Intensification of Biocatalytic Processes Towards Industrial Volumetric Productivities

21ST ISCHIA ADVANCED SCHOOL OF ORGANIC CHEMISTRY (IASOC)
IASOC2026 (September 17 – 21, 2026)

Leibniz Universität Hannover

INSTITUTE OF TECHNICAL CHEMISTRY TCI

AARHUS UNIVERSITY

BIOCATALYSIS & PROCESSING

Understanding the needs of efficient biocatalysis

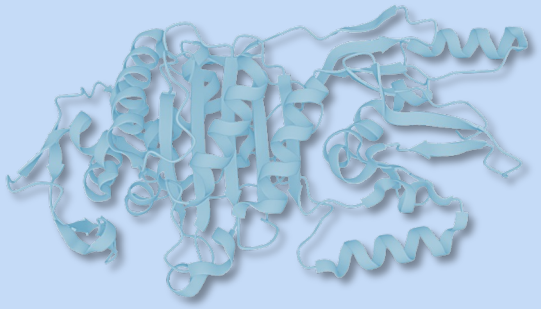
REACTION

- Enzyme kinetics
- Thermodynamics
- Reaction media
- Side reactions
- Cascades



BIOCATALYST

- Activity and stability
- Selectivity
- Form/Preparation



PROCESS

- Reactor type
- Catalyst recovery
- Downstream processing



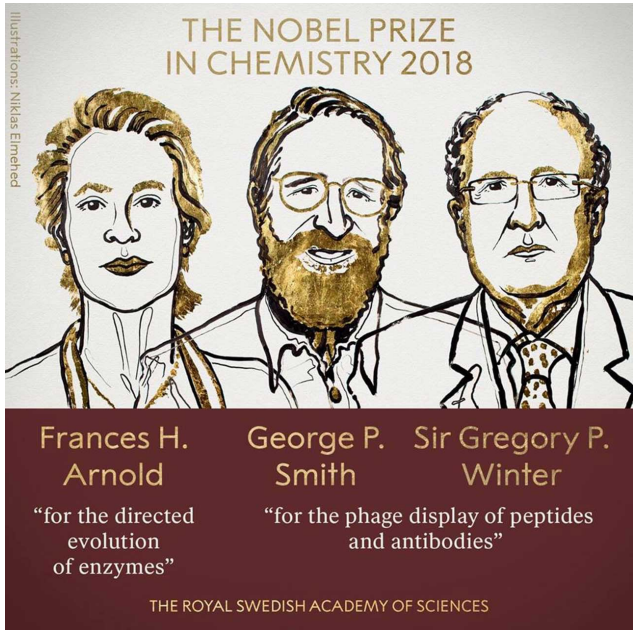
Online analytics

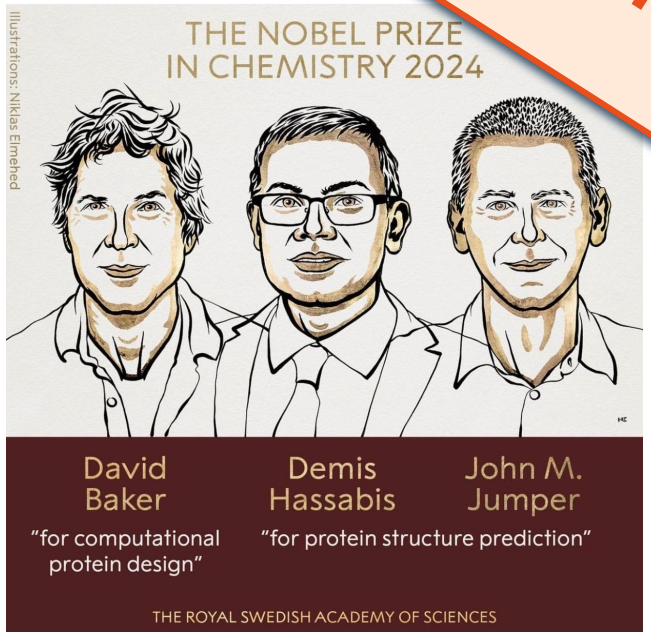
Process intensification challenges

BIOCATALYST

- limited specific activity under the require n conditions
- unfavourable kinetics at required con
- limited enzyme stability

to be addressable by Protein Engineering!





Process intensification challenges

BIOCATALYST

- limited specific activity under the require n conditions
- unfavourable kinetics at required con
- limited enzyme stability

NOT to be addressable by Protein Engineering!

REACTION

- unfavourable reaction thermodynamics
- problematic reactants
  - poor water solubility
  - high viscosity

The need of an efficient biocatalysis

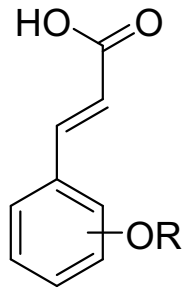
Parameters	Target values
Substrate conversion	≥95% (in 24 hours, ideally in one shift)
Volumetric productivity	>100 g/(L·d) >300 g/(L·d) (bulk chemicals)
Substrate-to-enzyme ratio	>50 g/g
Product-to-enzyme ratio	**Bulk chemicals:** • 700 – 2000 kg $_{product}$/kg $_{dry\ cell\ weight}$ • 5000 – 20,000 kg $_{product}$/kg $_{free\ enzyme}$ • 2000 – 10,000 kg $_{product}$/kg $_{immobilized\ enzyme}$ **Pharmaceuticals:** • 10 – 35 kg $_{product}$/kg $_{dry\ cell\ weight}$ • 100 – 250 kg $_{product}$/kg $_{free\ enzyme}$ • 50 – 100 kg $_{product}$/kg $_{immobilized\ enzyme}$
E-factor (Roger A. Sheldon, 1942–2025)	**Bulk chemicals:** • 1 – 5 kg $_{waste}$/kg $_{product}$ **Fine chemicals and speciality chemicals:** • Up to 500 kg $_{waste}$/kg $_{product}$ **Pharmaceuticals:** • Up to 4000 kg $_{waste}$/kg $_{product}$

G.W. Huisman, J. Liang, A. Krebber, *Curr. Opin. Chem. Biol.* 14 (**2010**) 122 – 129.
F. Hollmann, I.W.C.E. Arends, D. Holtmann, *Green Chem.* 13 (**2011**) 2285 – 2313.
P. Tufvesson, J. Lima-Ramos, M. Nordblad, J.M. Woodley, *Org. Process Res. Dev.* 15 (**2011**) 266 – 274.

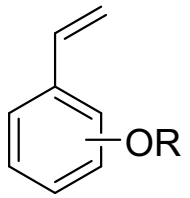
Background



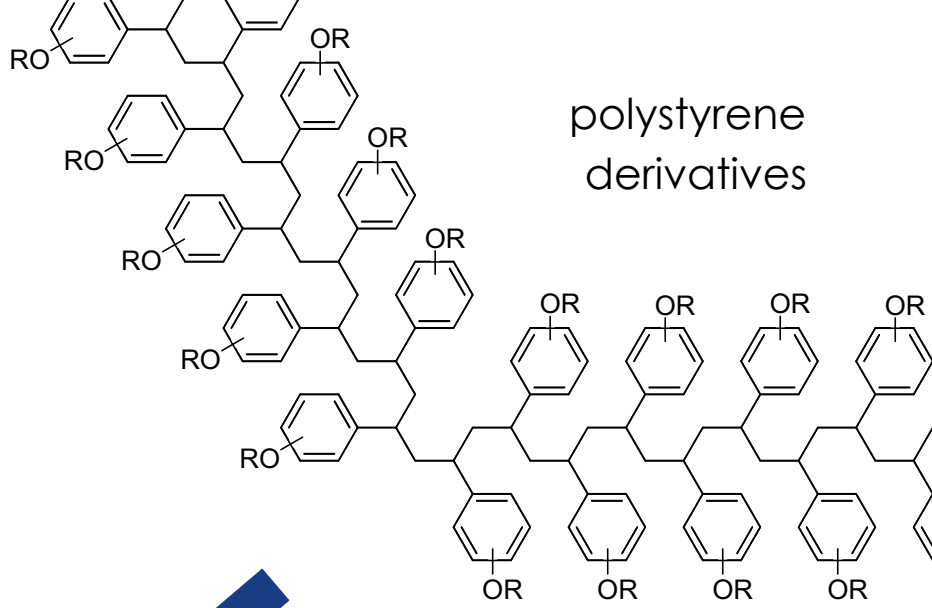
lignocellulosic biomass



phenolic acids



monomer



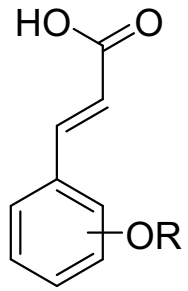
polystyrene derivatives

Background

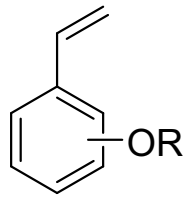


lignocellulosic biomass

[1]

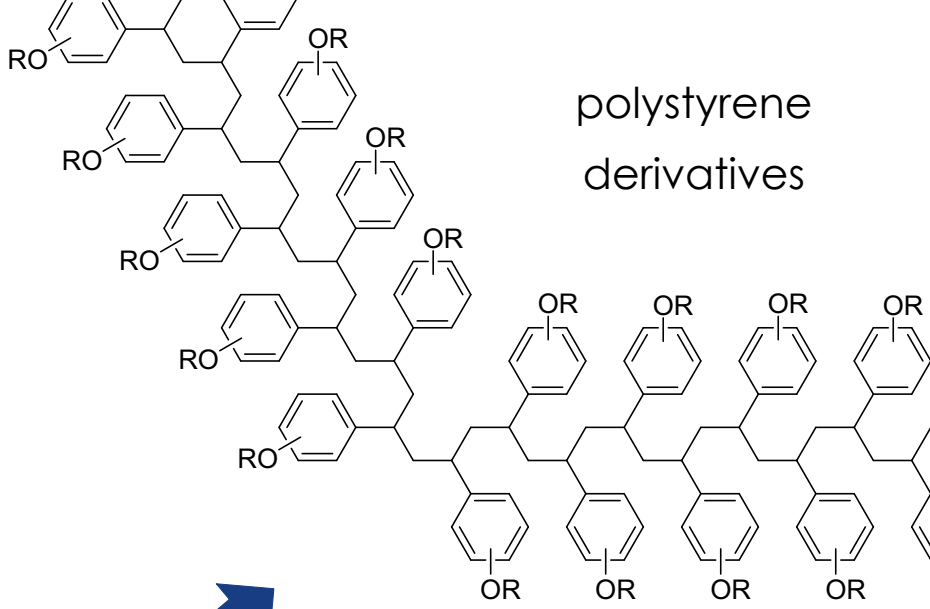


phenolic acids



monomer

[2]



polystyrene derivatives

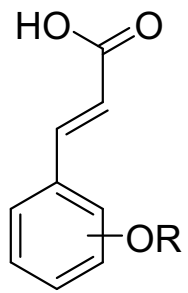
[1] (a) P. B. Dhamole, *et al.*, *Korean J. Chem. Eng.* (**2016**) 33, 1860 – 64.
(b) H. T. Truong, *et al.*, *Appl. Sci.* (**2017**) 7, 796.
(c) V. I. Timokhin, *et al.*, *ACS Sustain. Chem. Eng.* (**2020**) 8, 17427 – 38.

[2] (a) M. Messerschmidt, *et al.*, *Macromolecules* (**2008**) 41, 2821 – 31.
(b) H. Takeshima, *et al.*, *Macromolecules* (**2017**) 50, 4206 – 16.
(c) H. Takeshima, *et al.*, *ACS Sustain. Chem. Eng.* (**2018**) 6, 13681 – 86.
(d) H. Takeshima, *et al.*, *J. Polym. Sci.* (**2019**) 58, 91 – 100.
(e) J. van Schijndel, *et al.*, *Eur. Polym. J.* (**2020**) 125, 109534.

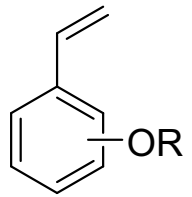
Background

lignocellulosic biomass

[1]



phenolic acids



monomer

[2]

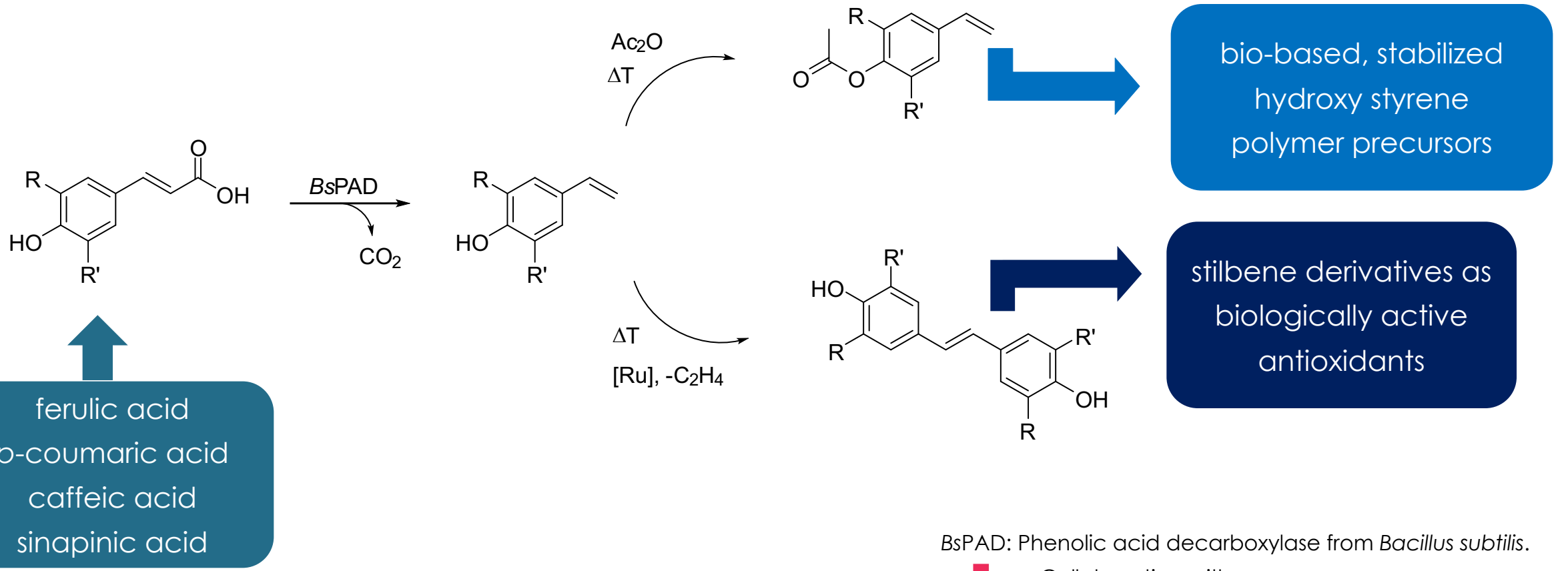
polystyrene derivatives

Chemoenzymatic cascades

The big picture



Philipp Petermeier



ferulic acid
p-coumaric acid
caffeic acid
sinapinic acid

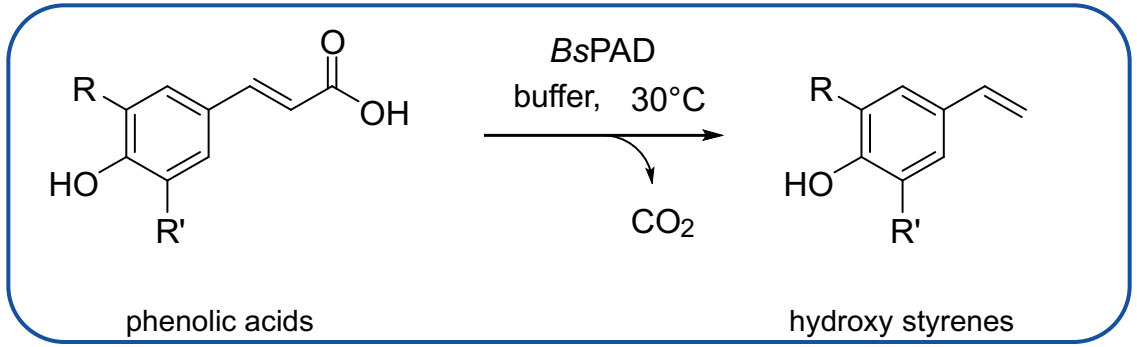
bio-based, stabilized hydroxy styrene polymer precursors

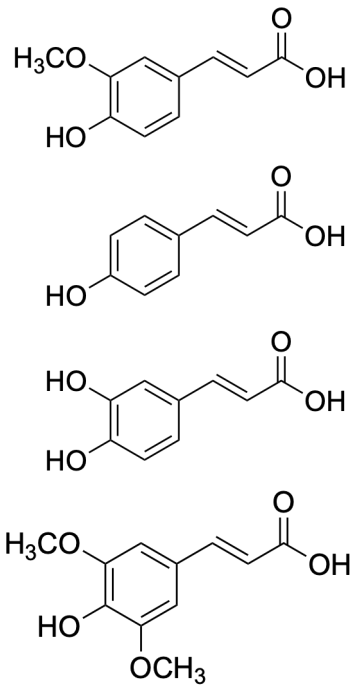
stilbene derivatives as biologically active antioxidants

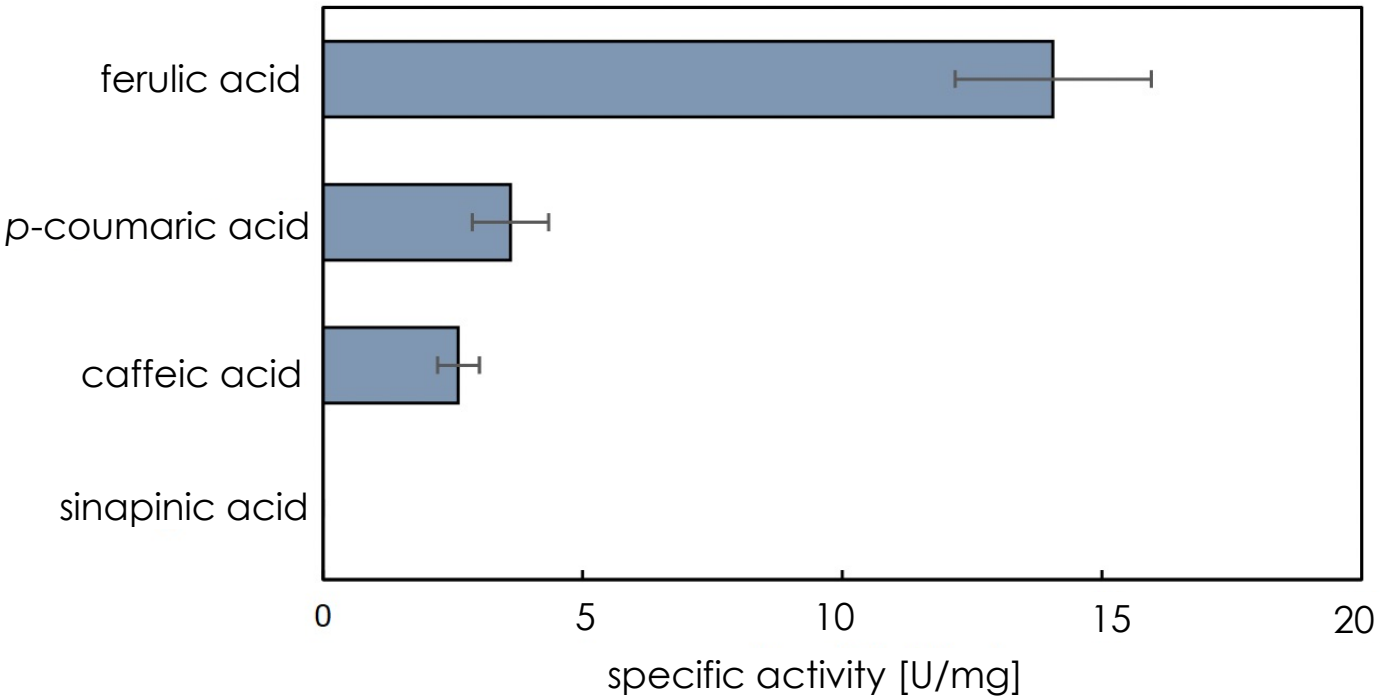
*Bs*PAD: Phenolic acid decarboxylase from *Bacillus subtilis*.

Collaboration with Prof. Dr. Robert Kourist

Biocatalytic decarboxylations









Bs PAD

phenolic acid decarboxylase

origin: *Bacillus subtilis*

PDB: 2P8G

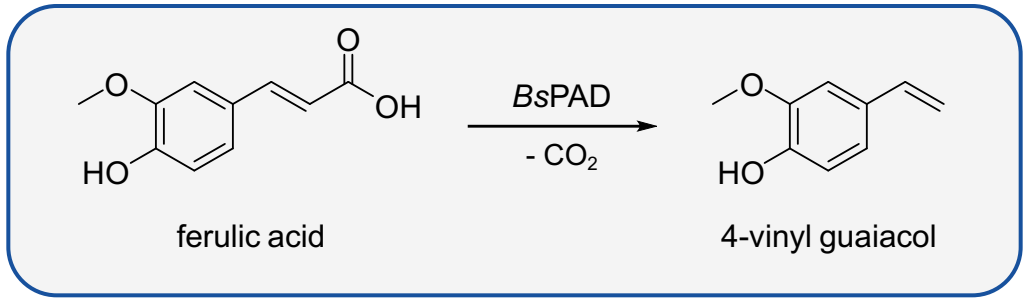
JCSG, 2007. https://www.rcsb.org/structure/2P8G: RCSB PDB.

Reaction conditions: 10 mM substrate, 50 mg/L lyophilized whole-cells, KPi buffer (50 mM, pH 6.0), 5 vol-% DMSO, 30 °C, 1,000 rpm.

Computational assessments



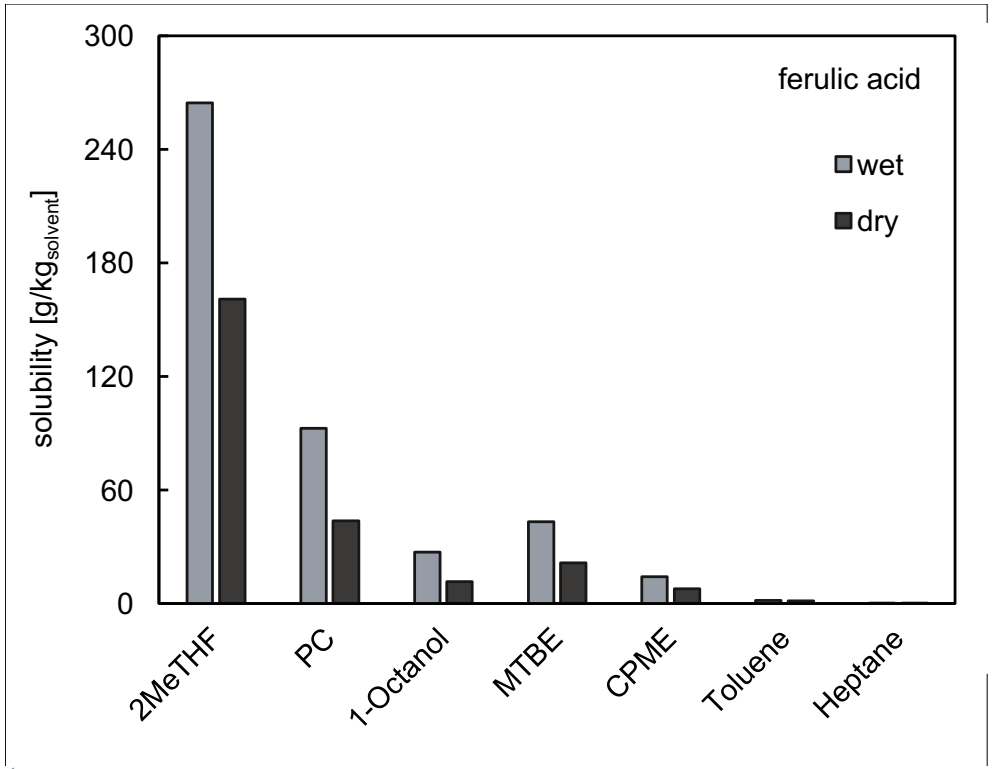
Jan Philipp Bittner
TUHH

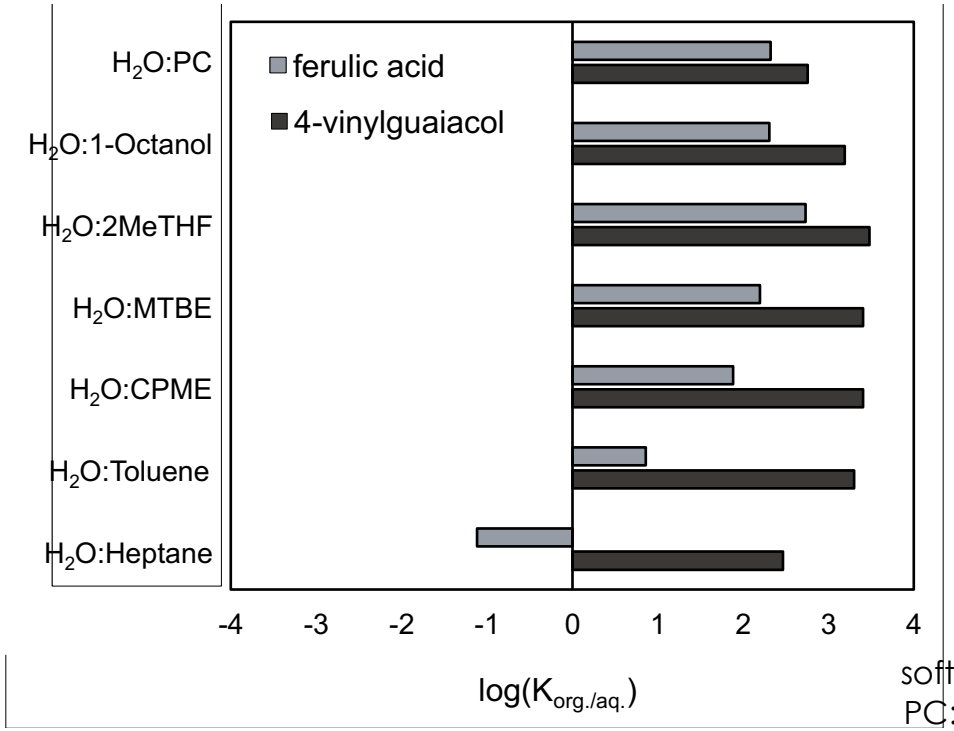


- solubility trends
- influence of co-solubilized water
- extraction efficiencies

conclusions:

- ➢ fed-batch process
- ➢ two-liquid phase system



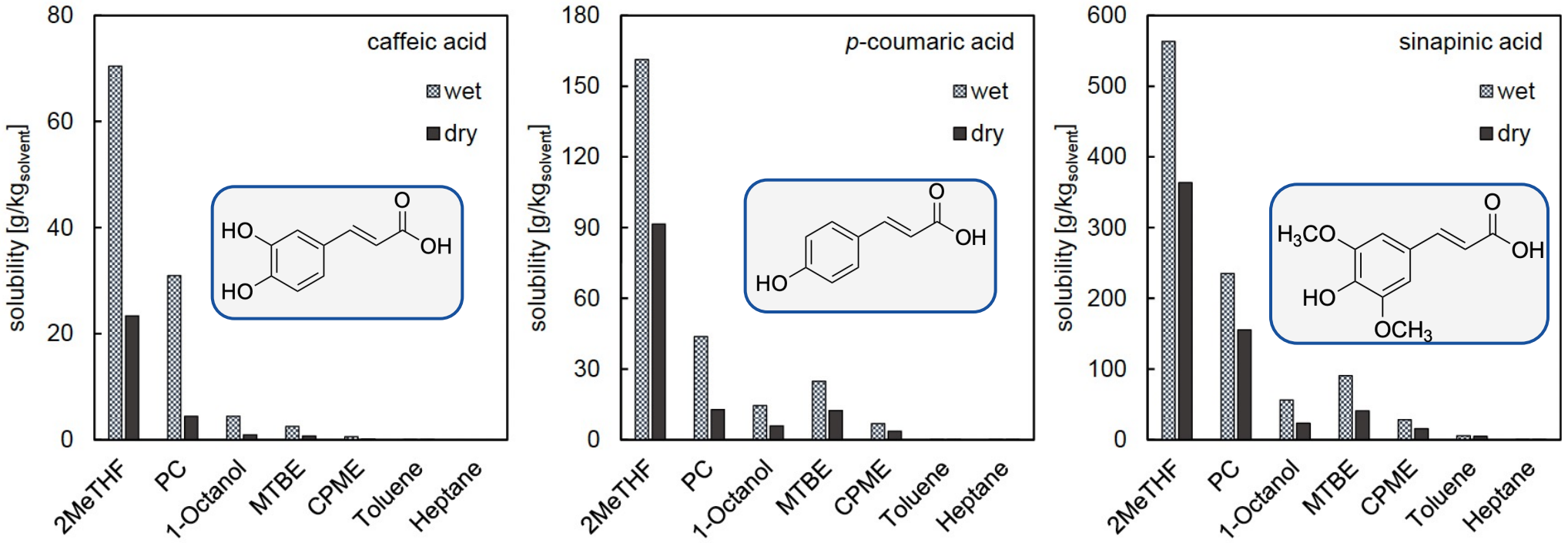


software: COSMOtherm v2020
PC: Propylene carbonate

Computational assessments



Jan Philipp Bittner
TUHH



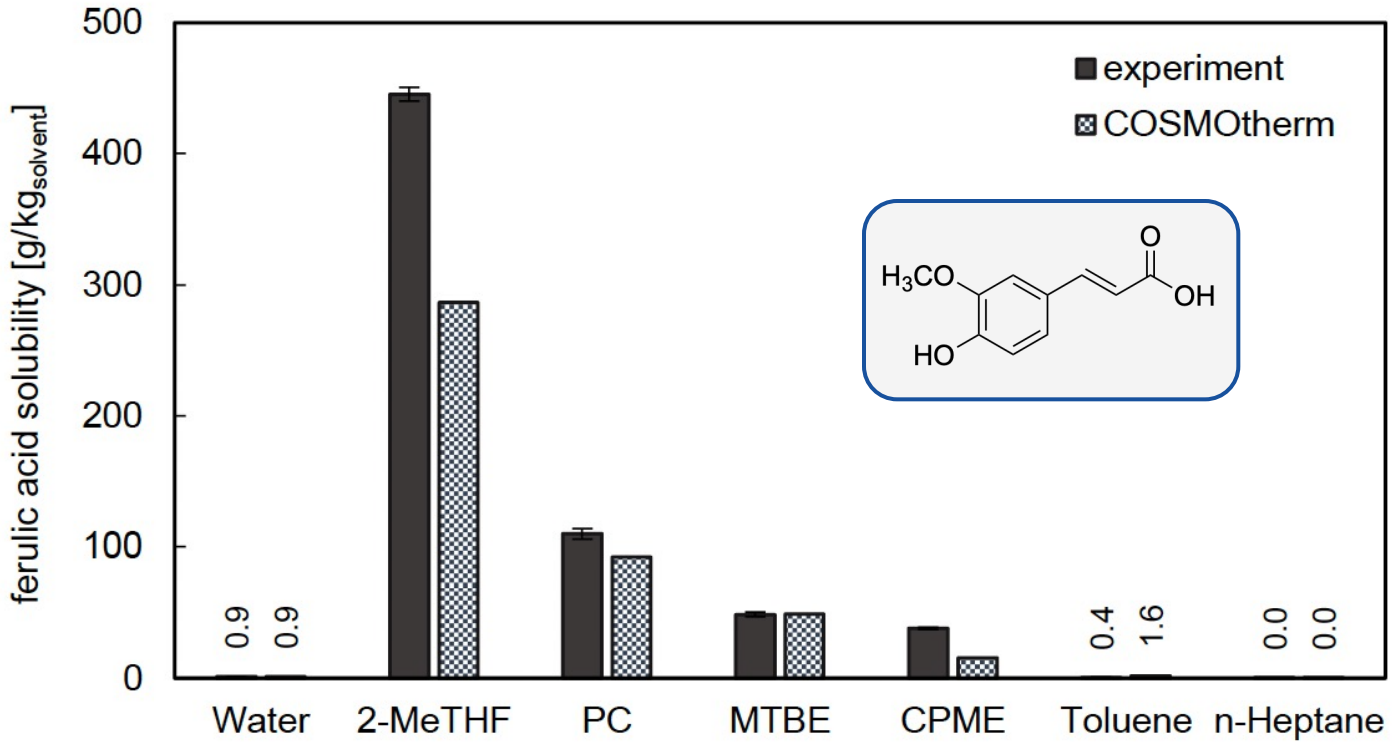
OUTCOME

(i) all substrates have drastically increased solubility in 2-MeTHF, PC, 1-octanol, MTBE, and CPME compared to water (~1 g L^{-1}),
(ii) all substrates are more soluble in polar organic solvents than in apolar ones,
(iii) hydroxy groups of substrates reduce their solubility in screened solvents, and
(iv) methoxy groups of substrates increase their solubility in screened solvents.

software: COSMOtherm v2020
PC: Propylene carbonate

*log*P values: 2-MeTHF, 1.9; PC, -0.4; MTBE, 0.9; CPME, 1.5; Toluene 2.6; *n*-heptane, 4.5.

Computational vs. experimental assessments

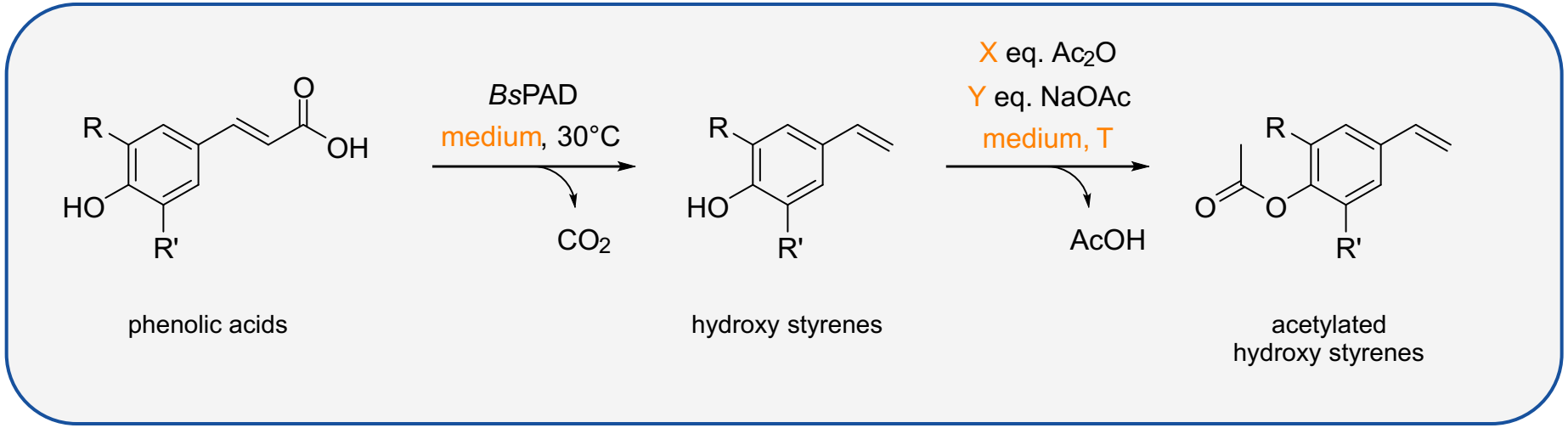


Comparison of calculated and experimentally determined solubilities of the model compound ferulic acid in selected solvents. Organic solvents were equilibrated over water at room temperature (24 h) and the solubility of ferulic acid was determined for a temperature of +30 °C.
*log*P values: 2-MeTHF, 1.9; PC, -0.4; MTBE, 0.9; CPME, 1.5; Toluene 2.6; *n*-heptane, 4.5.

software: COSMOtherm v2020
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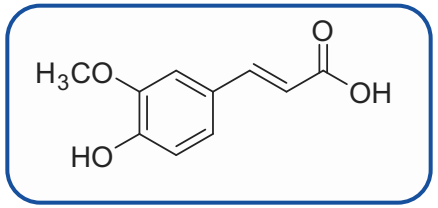
Chemoenzymatic cascade

Polymer precursors



*Bs*PAD: Phenolic acid decarboxylase from *Bacillus subtilis*.

Medium engineering for *BsPAD*



ferulic acid

KPi buffer pH 7.3 (500 mM)	44 ± 5%	
media	as received	wetted
ChCl:EG (1:2)		
ChCl:Ur (1:2)		
ChCl:Gly (1:2)		
Ethylene glycol (EG)		
Cyrene		
Dimethylformamide (DMF)		
Propylene carbonate (PC)		
Tetrahydrofuran (THF)		
2-MeTHF		
MTBE		
CPME		

Reaction conditions: 1.5 mL glass vials, 1.0 mL rxn. volume, 100 mM substrate, 100 mg/L cells, 1,000 rpm, 30 °C, 16 h, exp. triplicates.

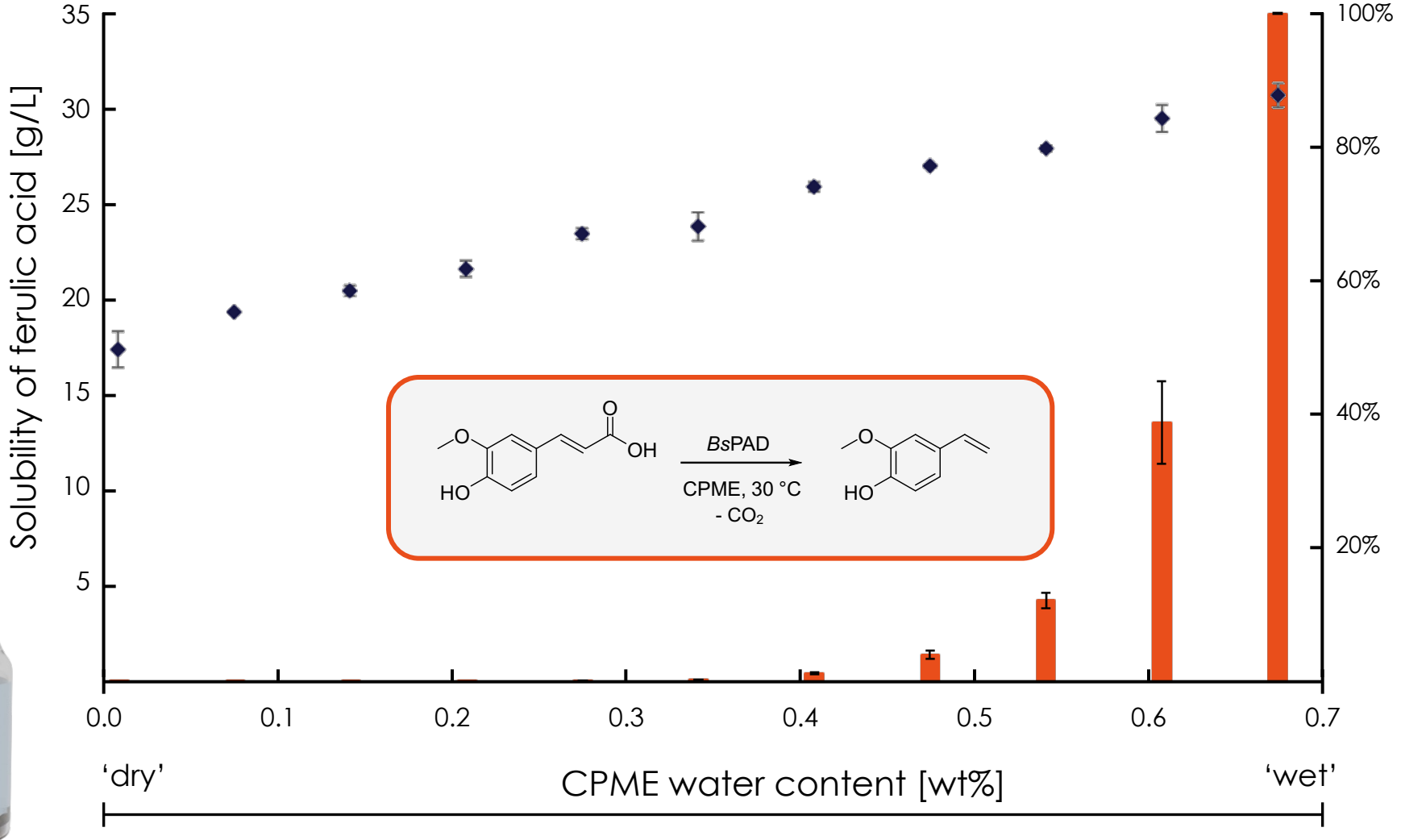
Wetted: Water saturated at room temperature (PC, MTBE, 2-MeTHF, CPME) or 5 vol-% water added (EG, Cyrene, DMF, THF, ChCl:EG, ChCl:Ur, ChCl:Gly); minor background reactions for: KPi buffer, ChCl:Ur (1:2), and PC.

BI CATALYSIS & PROCESSING

Medium engineering for *Bs*PAD

30 °C, 3 h
450 rpm





30 °C, 5.5 h
1,000 rpm
100 mM FA
100 mg/L cells

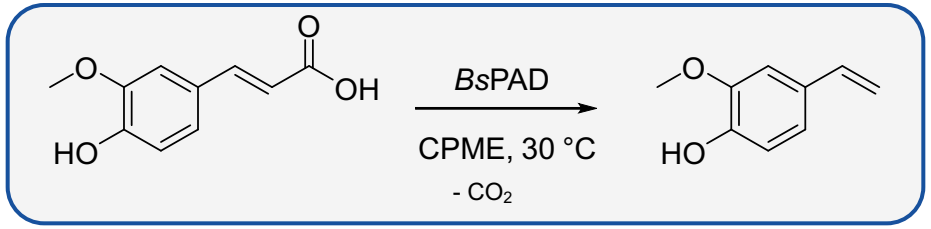


10% (m/v) mol. sieve 3A
25 °C, ≥ 24 h, no stirring

equilibrated over H_2O
25 °C, ≥ 24 h, no stirring

Enzyme activity & stability

CPME-water content



reaction progress in small scale:

1.5 mL glass vials

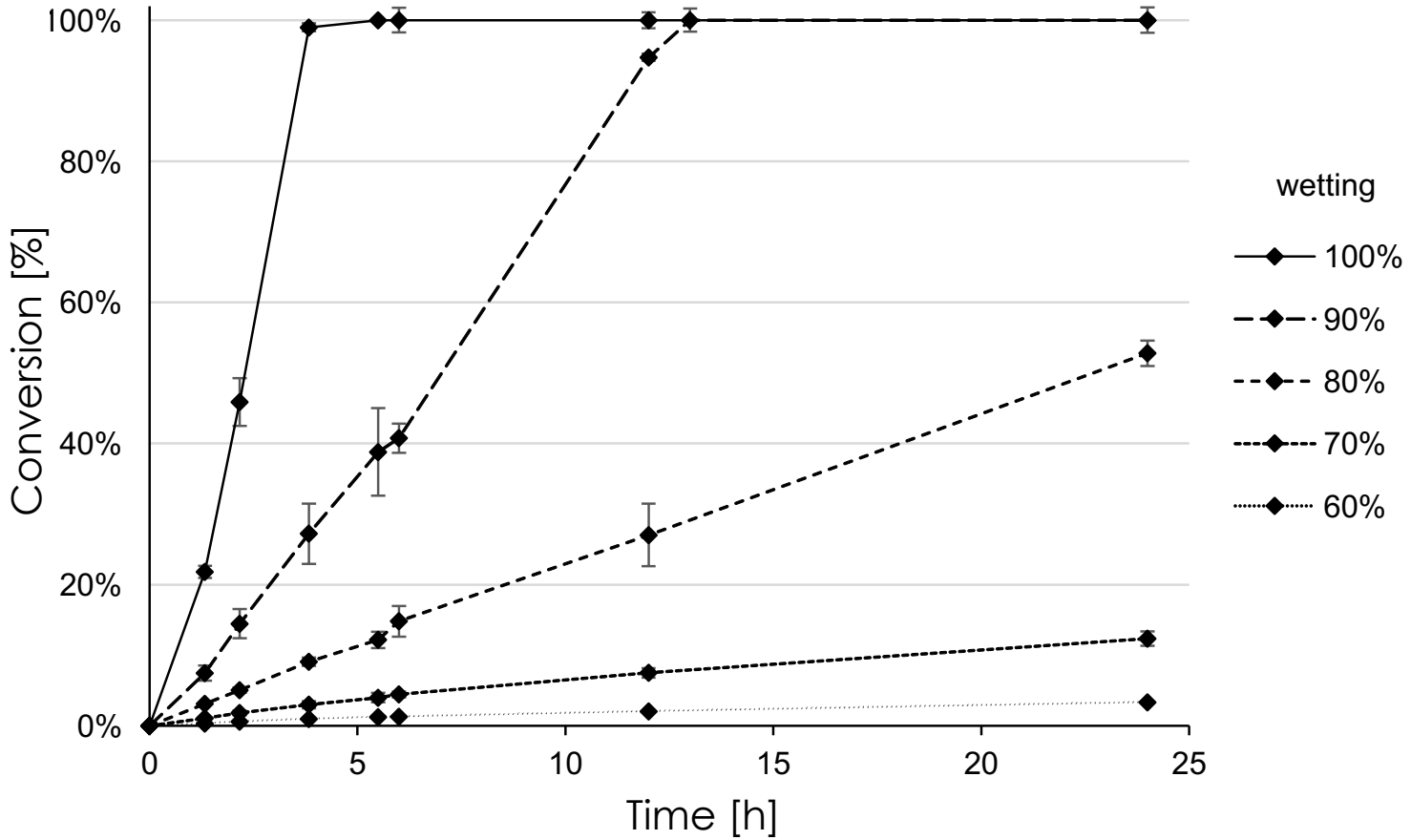
1.0 mL reaction volume

100 mM ferulic acid

100 mg/L cells

1,000 rpm

30 °C

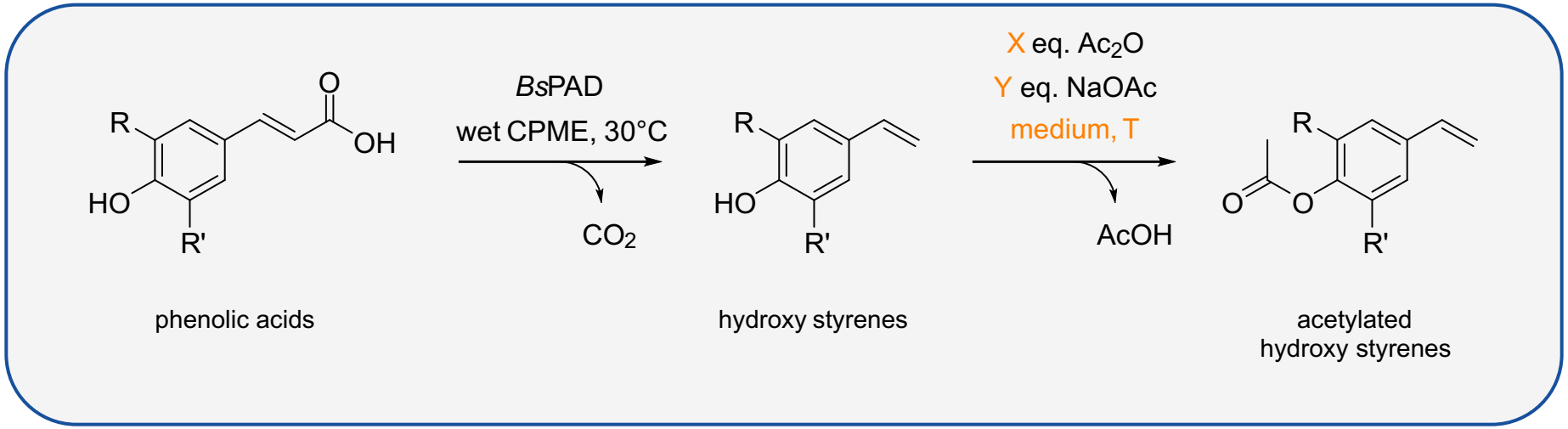


findings:

- activity depends on water content
- const. activity for wetting of 100 – 80%
- 70% wetting: k_d = 0.0409 h^{-1}; $t_{1/2}$ = 17.0 h
- 60% wetting: k_d = 0.0589 h^{-1}; $t_{1/2}$ = 11.8 h

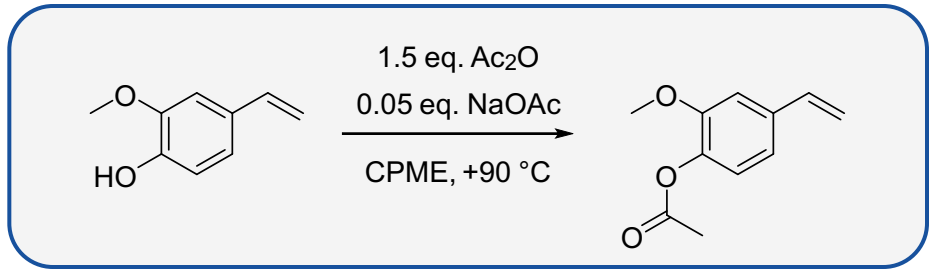
Chemoenzymatic cascade

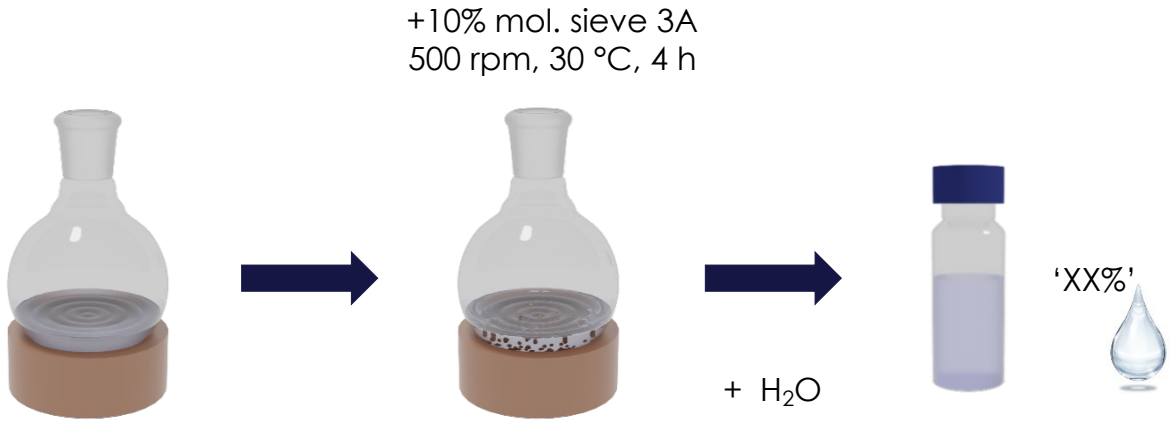
Polymer precursors



Same solvent for one-pot synthesis ?!

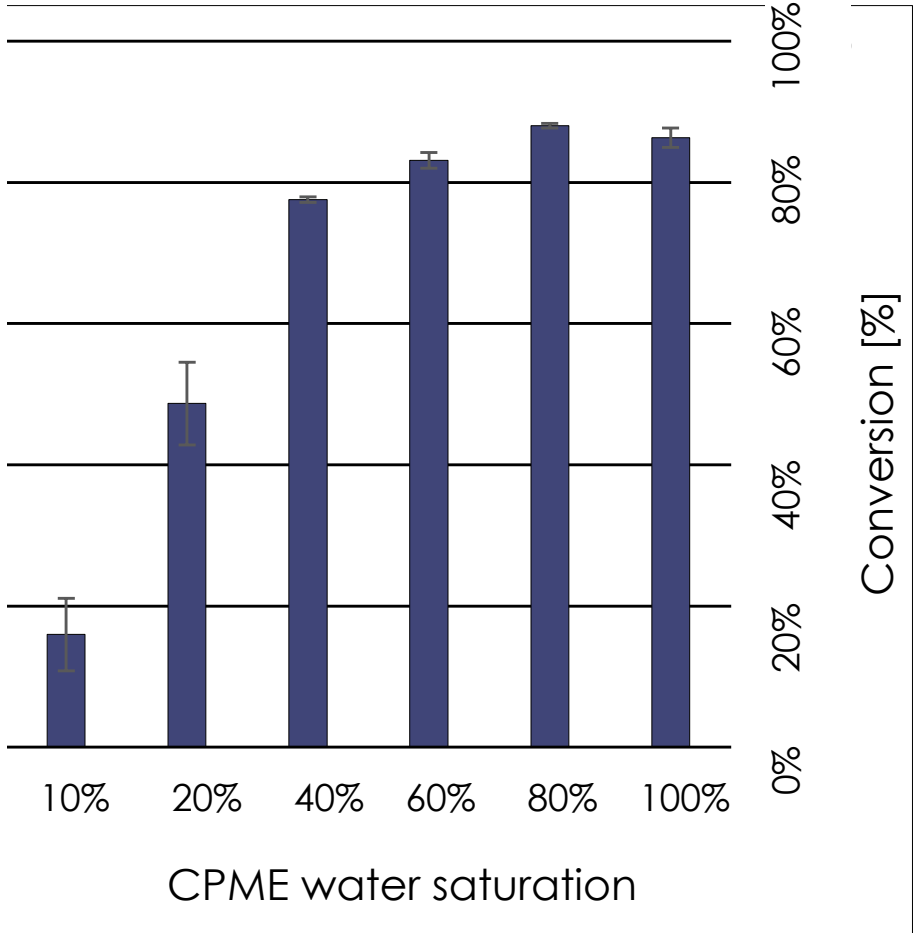
Acetylation





100 mM FA
300 mg/L cells
300 rpm, 30 °C, 3 h

150 mM Ac_2O
5 mM NaOAc
1,000 rpm, 90 °C, 1 h

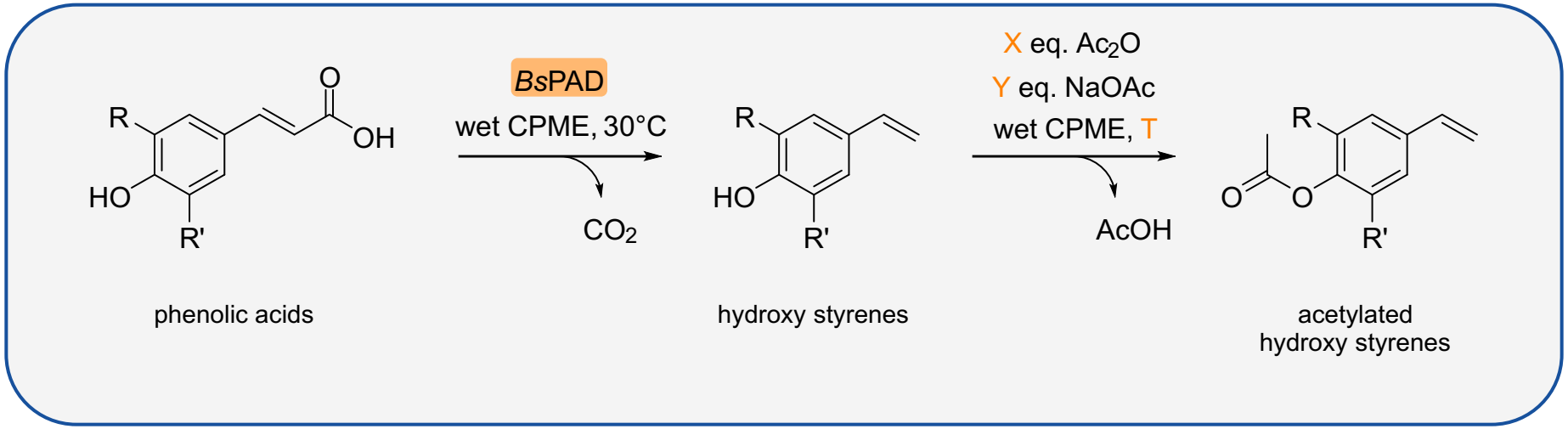


Reaction conditions: 1.5 mL glass vials, 1.0 mL rxn. volume, 100 mM 4-VG, 150 mM Ac_2O (1.5 eq.), 5 mM NaOAc (0.05 eq.), 1,000 rpm, 90 °C, 1 h, exp. duplicates. 100% water saturated CPME = wet CPME (0.7% w/w water).

BIOCATALYSIS & PROCESSING

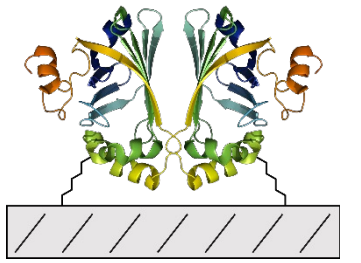
Chemoenzymatic cascade

Polymer precursors

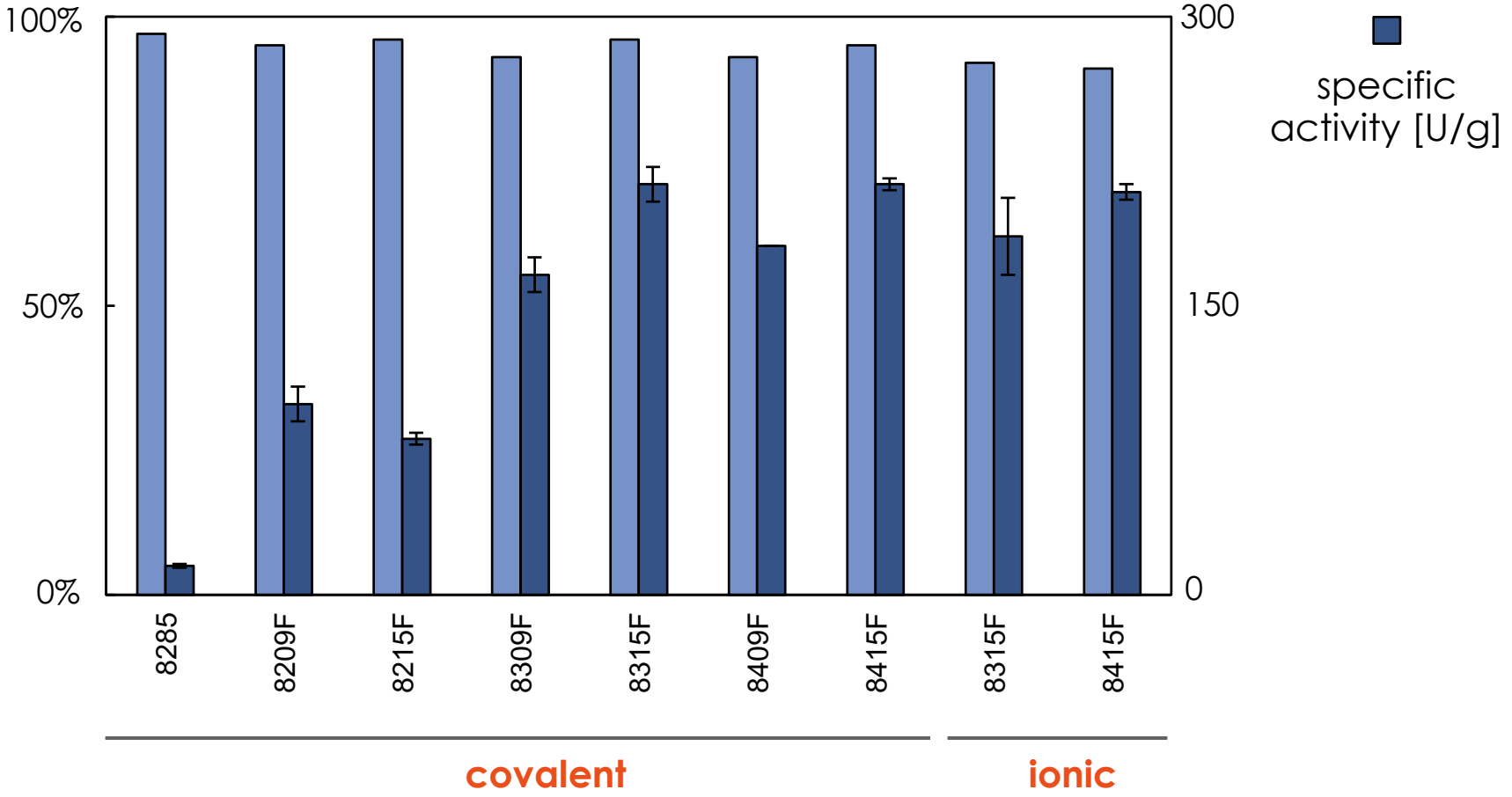


Motivation: Enzyme immobilization for a simple one-pot strategy

Enzyme immobilization



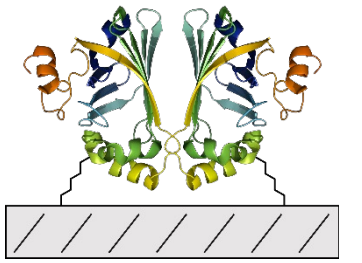
enzyme carrier resin (ECR)



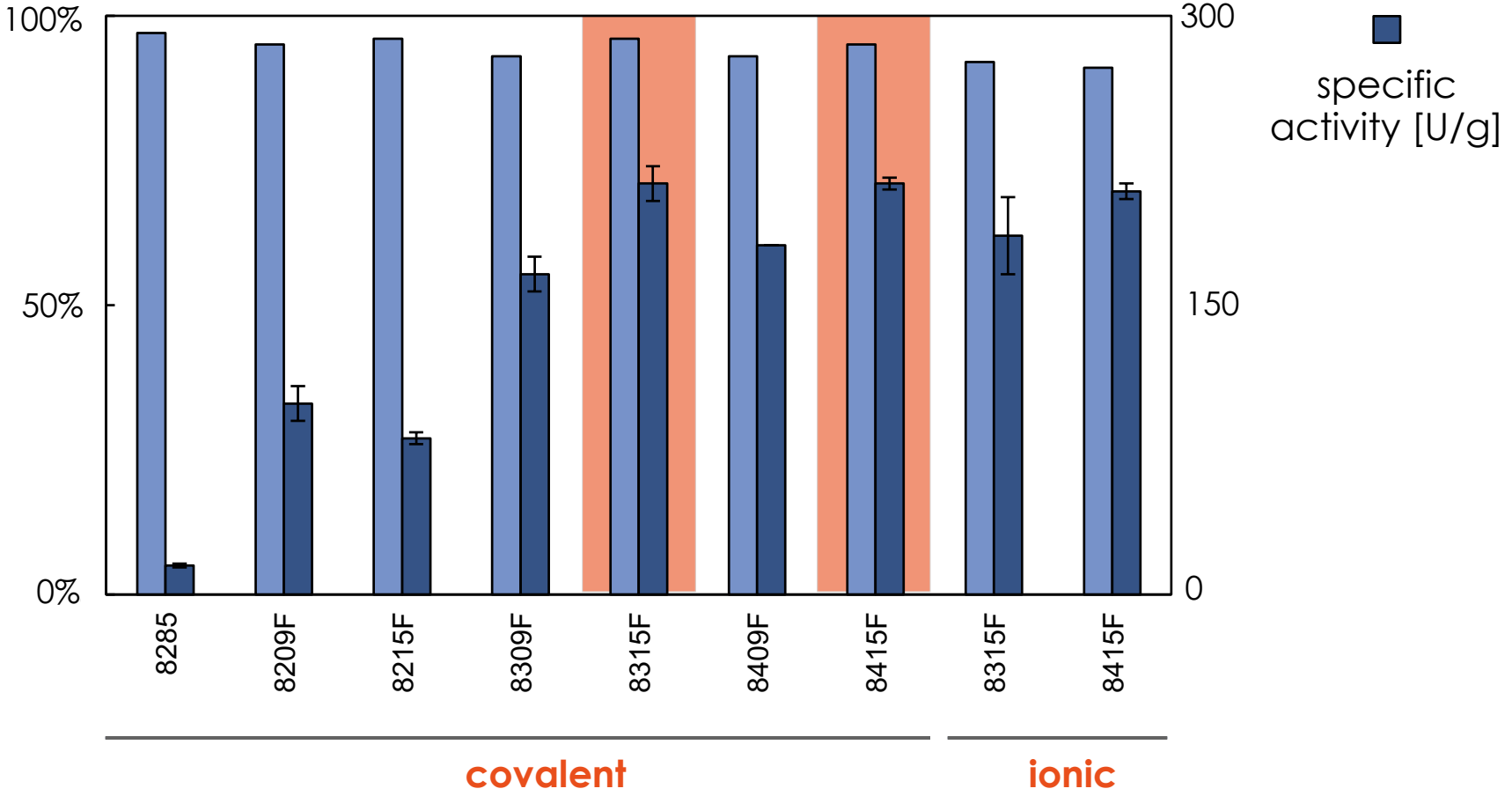
Immobilization buffers: KPi (1 M, pH 6.0) for epoxy carriers and KPi (50 mM, pH 6.0) for amino carriers // **Nominal protein load:** 50 mg / g carrier.
Activity assay: 10 mM ferulic acid in KPi buffer (50 mM, pH 6.0), either 50 mg/L CFE or 3 g/L immo-*Bs*PAD, 5% (v/v) DMSO, 30 °C, and 1000 rpm.

P. Petermeier, J.P. Bittner, S. Müller, E. Byström, S. Kara, *Green Chemistry* (**2022**) 24, 6889 – 6899.

Enzyme immobilization



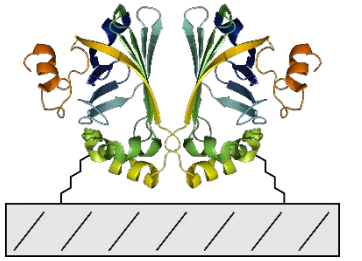
enzyme carrier resin (ECR)



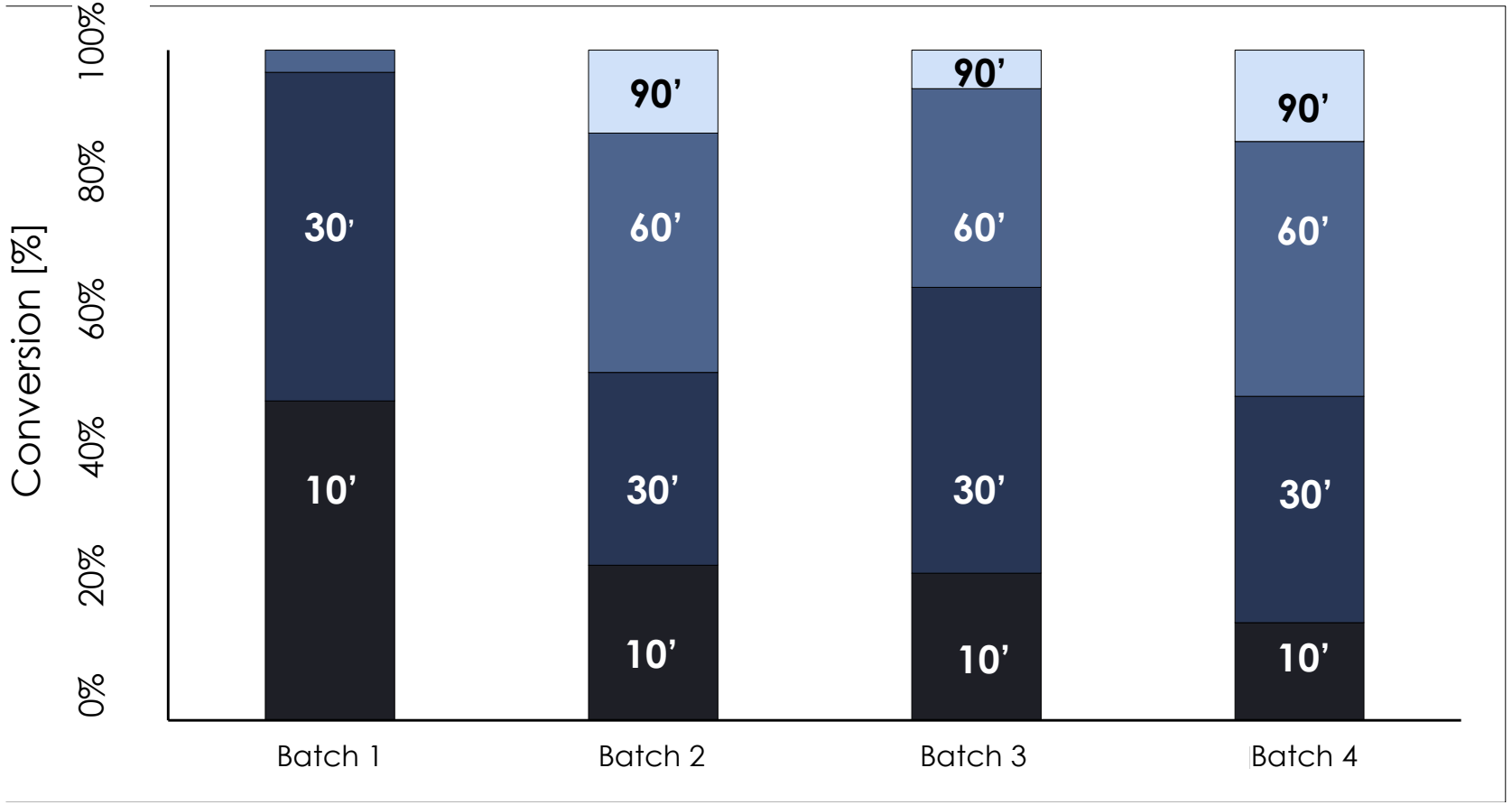
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P. Petermeier, J.P. Bittner, S. Müller, E. Byström, S. Kara, *Green Chemistry* (**2022**) 24, 6889 – 6899.

Reusability of *Bs*PAD-8415F



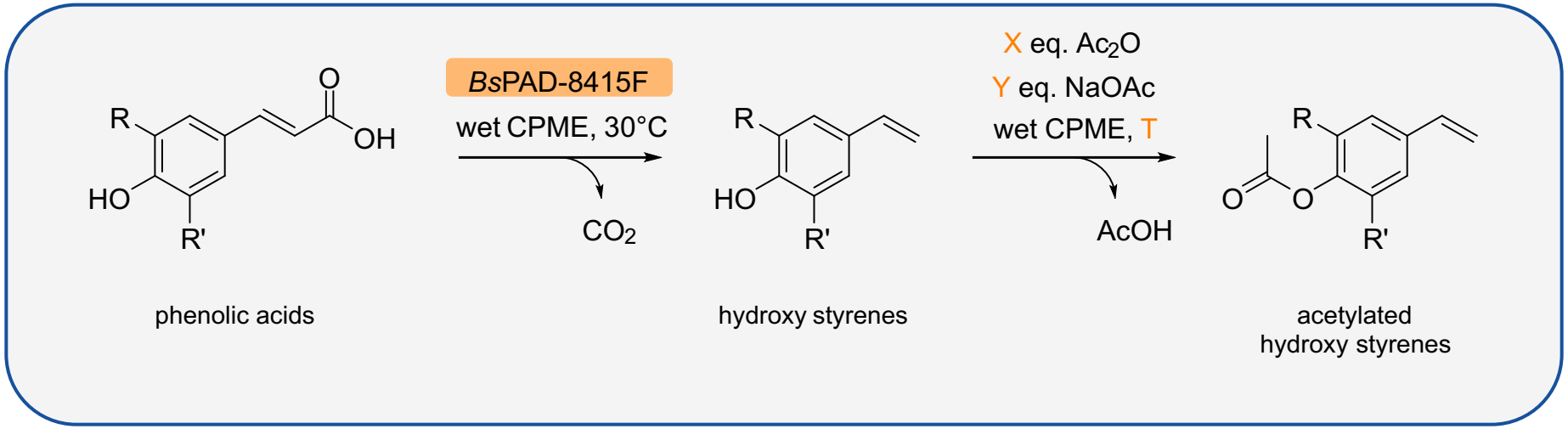
Purolite®
An Ecolab Company



Reaction conditions: 2 mL microcentrifuge tubes, 1.5 mL rxn. volume, 100 mM substrate, 4 g/L *Bs*PAD-8415F, wet CPME, 1,000 rpm, 30 °C, exp. duplicates.

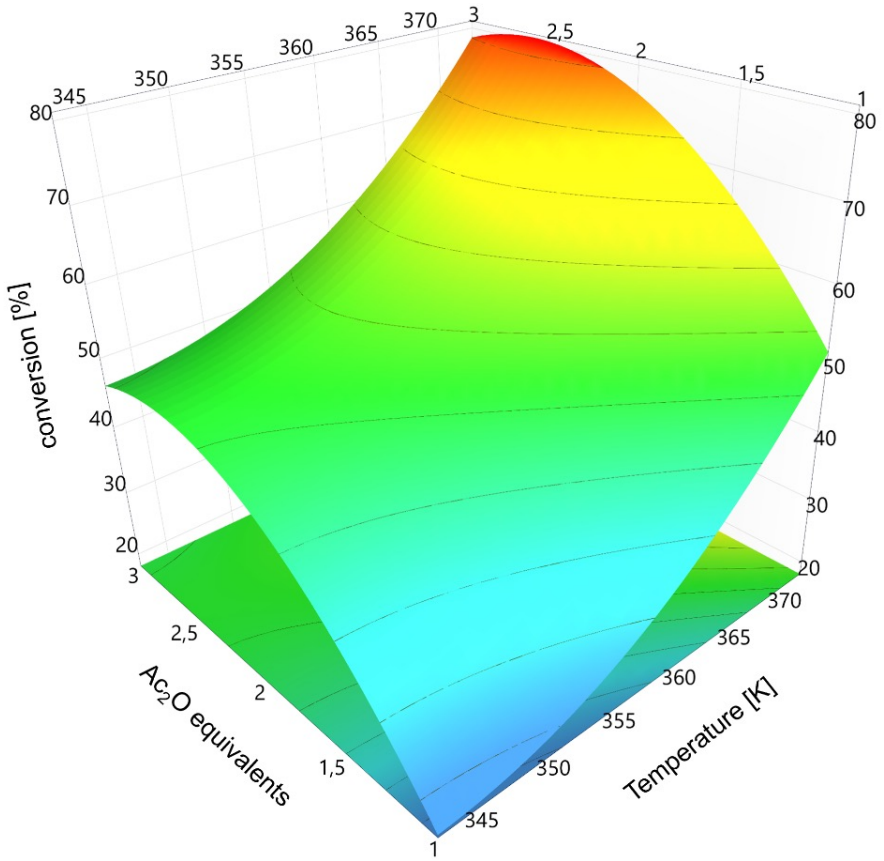
Chemoenzymatic cascades

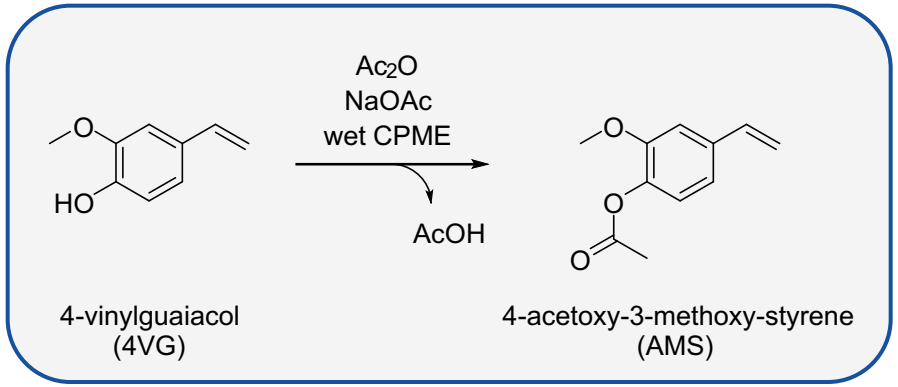
Polymer precursors



Acetylation tailoring

- DoE in MODDE®13 Temperature: 70 – 100 °C NaOAc: 0.02 – 0.08 eq. Ac_2O: 1 – 3 eq.
- 20 min reaction time:



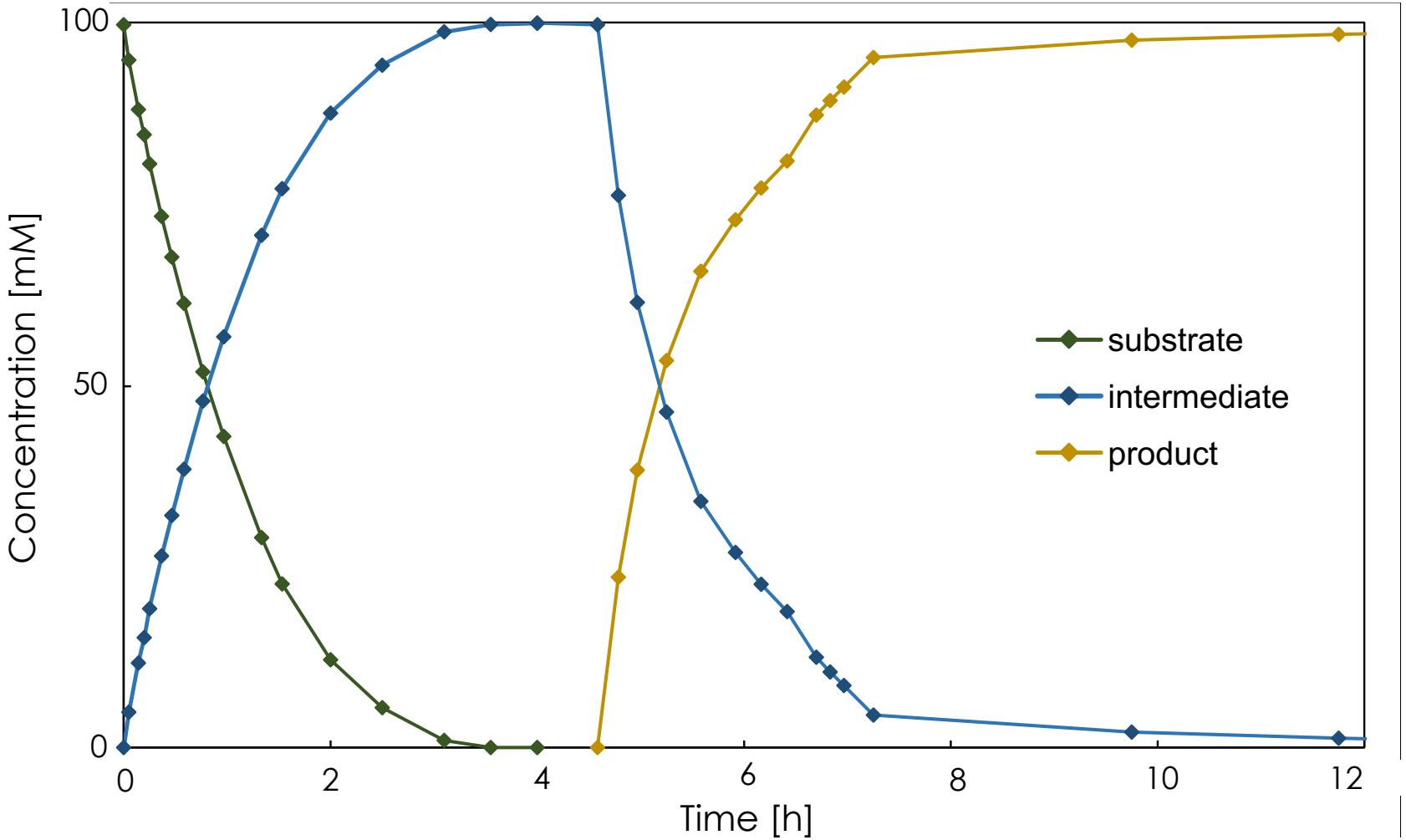


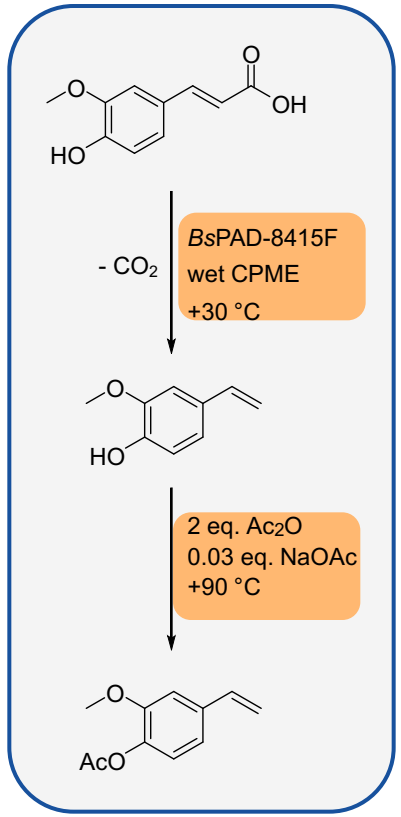
Conclusion:

- Maximize temperature within operational limits
- Minimize the use of the catalyst NaOAc
- Limit the acetylation agent Ac_2O to 2.0 – 2.5 eq.

Scale: 1 Liter



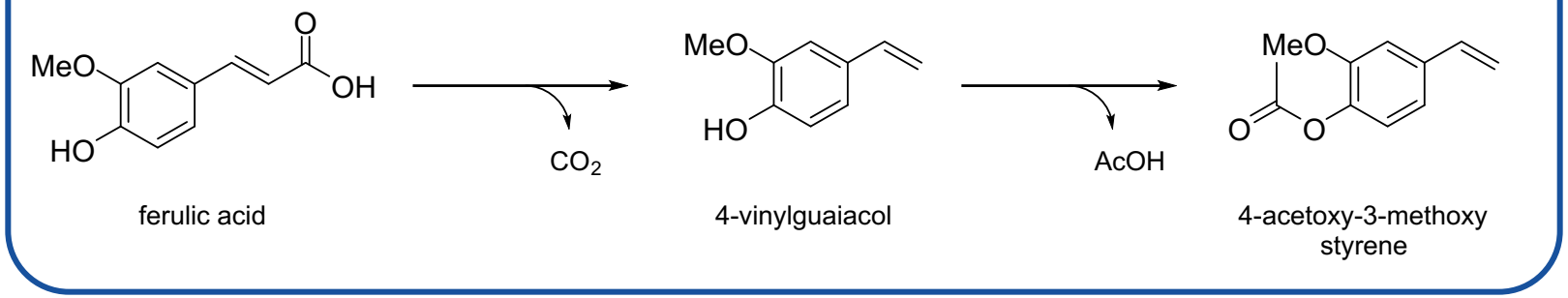




Reaction conditions #1: 100 mM ferulic acid, water saturated CPME, 5 g/L *Bs*PAD-8415F, 10 g/L 8415F, 300 rpm, and 30 °C.
Reaction conditions #2: 200 mM Ac_2O (2 eq.), 3 mM NaOAc (0.03 eq.), 500 rpm, and 90 °C.

P. Petermeier, J.P. Bittner, S. Müller, E. Byström, S. Kara, *Green Chemistry* (**2022**) 24, 6889 – 6899.

Preparative scale



ferulic acid — 4-vinylguaiacol — 4-acetoxy-3-methoxy styrene

- **Reaction progress:**
  - ✓ 1st step: >99.9% (3.5 h)
  - ✓ 2nd step: >98% (5 h)
  - ✓ Product titer: 19 g/L
  - ✓ Volumetric productivity: 38 g/(L·d)

- **18.3 g isolated final product**
  - ✓ 96% yield
  - ✓ 99% HPLC purity

Henkel

Collaboration with
Henkel Adhesive Research / Bio-Renewables.

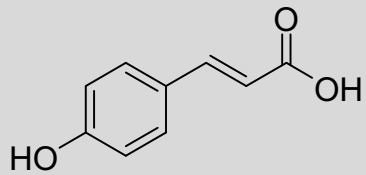


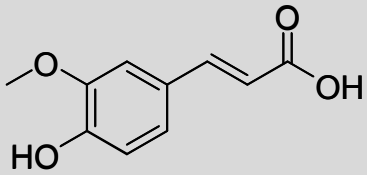
RBR

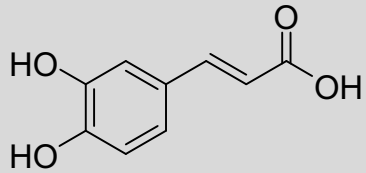
Rotating Bed Reactor

P. Petermeier, J.P. Bittner, S. Müller, E. Byström, S. Kara, *Green Chemistry* (**2022**) 24, 6889 – 6899.

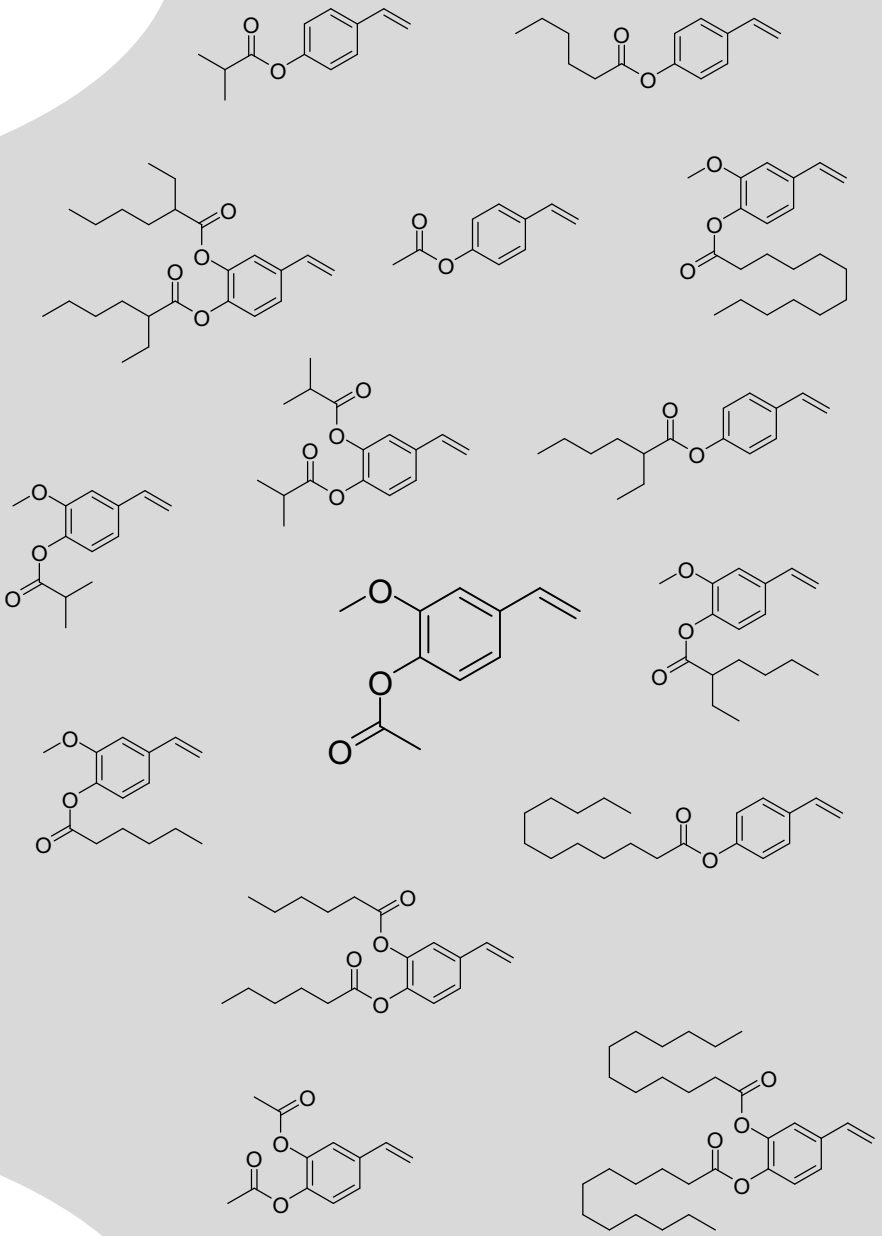
Process modularity





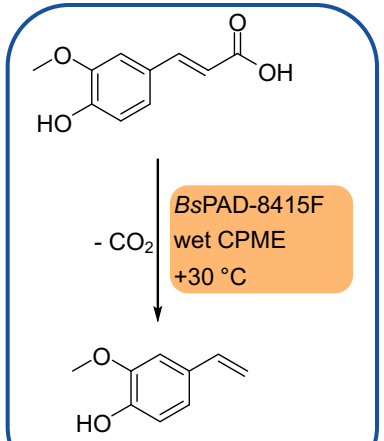


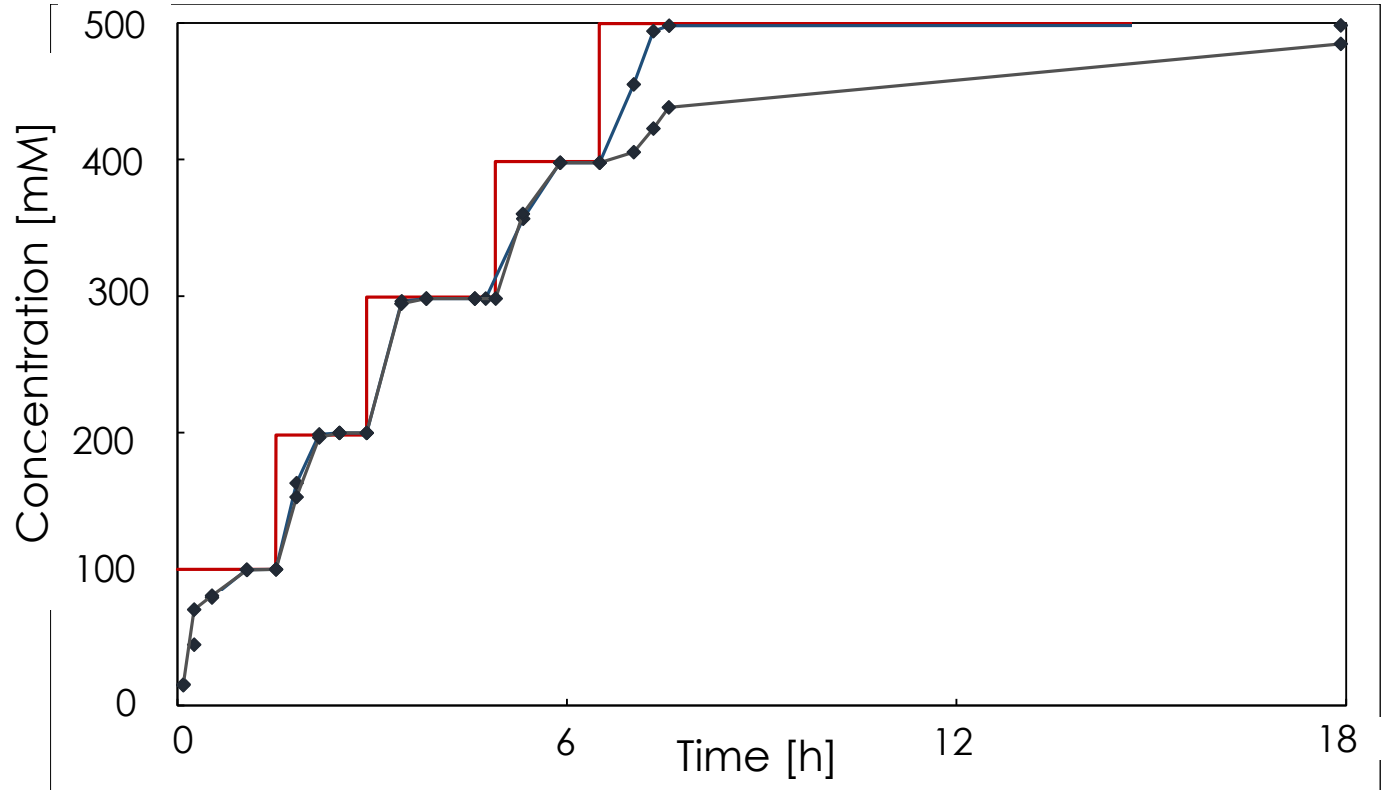




Process intensification

fed-batch strategy



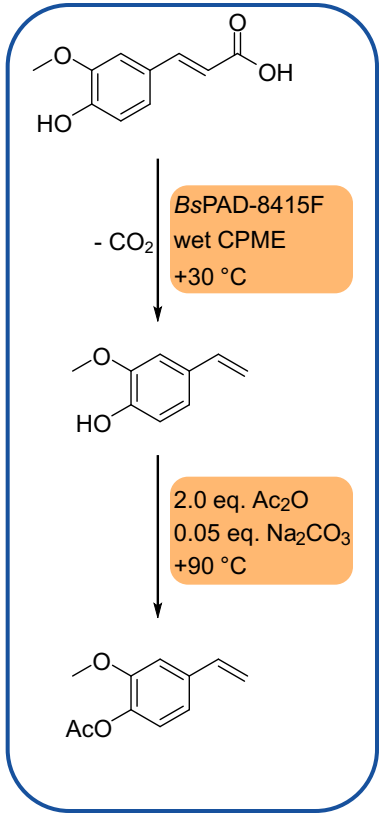


- 5.7 g/L *Bs* PAD-8415F
- 5.6 g/L ECR8415F
- H_2O saturated
- +2 µL H_2O

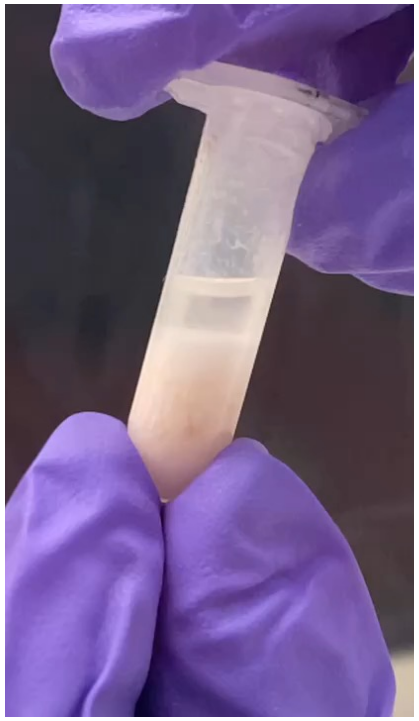
✓ **492 mM product**

P. Petermeier, J.P. Bittner, T. Jonsson, P. Domínguez de María, E. Byström, S. Kara, *Nature Communications Chemistry* (**2024**) 7, 57.

Process intensification
All-in strategy



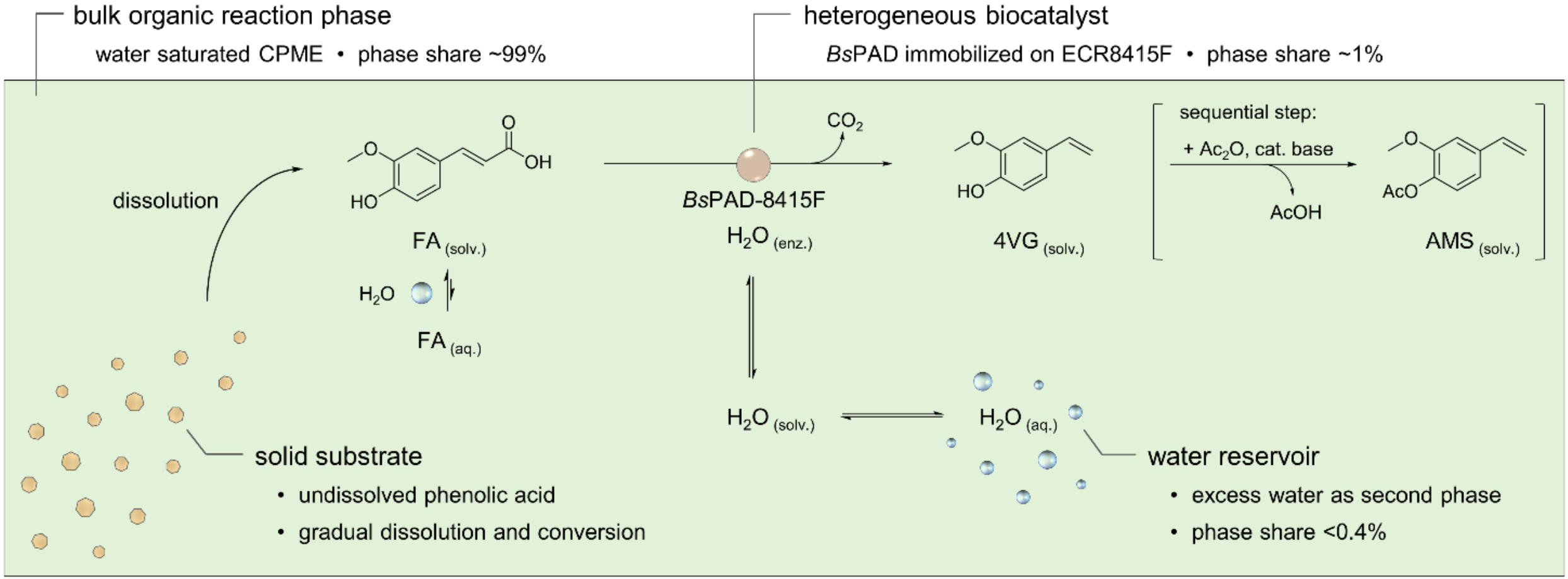
substrate [mol/L]	*Bs* PAD-8415F [g/L]	8415F [g/L]	H_2O [mL/L]	decarbox. conv. [%]	rxn. time [h]	productivity [mmol/g_{cat}/h]	acetylation conv. [%]	rxn. time [h]
0.1	5	0	0	> 99%	0.7	28.4 ± 1.5	97%	2.0
0.1	5	0	4	> 99%	2.0	9.7 ± 0.3	96%	2.0
0.3	10	0	4	> 99%	1.5	19.1 ± 1.0	> 99%	0.8
0.3	10	11	4	> 99%	1.1	25.5 ± 1.8	> 99%	0.8
0.5	11	12	4	> 99%	1.8	24.8 ± 0.1	> 99%	0.5
0.5	11	17	4	> 99%	1.8	25.5 ± 0.1	> 99%	0.4
1.0	10	18	4	> 99%	4.1	23.6 ± 0.5	> 99%	0.2
1.0	21	17	8	> 99%	1.7	27.1 ± 0.8	> 99%	0.2
1.5	31	20	10	> 99%	2.0	24.0 ± 0.3	> 99%	0.2
2.0	40	30	14	> 99%	2.0	24.8 ± 0.3	> 99%	0.1



P. Petermeier, J.P. Bittner, T. Jonsson, P. Domínguez de María, E. Byström, S. Kara, *Nature Communications Chemistry* (**2024**) 7, 57.

Reaction conditions: 1 mL water-saturated CPME, 30 °C, 1000 rpm; for subsequent acetylation, the supernatants were subjected to: +2.0 eq. Ac_2O, +0.05 eq. Na_2CO_3, 90 °C, 1000 rpm.

Process intensification
Overview of the reaction medium



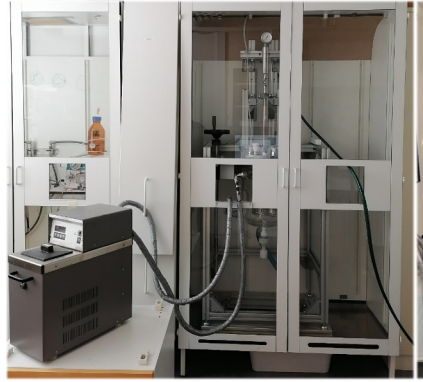
P. Petermeier, P. Domínguez de María, E. Byström, S. Kara, ACS *Sustainable Chemistry & Engineering* (**2024**) 12(34):12869 – 12878.

The need of an efficient biocatalysis

Parameters	Target values
Substrate conversion	≥95% (in 24 hours, ideally in one shift)
Volumetric productivity	>100 g/(L·d) >300 g/(L·d) (bulk chemicals)
Substrate-to-enzyme ratio	>50 g/g
Product-to-enzyme ratio	**Bulk chemicals:** • 700 – 2000 kg $_{product}$/kg $_{dry\ cell\ weight}$ • 5000 – 20,000 kg $_{product}$/kg $_{free\ enzyme}$ • 2000 – 10,000 kg $_{product}$/kg $_{immobilized\ enzyme}$ **Pharmaceuticals:** • 10 – 35 kg $_{product}$/kg $_{dry\ cell\ weight}$ • 100 – 250 kg $_{product}$/kg $_{free\ enzyme}$ • 50 – 100 kg $_{product}$/kg $_{immobilized\ enzyme}$
E-factor (Roger A. Sheldon, 1942–2025)	**Bulk chemicals:** • 1 – 5 kg $_{waste}$/kg $_{product}$ **Fine chemicals and speciality chemicals:** • Up to 500 kg $_{waste}$/kg $_{product}$ **Pharmaceuticals:** • Up to 4000 kg $_{waste}$/kg $_{product}$

G.W. Huisman, J. Liang, A. Krebber, *Curr. Opin. Chem. Biol.* 14 (**2010**) 122 – 129.
F. Hollmann, I.W.C.E. Arends, D. Holtmann, *Green Chem.* 13 (**2011**) 2285 – 2313.
P. Tufvesson, J. Lima-Ramos, M. Nordblad, J.M. Woodley, *Org. Process Res. Dev.* 15 (**2011**) 266 – 274.

Upscaling



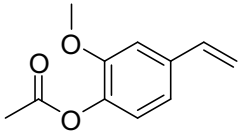
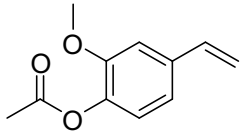
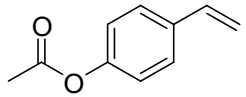
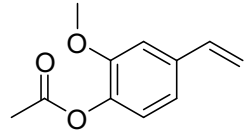

start of decarboxylation

end of decarboxylation

acetylation

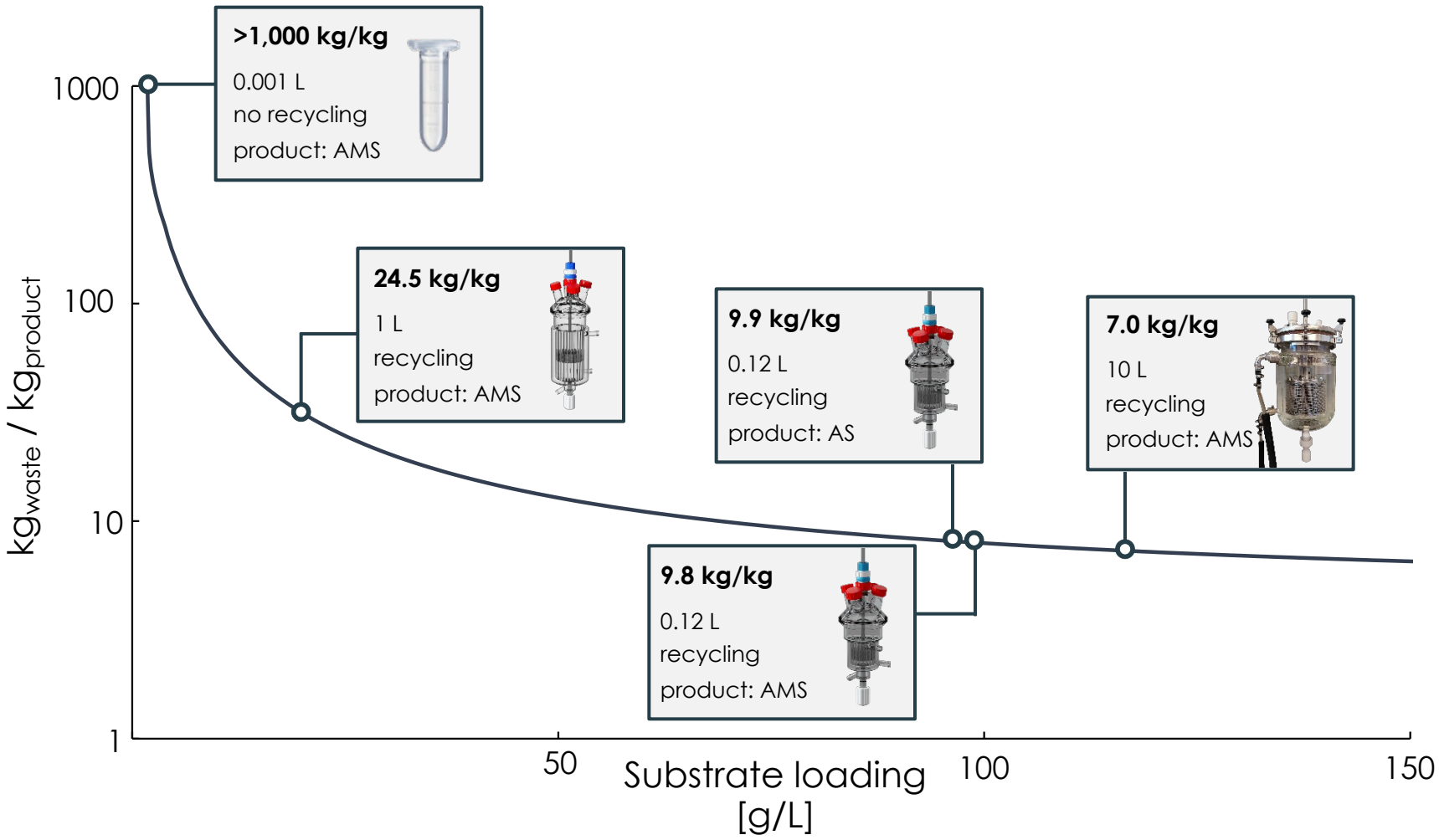
quench

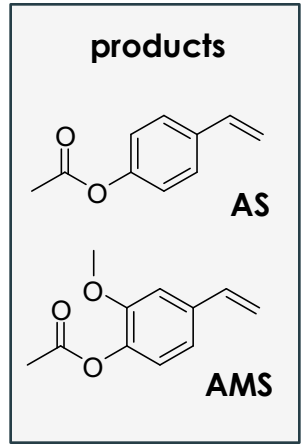
after quench

Reactor	SpinChem S3	SpinChem S2	SpinChem S2	SpinChem S4
Reaction volume	1 L	0.12 L	0.12 L	10 L
Product				
Product titer	100 mM	500 mM	500 mM	630 mM
Isolated yield	18.3 g (96%)	10.8 g (93%)	9.3 g (95%)	1060 g (97%)
Purity (HPLC)	99%	98.4%	99.9%	99.4%
Purity (^{1}H NMR)	~95%	~99%	~99%	~98%
Volumetric productivity	38 g/(L·d)	408 g/(L·d)	204 g/(L·d)	288 g/(L·d)

P. Petermeier, P. Domínguez de María, E. Byström, S. Kara, ACS *Sustainable Chemistry & Engineering* (**2024**) 12(34):12869 – 12878.

E-Factor: kg waste / kg product



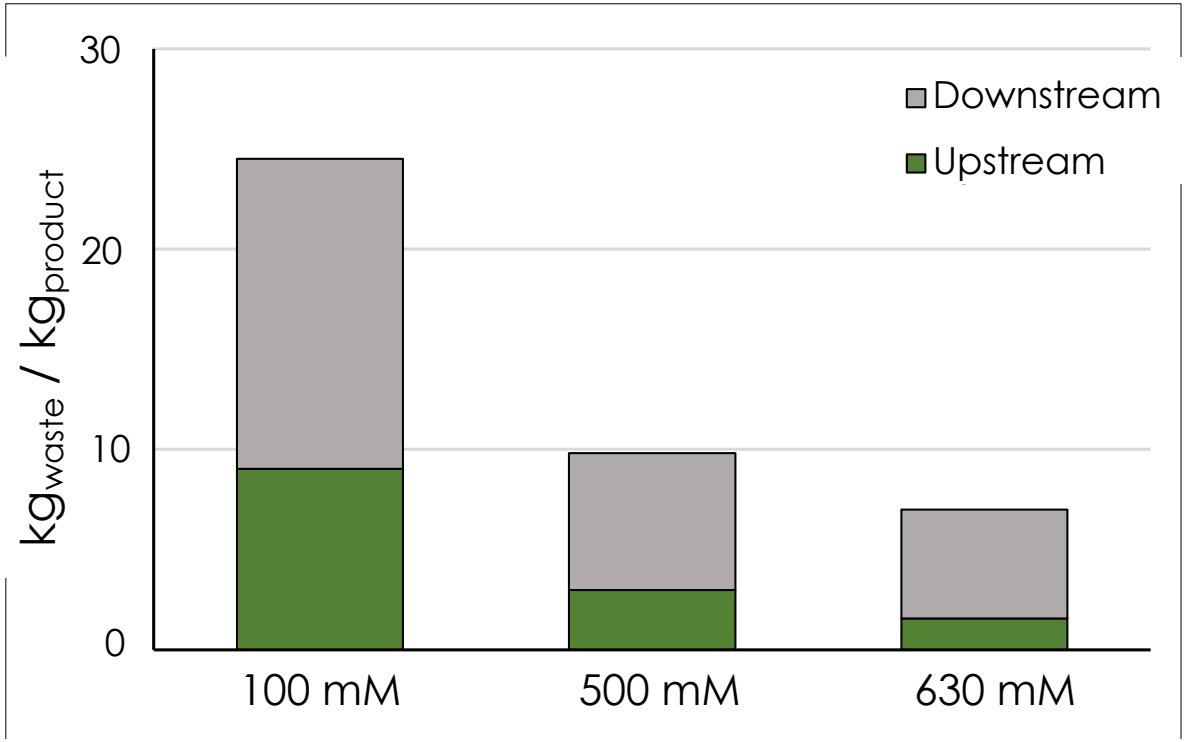


E-Factor: kg $_{waste}$ / kg $_{product}$

waste
unrecovered materials
recovered, not reusable materials
no waste
recovered & reusable materials

Conclusions:

- downstream processing is the main origin of the waste



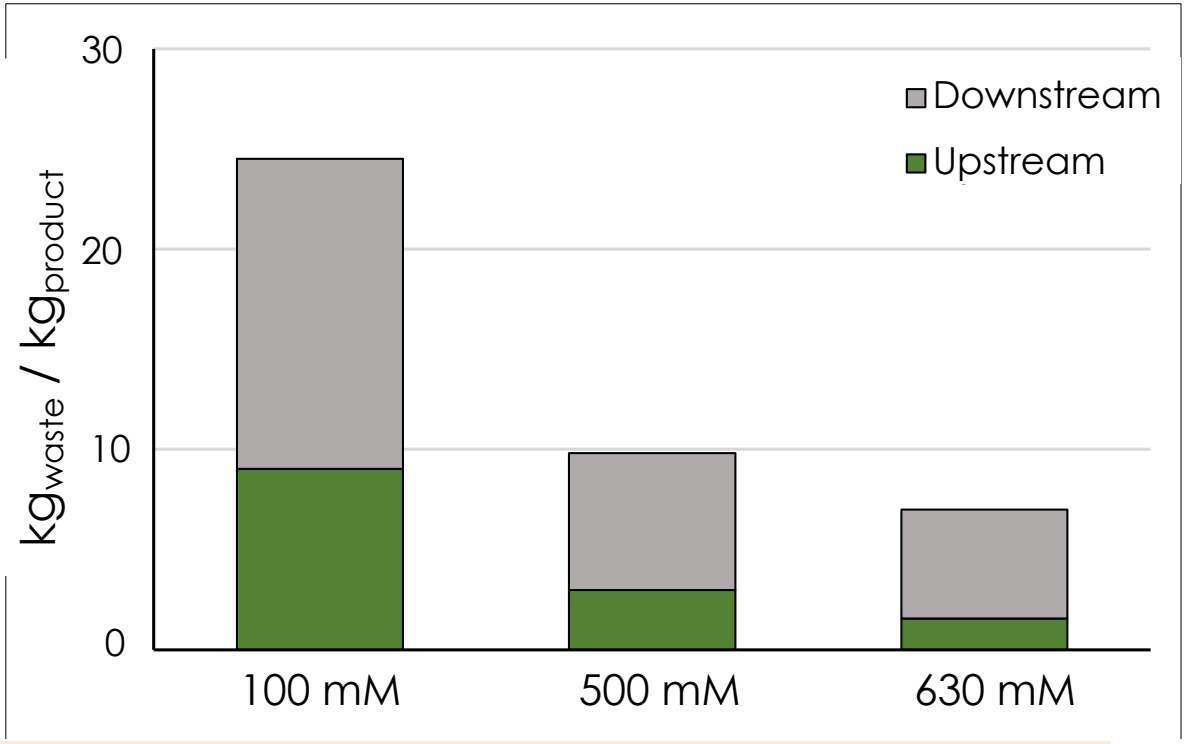
P. Petermeier, P. Domínguez de María, E. Byström, S. Kara, ACS *Sustainable Chemistry & Engineering* (**2024**) 12(34):12869 – 12878.

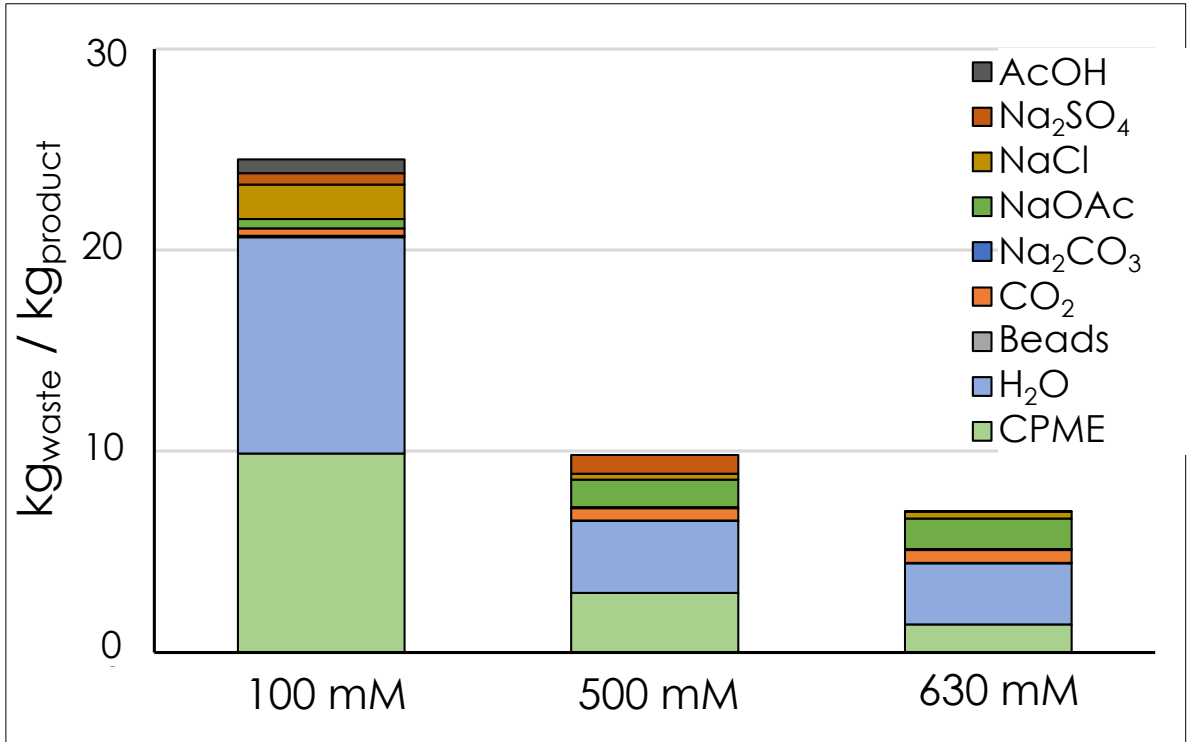
E-Factor: kg $_{waste}$ / kg $_{product}$

waste
unrecovered materials
recovered, not reusable materials
no waste
recovered & reusable materials

Conclusions:

- downstream processing is the main origin of the waste
- solvent & H_2O are the main contributors





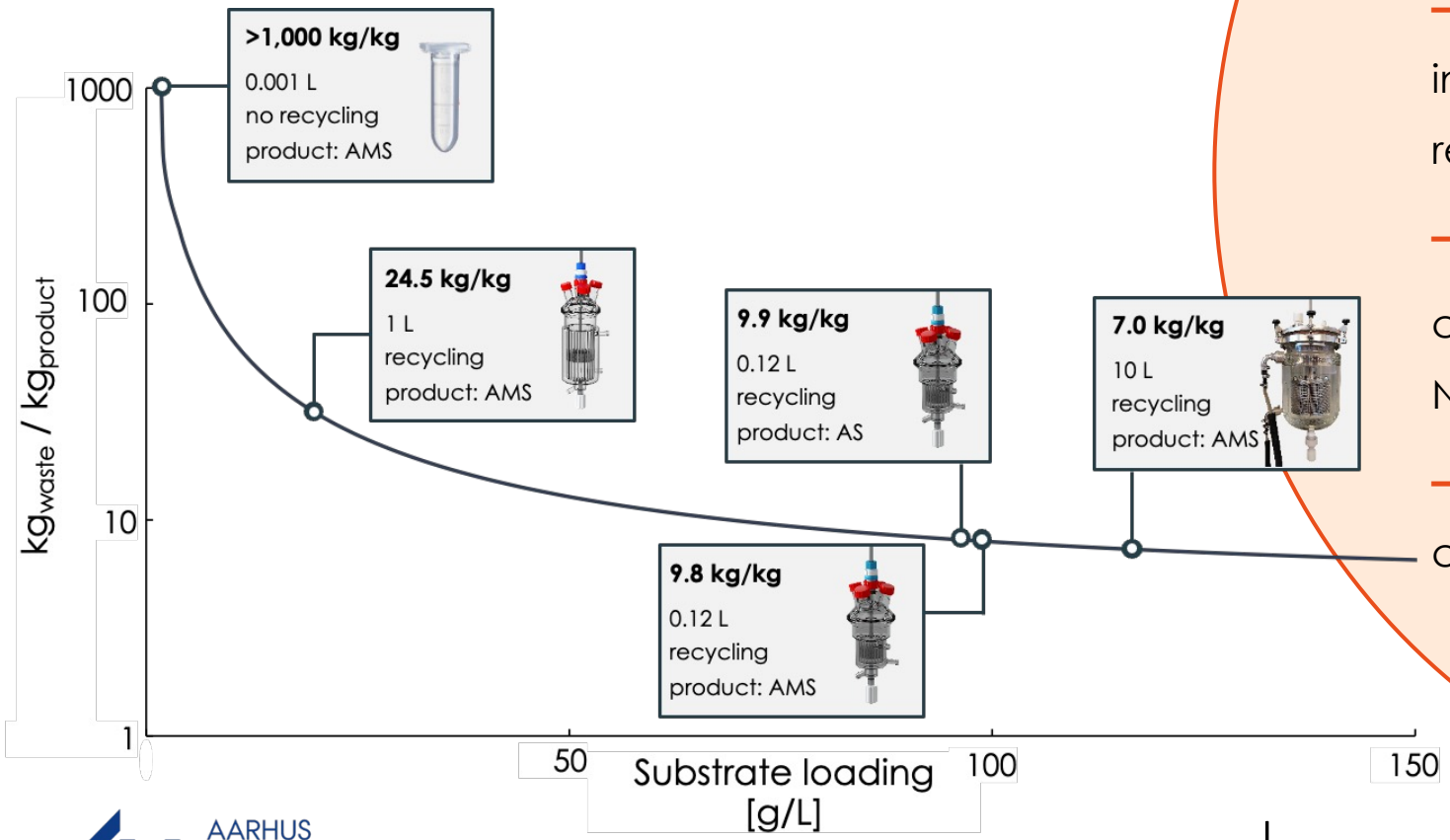
scale	1 L	0.12 L	10 L
CPME recovery	84%	75%	84%

Contaminated water:
water-saturated reaction medium, quenching, and washing.

E-Factor: kg waste / kg product

Conclusions:

- downstream processing is the main origin of the waste
- solvent & H_2O are the main contributors



further possible reductions by:

–3.0 kg/kg in-house wastewater treatment facilitating water recycling.

–1.4 kg/kg complete solvent recovery enabled by an integrated and automated reactor system, solvent recycling.

–0.6 kg/kg gas scrubbing of reactor exhaust gases to capture the released CO_2 to potentially recycle it as a Na_2CO_3 catalyst or quenching agent.

–0.3 kg/kg direct recovery and reuse of the sodium chloride solution used during the workup.

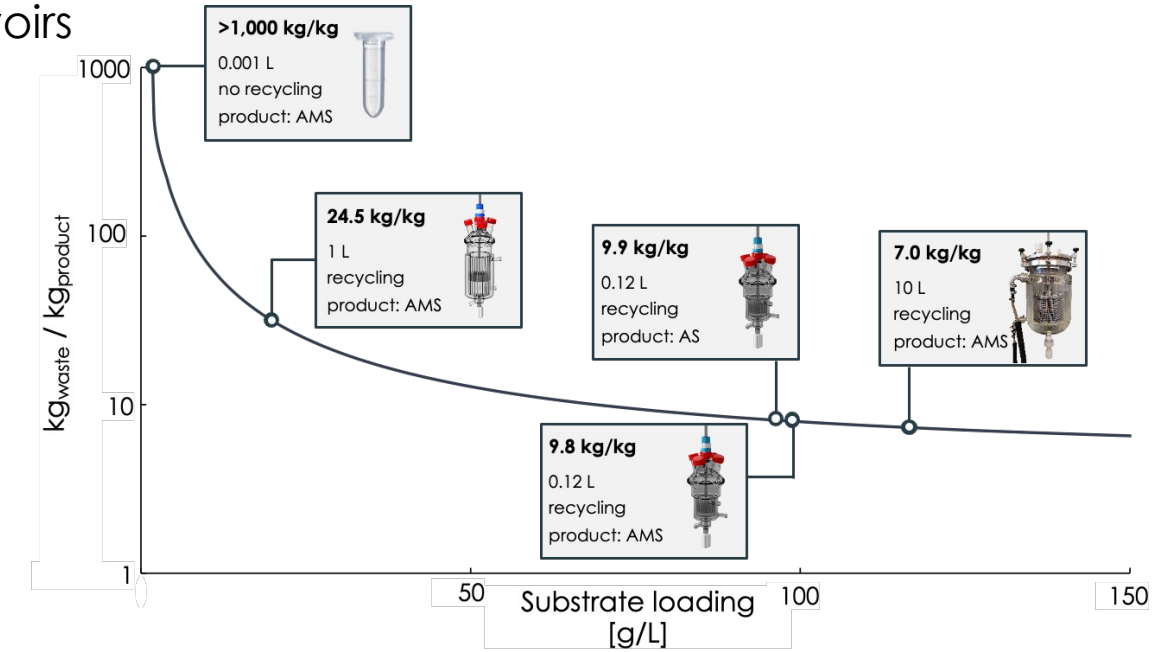
< 2 kg/kg

Summary

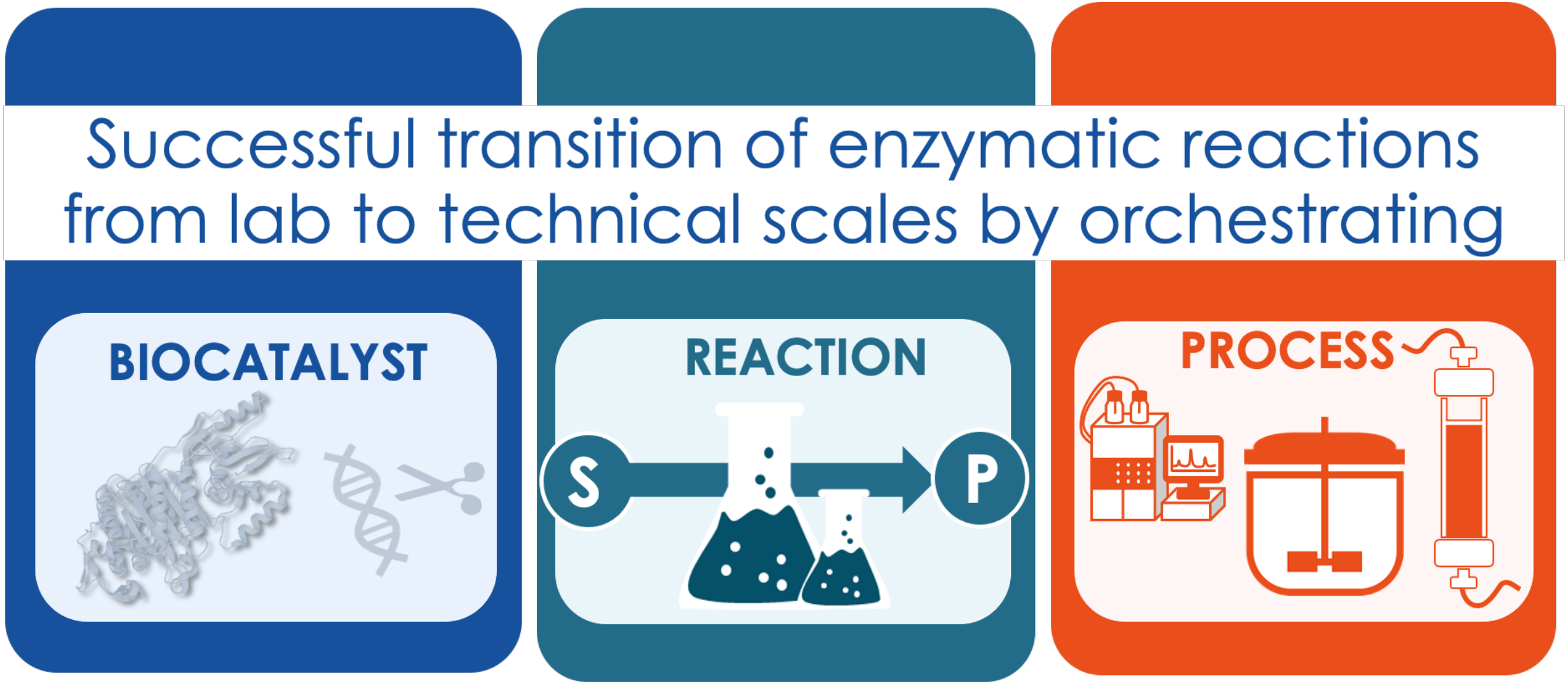
- ✓ Identification of solvent system for complete cascade
- ✓ Stable, active, and reusable immobilized enzyme
- ✓ Optimized acylation conditions
- ✓ Modular platform process (15 different acetylation products)
- ✓ Intensification (2.0 M; 400 g/L) *via* introduced water reservoirs
- ✓ Upscaled to 1 kg product in a 10 L reactor
- ✓ Systematically lowered E-factor
  (from 1,000 to 7.0 $kg_{product}/kg_{waste}$…possibility to go down)



1.5 mL (up to 2 M; 400 g/L) | 0.12 – 1 L | 10 L (630 mM; 120 g/L)



Successful transition of enzymatic reactions from lab to technical scales by orchestrating



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AARHUS UNIVERSITY

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Erik Löfgren
Dr. Pablo Domínguez de María
Dr. Alessandra Basso
Dr. Simona Serban
Dr. Andreas Taden

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P. Petermeier et al., *Green Chemistry* (**2022**) 24, 6889 – 6899.
P. Petermeier et al., *Nature Communications Chemistry* (**2024**) 7, 57.
P. Petermeier et al., ACS *Sustainable Chemistry & Engineering* (**2024**) 12(34):12869 – 12878.

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TUHH Technische Universität Hamburg
TU Graz

(2020 – 2024)

INTERfaces

Innovative Training Network – European Industrial Doctorates

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INDEPENDENT RESEARCH FUND DENMARK

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DECADES

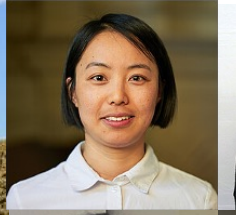
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FOR 5730

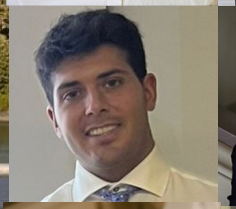
ELEGANCE

B3PO
Better Biobased Polymers

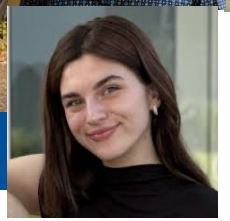
NET4FLOW
Advanced Processes











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Dr. Christopher Grimm
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