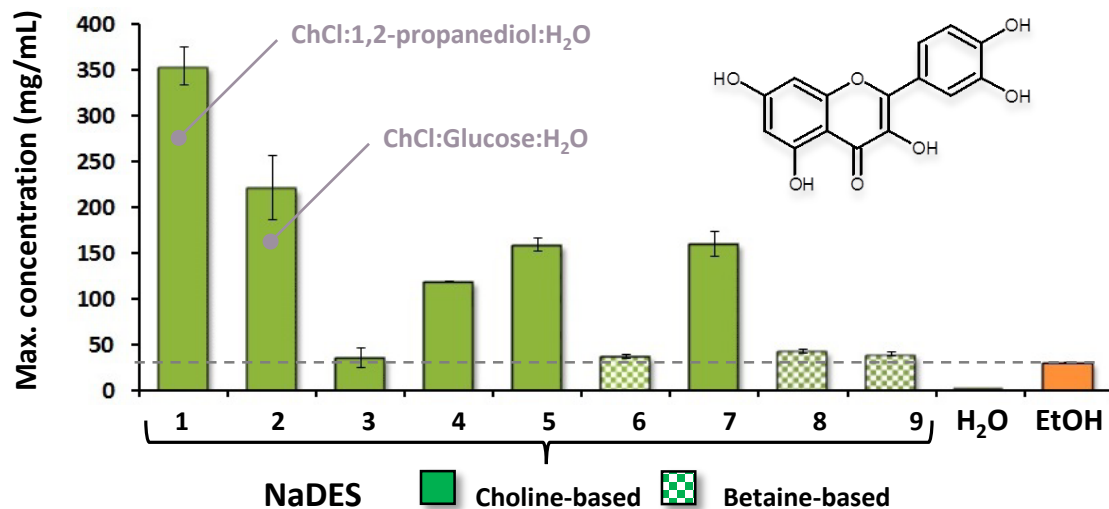
(Na)DES may be advantageous due to their high solubilizing capacity of phenolic compounds

Quercetin

**Best Solubility in NaDES made of
ChCl**

* 12 fold more soluble in this NADES than in ethanol

* 1500 fold more soluble in NADES than in water

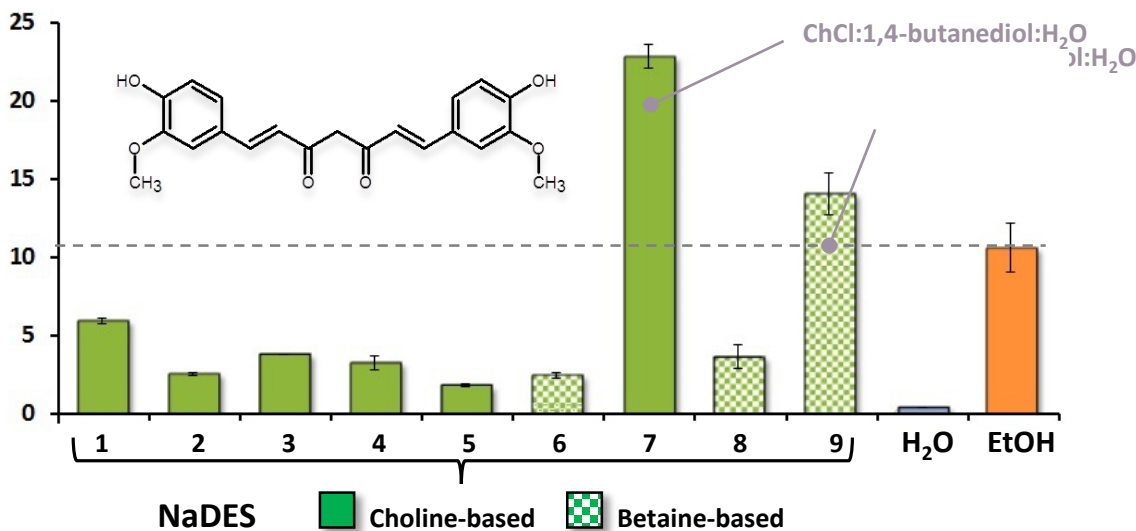


Curcumin

**Best Solubility in NaDES made of
butanediol**

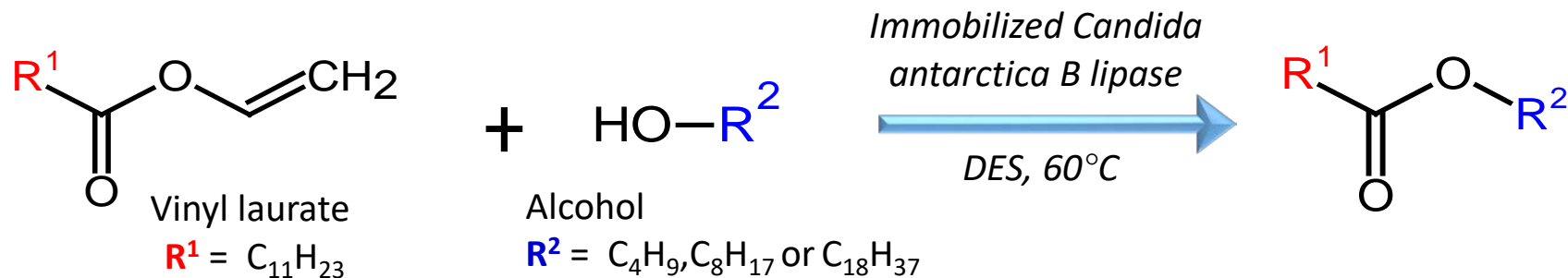
* 12 fold more soluble in this NADES than in ethanol

* 50 fold more soluble in NADES than in water



(Na)DES AS NEW MEDIA FOR LIPASE-CATALYZED REACTIONS: SYNTHESIS OF PHENOLIPIDS

Model reaction to assess lipase activity in selected (Na)DES



Composition of screened DES

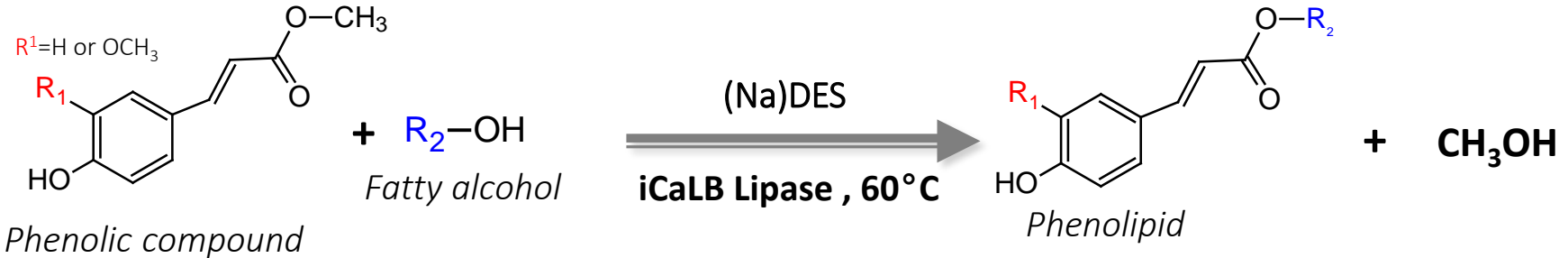
DES	Ammonium salts	Hydrogen-bond donors	Molar ratios
ChCl:U	Choline chloride	Urea	1:2
ChCl:gly	Choline chloride	Glycerol	1:2
ChCl:Ox	Choline chloride	Oxalic acid	1:1
ChCl:Mo	Choline chloride	Malonic acid	1:1
ChCl:EG	Choline chloride	Ethylene glycol	1:2
EAC:U	Ethylammonium chloride	Urea	1:2
EAC:gly	Ethylammonium chloride	Glycerol	1:2

(Na)DES AS NEW MEDIA FOR LIPASE-CATALYZED REACTIONS: SYNTHESIS OF PHENOLIPIDS

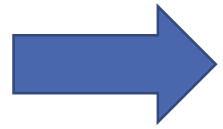
16 h, 60° C	1-Butanol	1-Octanol	1-Octadecanol
SOLVENT	Conversion (%)	Conversion (%)	Conversion (%)
Toluene	100	100	100
EAC : U	94	10	9
EAC : Gly	3	4	4
ChCl : EG *	33	8	10
ChCl : Ox *	10	43	41
ChCl : Mo *	5	36	34
ChCl : U	100	100	100
ChCl : Gly	100	100	100

* Side reaction observed: reactivity from the hydrogen-bond donor

(Na)DES AS NEW MEDIA FOR LIPASE-CATALYZED REACTIONS: SYNTHESIS OF PHENOLIPIDS



(Na)DES = Choline Chloride/Urea (1:2) or Choline Chloride/Glycerol (1:2)

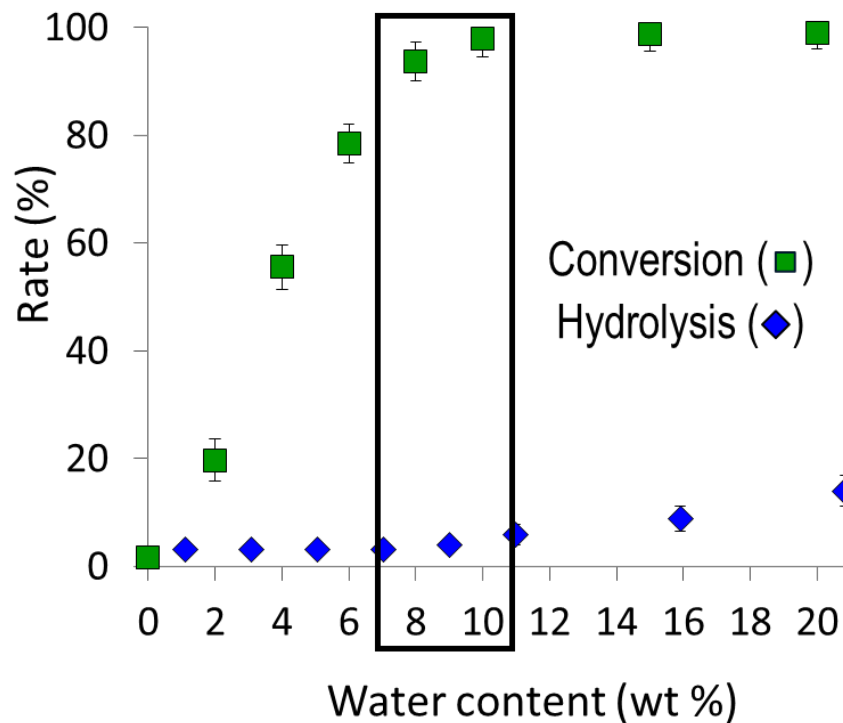


Very low conversion rate (<10%) in pure (Na)DES)

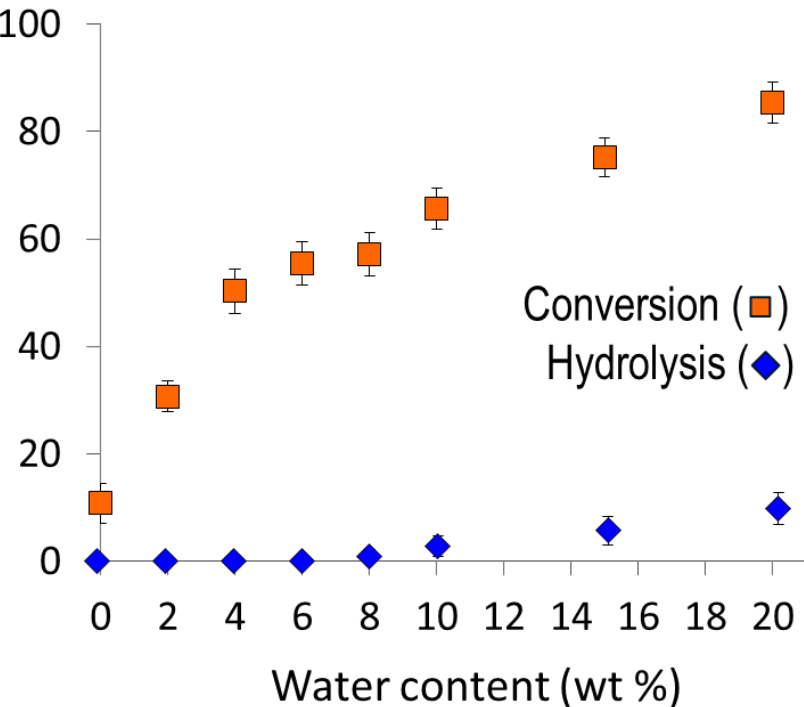
- Addition of **water is essential** and offers several advantages:
 - reduced viscosity and improved mass transfer
 - significant increase in lipase's initial activity
 - significant increase in the final conversion rate.


(Na)DES AS NEW MEDIA FOR LIPASE-CATALYZED REACTIONS: SYNTHESIS OF PHENOLIPIDS

In ChCl:U with water



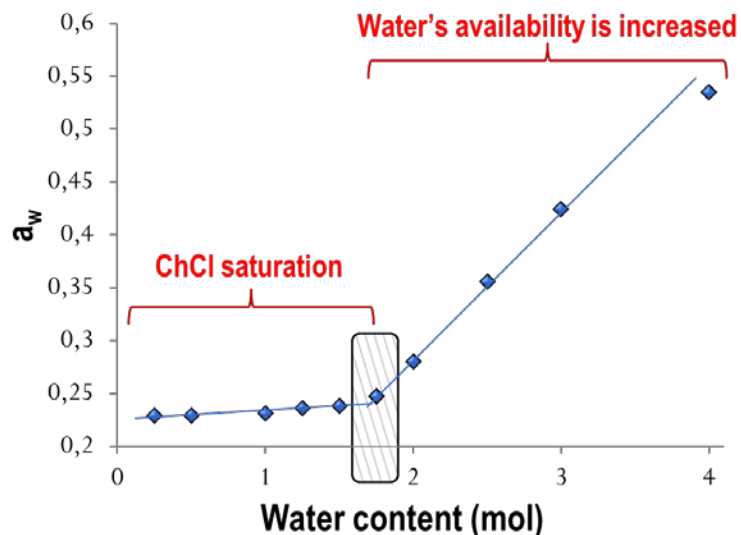
In ChCl:Gly with water



- 
- Max yield in phenolipids (~92%)
 - Good conversion rate and limited hydrolysis even at high water contents

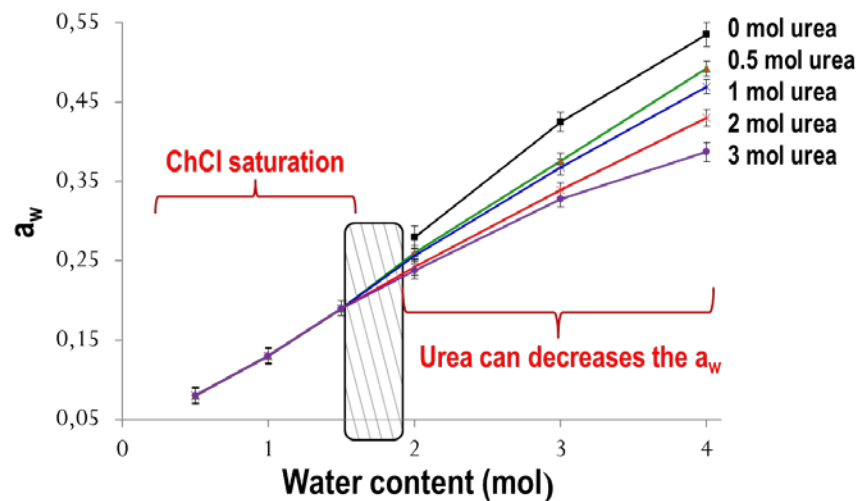
Effect of micro aqueous environment on lipase efficiency in (Na)DES

Effect of ChCl (1mol) on a_w



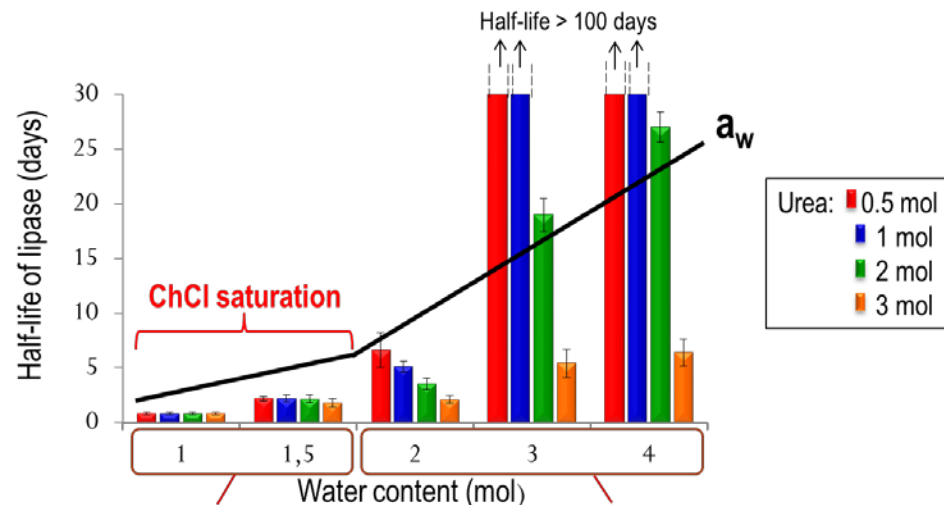
a_w is governed by the strong interactions of water with ChCl to a threshold corresponding to ChCl saturation

Effect of in the ternary mixture ChCl(1mol):water:urea on a_w



After ChCl saturation, the HBD (urea) allows to limit the phenomenon of a_w raising

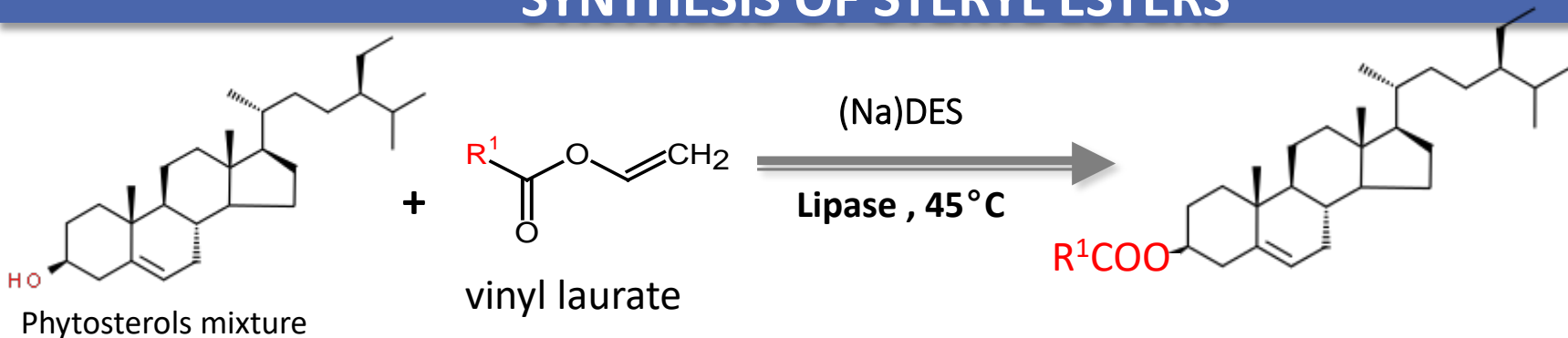
Residual activity of iCALB in the ternary mixture ChCl(1mol):water:urea



mainly due to the ChCl (strong association with water, very low a_w , low lipase's hydration)

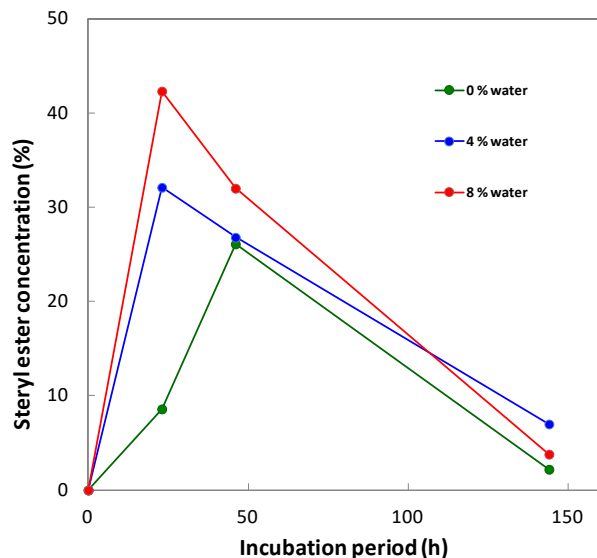
mainly due to urea (denaturing effect + decreasing in a_w)

(Na)DES AS NEW MEDIUMS FOR LIPASE-CATALYZED REACTIONS: SYNTHESIS OF STERYL ESTERS

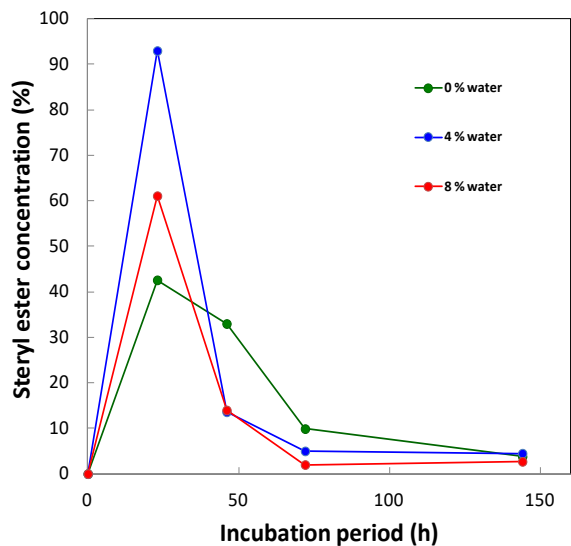


- (Na)DES = ChCl/butenediol (1:3), ChCl/glycerol (1:2), ChCl/isorbide 1:2)
- Lipases: *Candida antarctica* or *Candida rugosa*, 5%
- Water addition: 0, 4 and 8 %

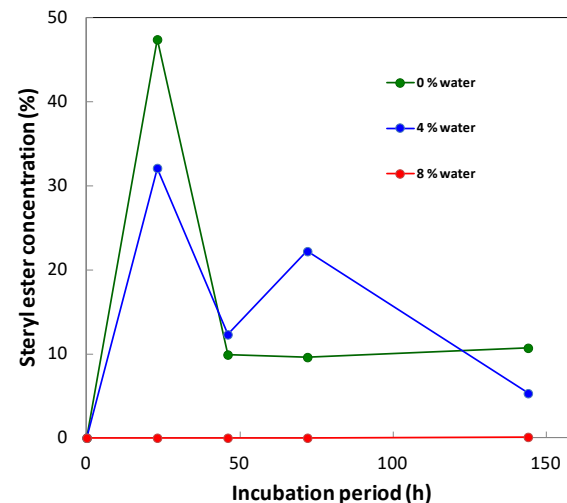
DES 1 (ChCl/Butanediol)



DES 2 (ChCl/Glycerol)



DES 3 (ChCl/Isosorbide)

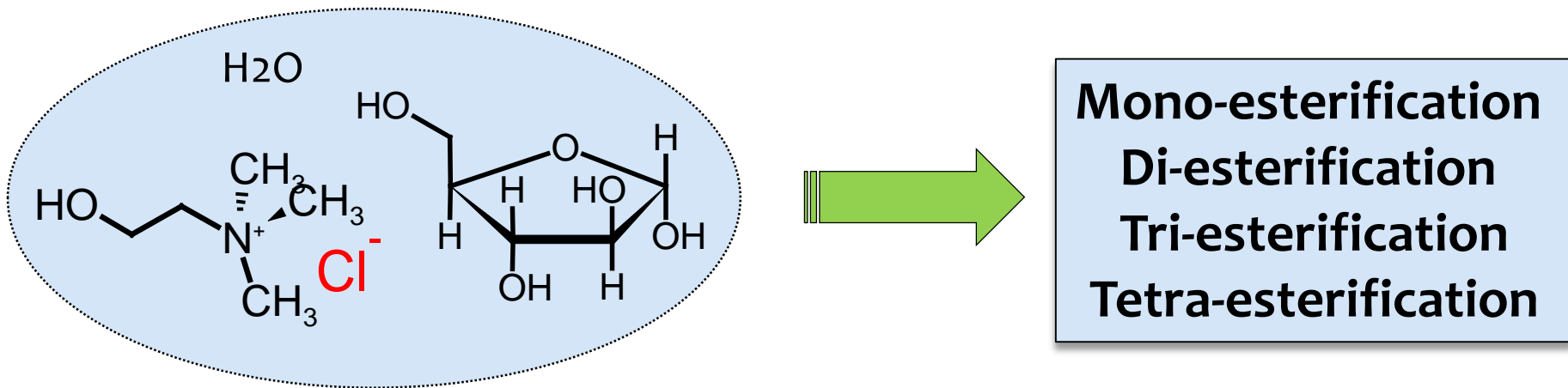


A maximum phytosteryl ester concentration was achieved after 24 h. A conversion close to completion was achieved in DES 2 (ChCl/Glycerol) containing 4% water. The decrease in the steryl esters concentration after 24 h reaction suggests the occurrence of competitive reactions: Esterification between free polyols and vinyl laurate ? Hydrolysis of steryl esters?

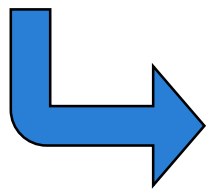


(Na)DES AS NEW MEDIUMS FOR LIPASE-CATALYZED REACTIONS: SYNTHESIS OF SUGAR ESTERS

Lipase-catalyzed reactions of sugar in sugar-based deep eutectic mixture



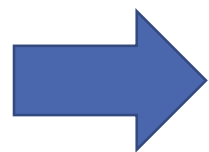
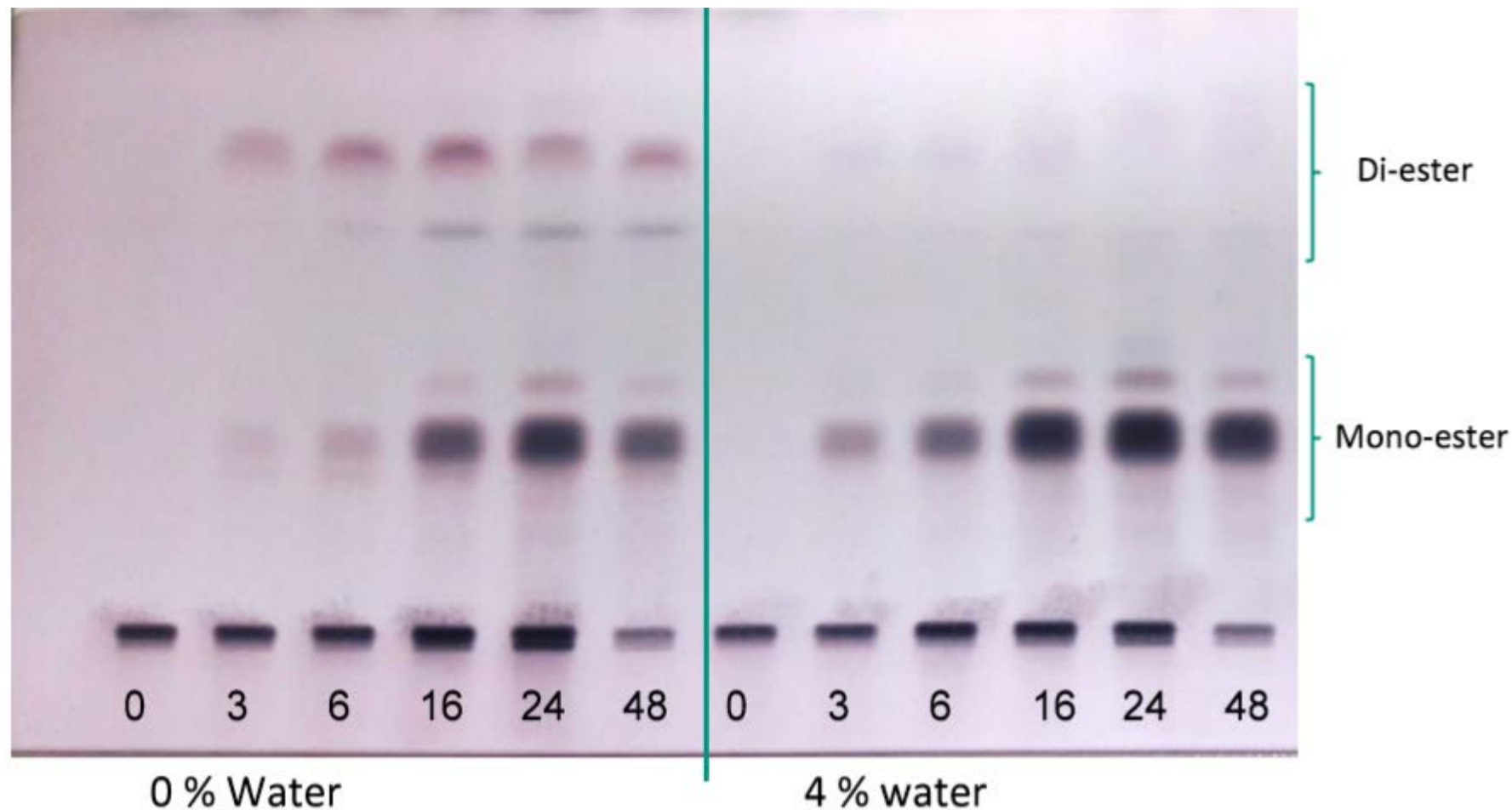
Sugar-based deep eutectic mixture



Effect of water content, a_w , viscosity, mole ratio, T° , etc.
Regioselective esterification

(Na)DES AS NEW MEDIUMS FOR LIPASE-CATALYZED REACTIONS: SYNTHESIS OF SUGAR ESTERS

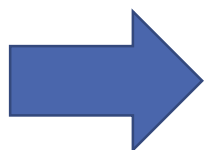
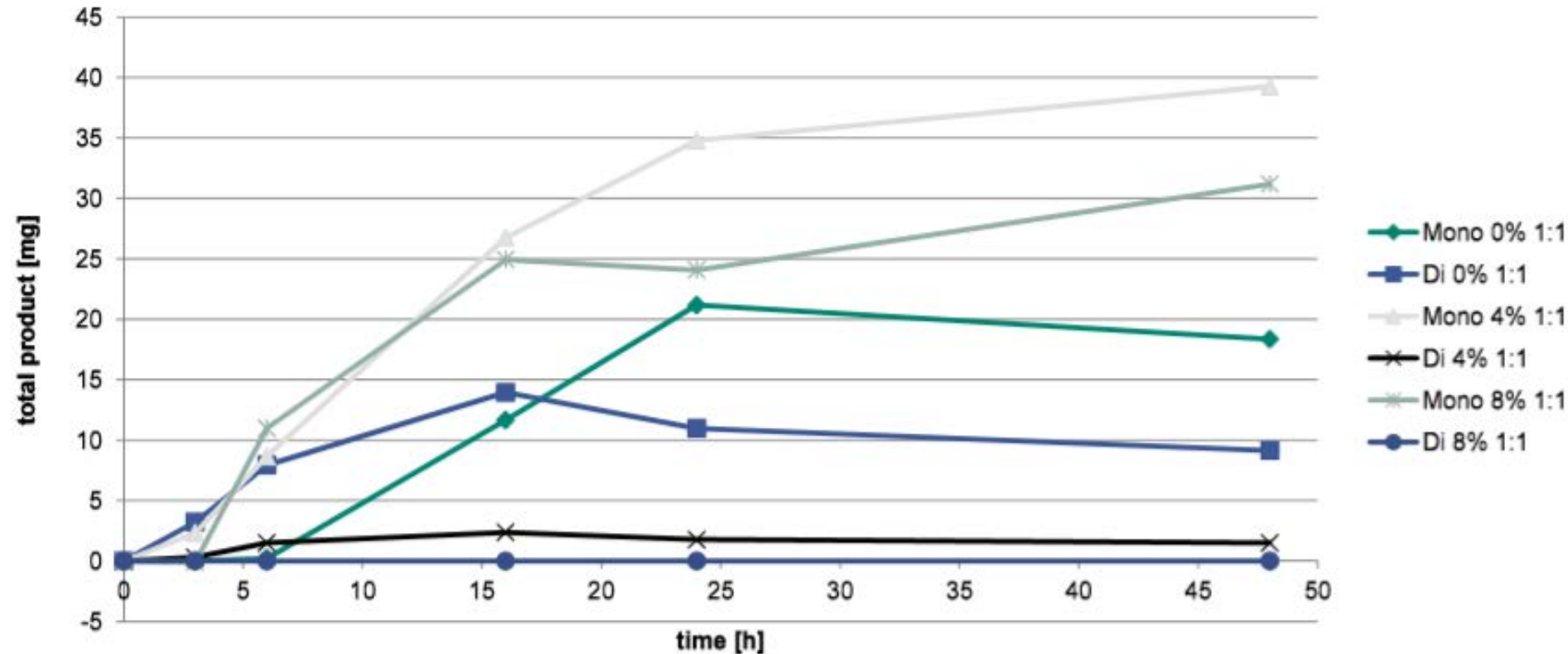
(Na)DES = Choline Chloride/Arabinose (1:1)



Depending of amounts of added water, product composition is changing from a mixture of di and mono esters to mono ester only.

(Na)DES AS NEW MEDIUMS FOR LIPASE-CATALYZED REACTIONS: SYNTHESIS OF SUGAR ESTERS

(Na)DES = Choline Chloride/Arabinose (1:1)

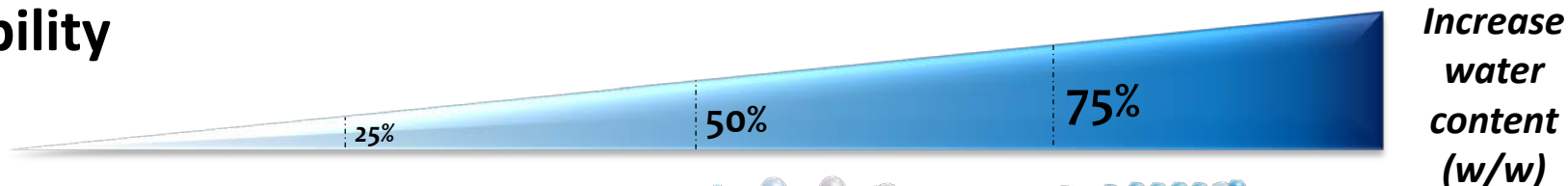


- Addition of water accelerates the reaction
- Addition of water reduces the formation of di-laurates

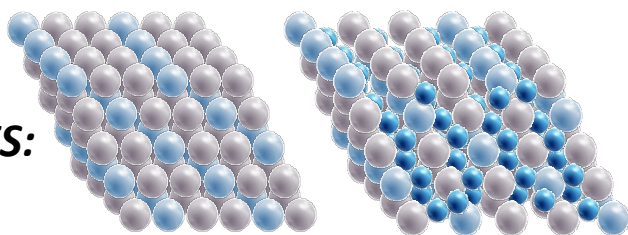
CONCLUSION FOR (Na)DES AS NEW MEDIUMS FOR LIPASE-CATALYZED REACTIONS



Water effect: Interactions of (Na)DES with water are of crucial importance to guarantee reaction efficiency and enzyme stability



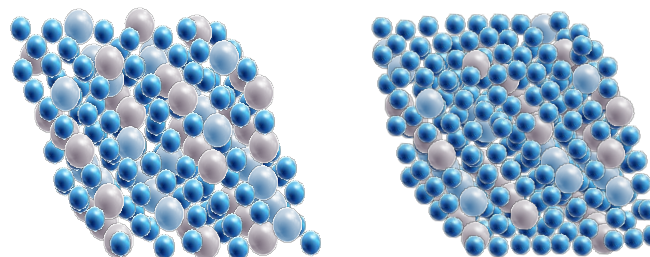
NaDES:



“superstructure” = NaDES drives physical properties

< 25% water:

Self-diffusion data for water strongly suggest that the liquids are not homogeneous and contain distinct microscopic water-rich phases. Separate micro-phases could be responsible for some of the surprising observations



Water drives physical properties

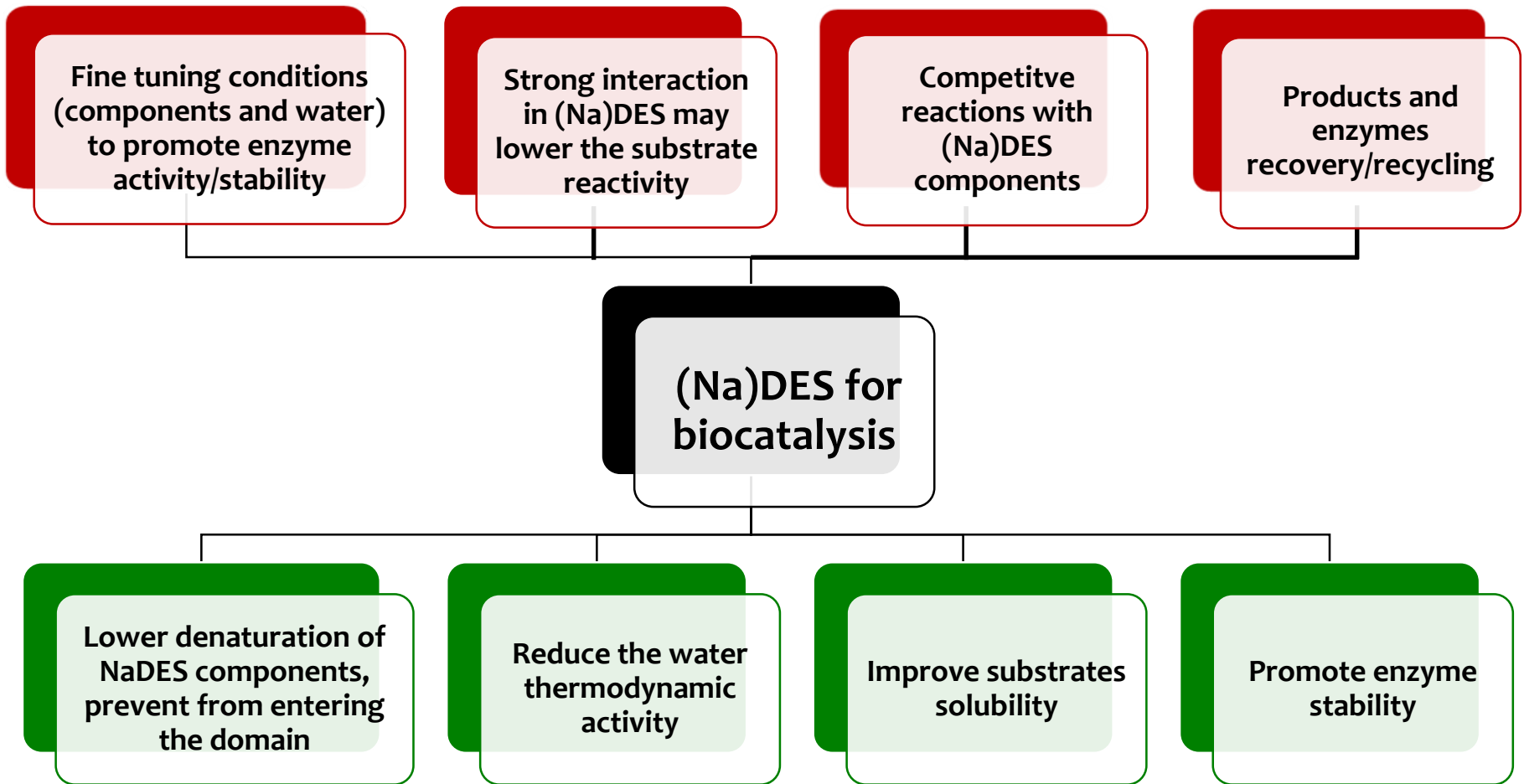
~50% water:

NaDES' effect (partner interactions) disappear in favor of water properties (evidence by NMR 2, FTIR, molecular dynamics simulations or other physicochemical assessment)

Strong NaDES dilution:

activity of constituents dissolved in water

CONCLUSION FOR (Na)DES AS NEW MEDIUMS FOR LIPASE-CATALYZED REACTIONS





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THANK YOU FOR YOUR ATTENTION !

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