Chemo-enzymatic conversions of biomass constituents

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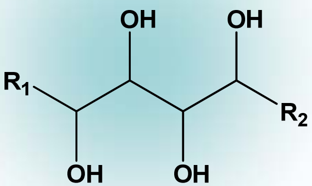
Dechema Praxisforum Enzymes for Industrial Applications 2016, Frankfurt

Nature‘s Chemical Diversity for the Diversity of Chemical Products

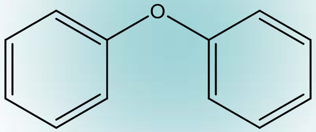
Biomass

$(C_{13}H_{21}O_{10})_n$

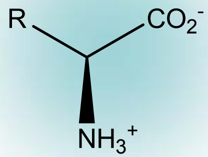
Carbohydrates



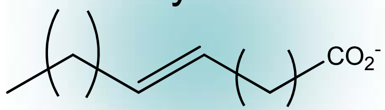
Lignin



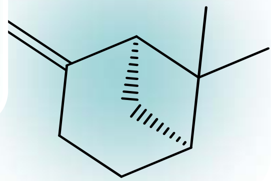
Amino acids

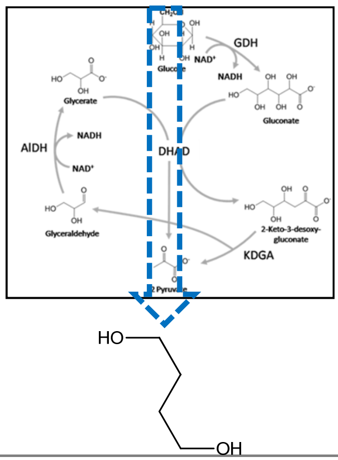


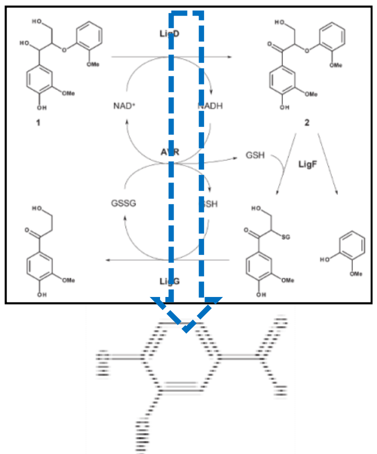
Fatty acids

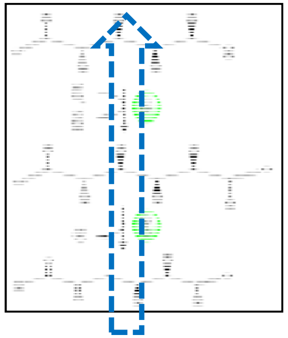


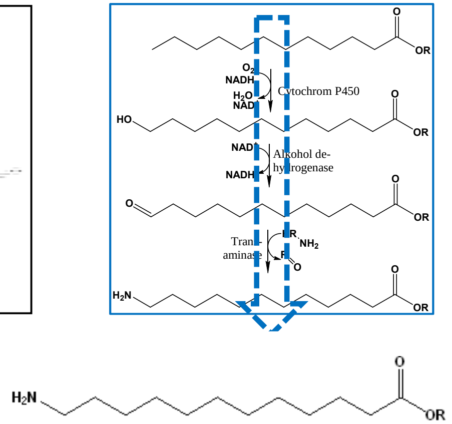
Terpene

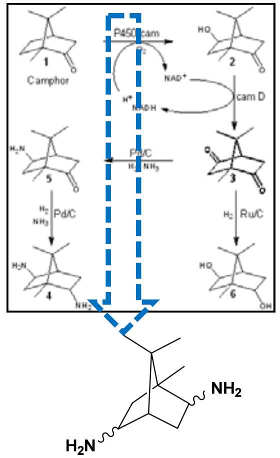












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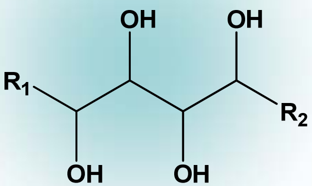

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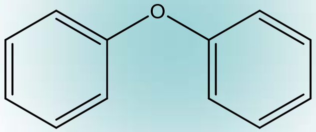
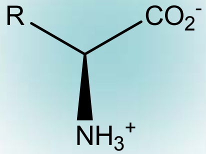
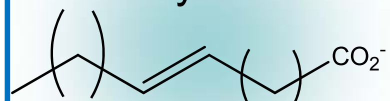


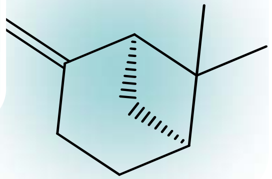
Amino acids



Fatty acids



Terpene

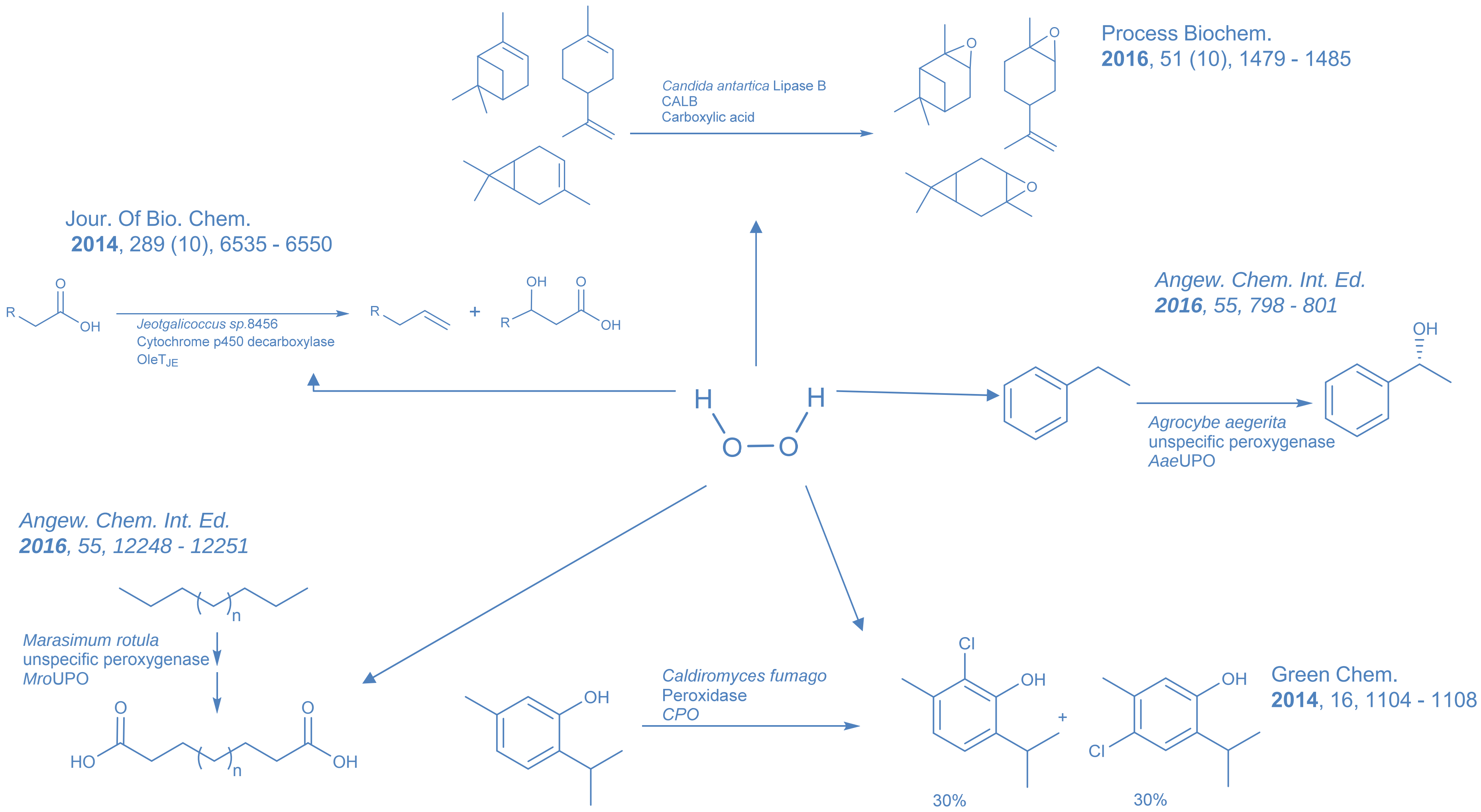


Over-functionalized

Under-functionalized

=> Oxidation

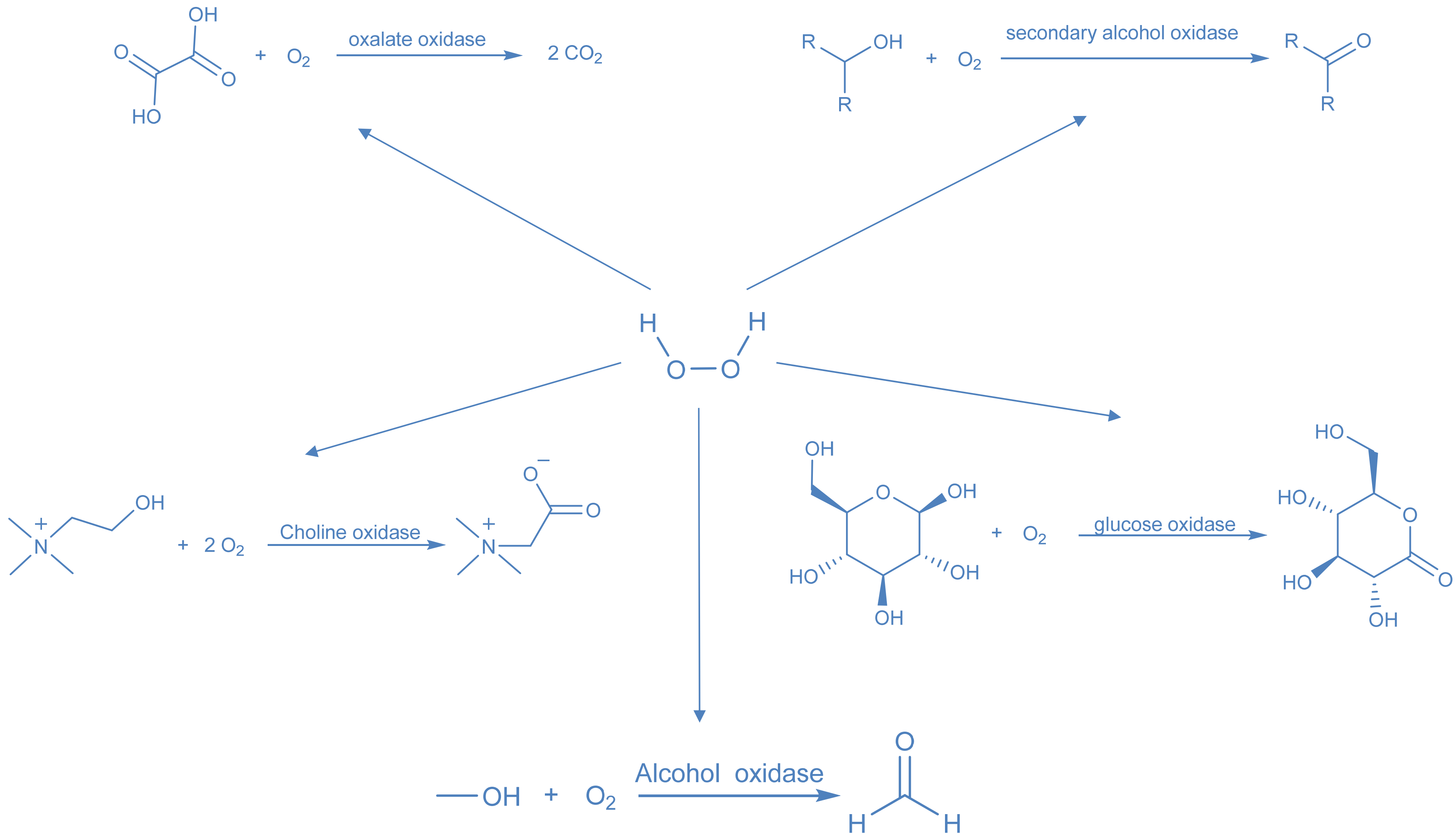
Enzymatic reactions dependent on H_2O_2



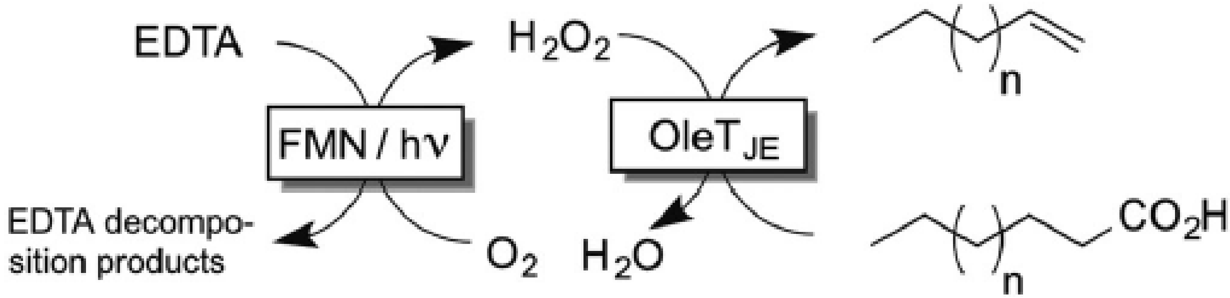
H_2O_2 synthesis methods

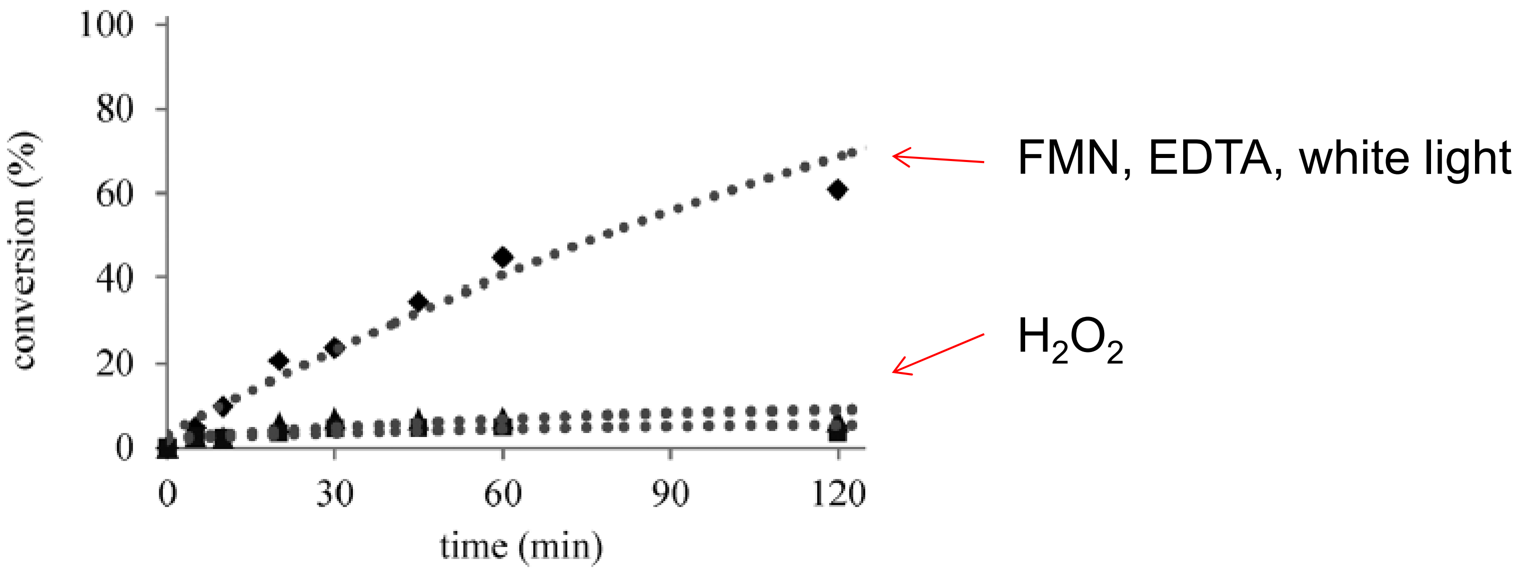
- Chemical
  - Anthraquinone autooxidation process
  - Shell 2-propanol process
  - Direct synthesis: H_2 and O_2
- Electrochemical
  - Degussa-Wissenstein Process
  - Münchner Process
- Photocatalytic
  - Carbon nitride catalysts
  - Flavin dependent generation
- Enzymatic
  - Oxidase (E.C 1.1.3.X)

Enzymatic H_2O_2 formation



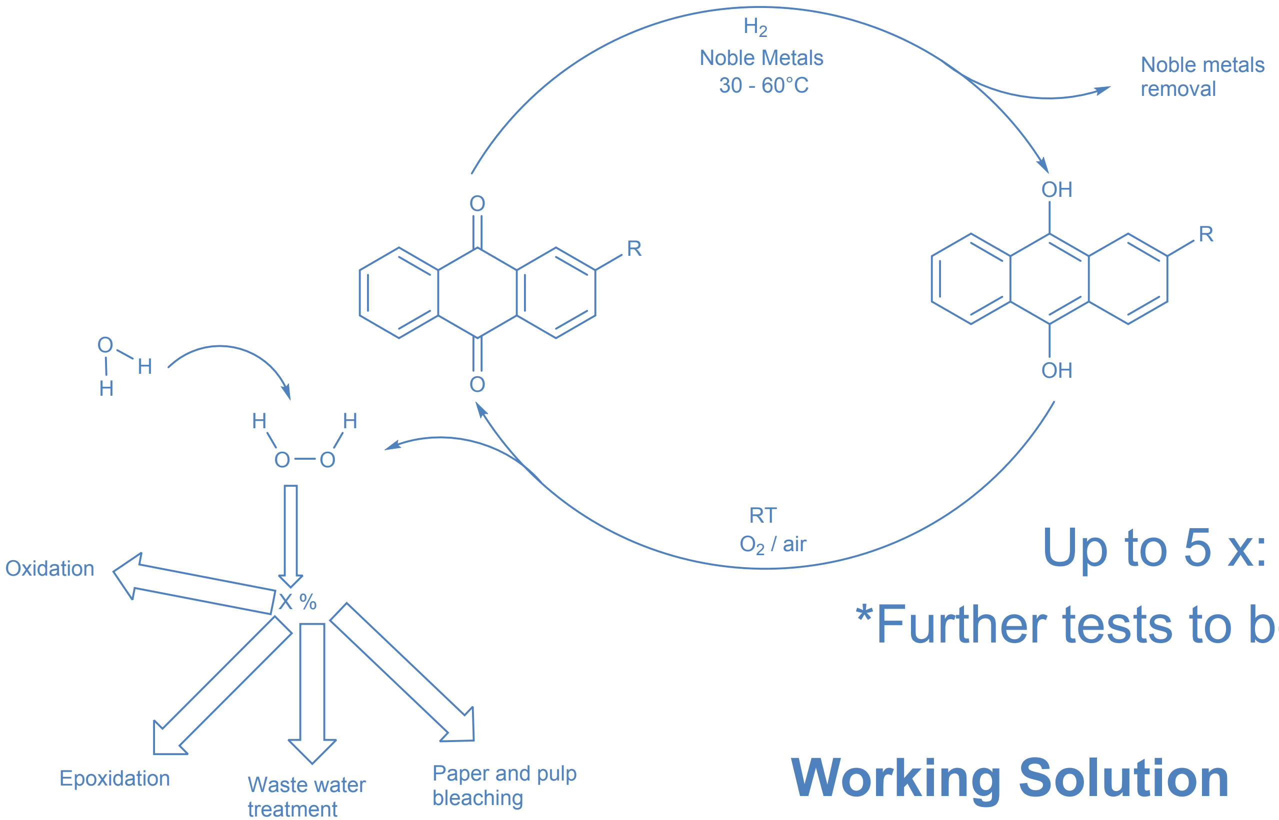
Oxidative Decarboxylation for long-chain Olefins





Zachos, S.K. Gaßmeyer, D. Bauer, V. Sieber, F. Hollmann, Robert Kourist, Photobiocatalytic decarboxylation for olefin synthesis (2014) *Chemical Communications*, DOI: 10.1039/C4CC07276F

Anthraquinone /Autooxidation Process



Pressure: 1 – 1.1 bar

Temperature: 45 - 60°C

H_2O_2: 92 – 100 %

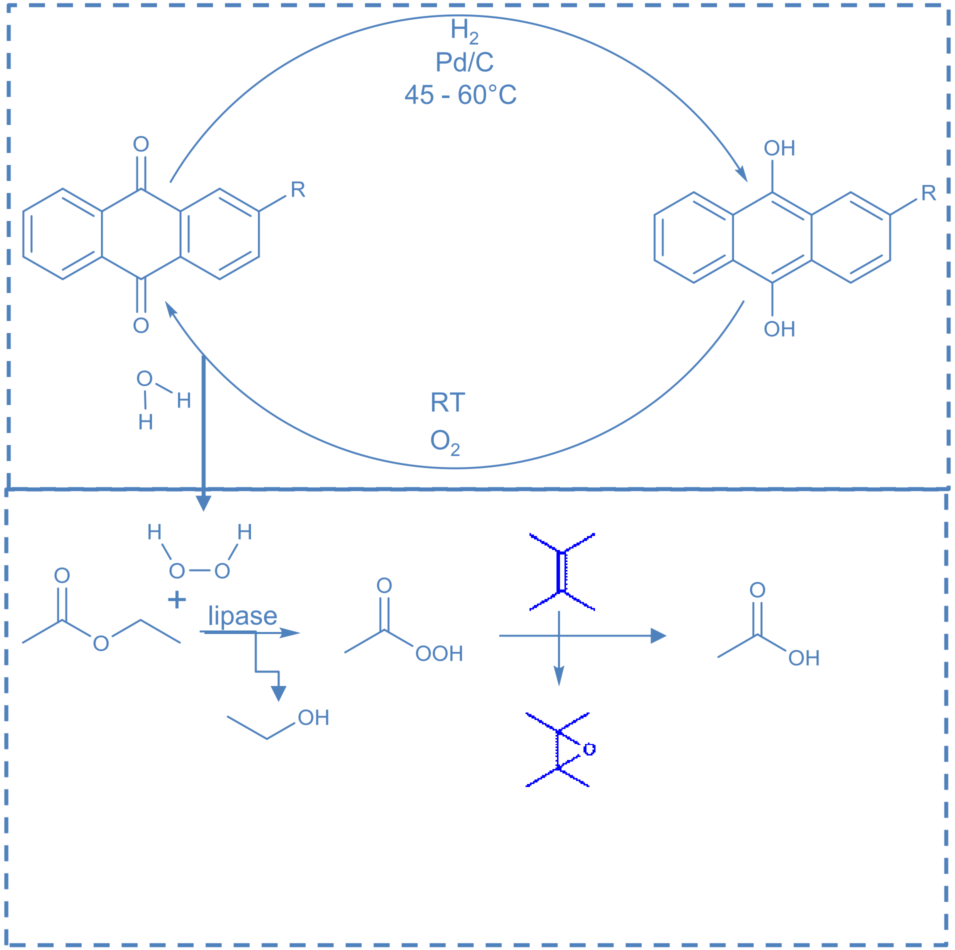
Up to 5 x: no catalyst inactivation*

*Further tests to be done with recyclability

Working Solution

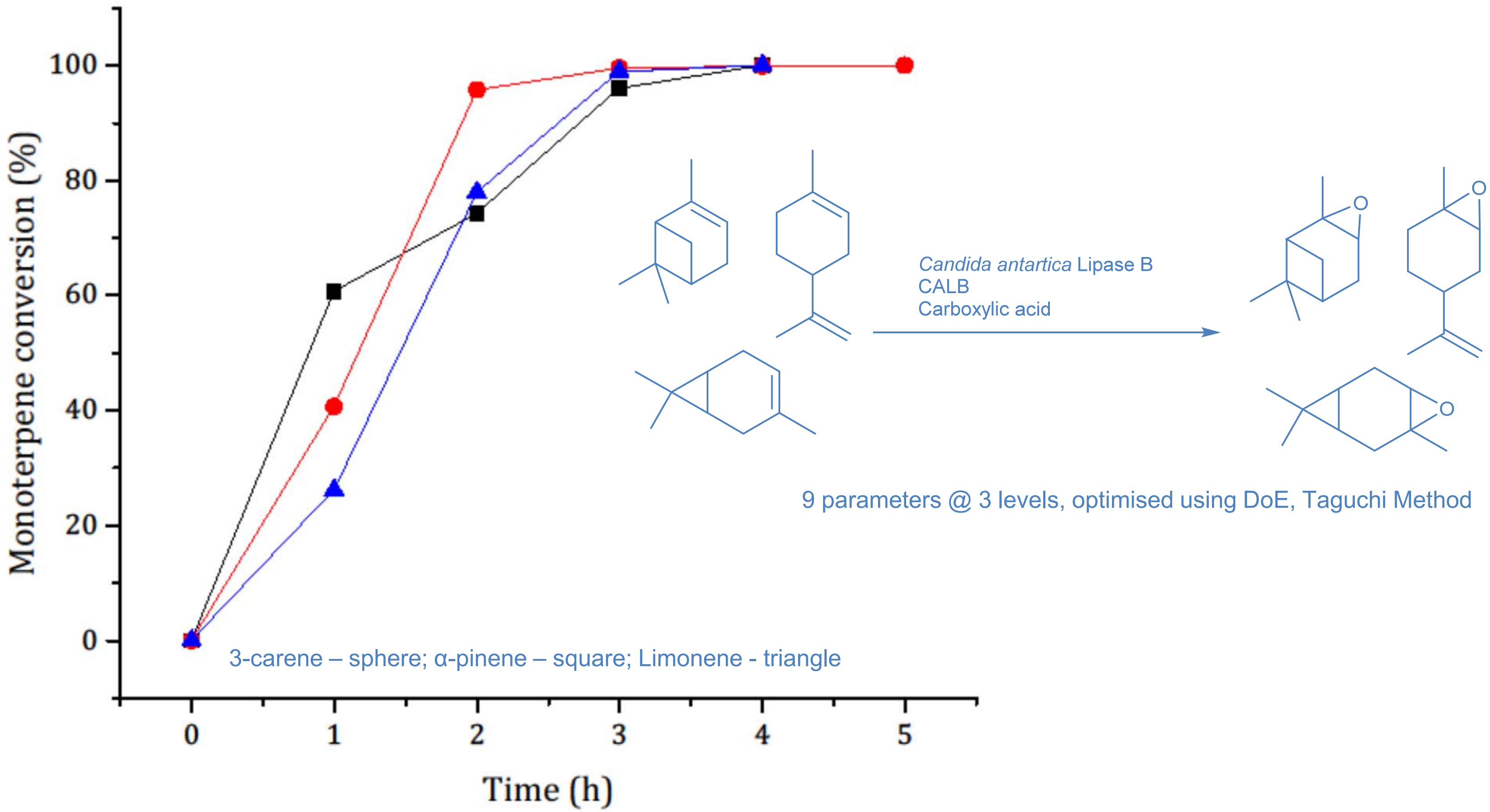
60% mesitylene & 40% tributyl phosphate

Combined Approach – 1.0



- Semi-batch process
- 60% toluene + 40% ethyl acetate
- Ethyl acetate hydrolysis to peroxyacetic acid and ethanol
- 75 – 85% epoxide yield
- Disadvantage
  – Only single run possible
  – Fresh solvent for every run
- Modified approach developed

Epoxidation of 3-carene, limonene and α-pinene



9 parameters @ 3 levels, optimised using DoE, Taguchi Method

Biosurfactants

Petroleum → **Process** → **Petro-based surfactants**

Synthesis: often high carbon footprint; products show limited biodegradation

Renewable resources

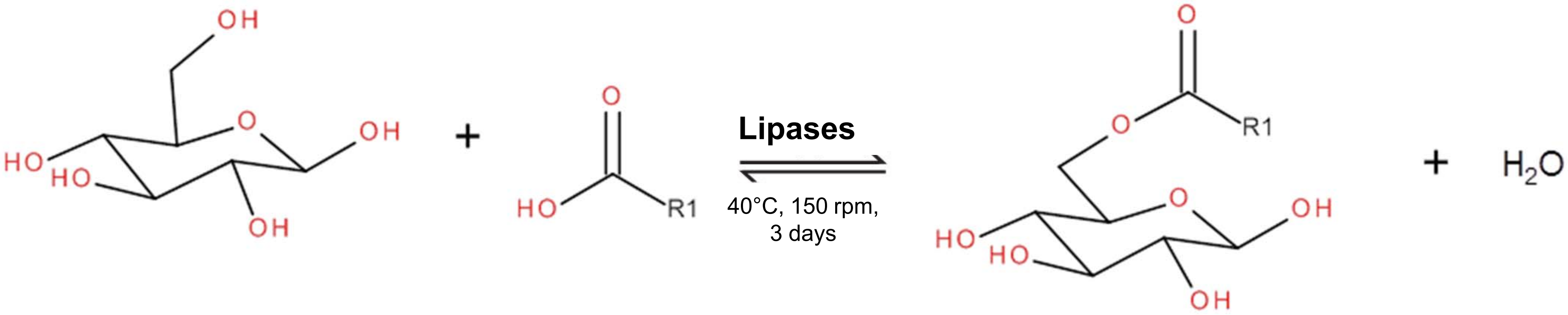
- Plant biomass
  - Terpenes
  - Sugars, fatty acids
- Animal biomass
  - Amino sugars

→ **Biocatalysis** →

Biosurfactants

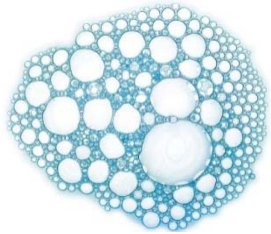
- Bio-based, biodegradable

Biosurfactants – Amphiphilic Sugar Esters



- **lipases (EC 3.1.1.3)** catalyze the formation esters in organic solvent
- esterification of primary and secondary alcohols possible
- waster has to be removed during course of reaction to prevent ester hydrolysis
- challenge: system contains hydrophilic, hydrophobic and amphiphilic components

Lubricants and Bio-based Surfactants from Renewable Resources



- Synthesis of new biosurfactants from vegetable oils, fatty acids, terpenes and sugars

- Characterization of novel surfactants via TLC, HPLC-MS, NMR and application tests

- Screening for enzyme/ substrate combinations
- Product analysis *via* TLC, HPLC-MS and NMR

→

- Optimization of reaction parameters

→

- Product isolation and purification

→

- Application tests

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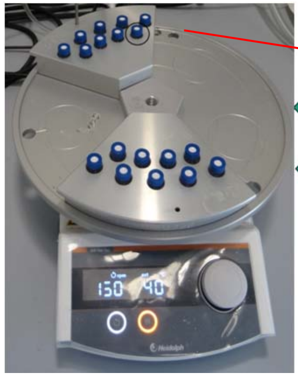
Enzymatic Synthesis of Surfactants - Procedure

- Screening of enzymes and substrates:



Thermal plate

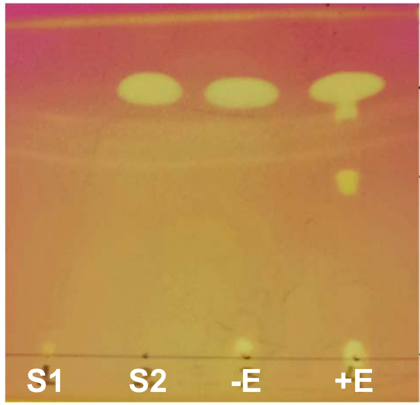
1 mL vials

Solution containing fatty acid and sugar

Molecular sieve and stirrer

Enzyme beads

TLC



Fatty acid

Product (Biosurfactant)

Sugar

Up-scaling to multi-gram scale:

Labmax

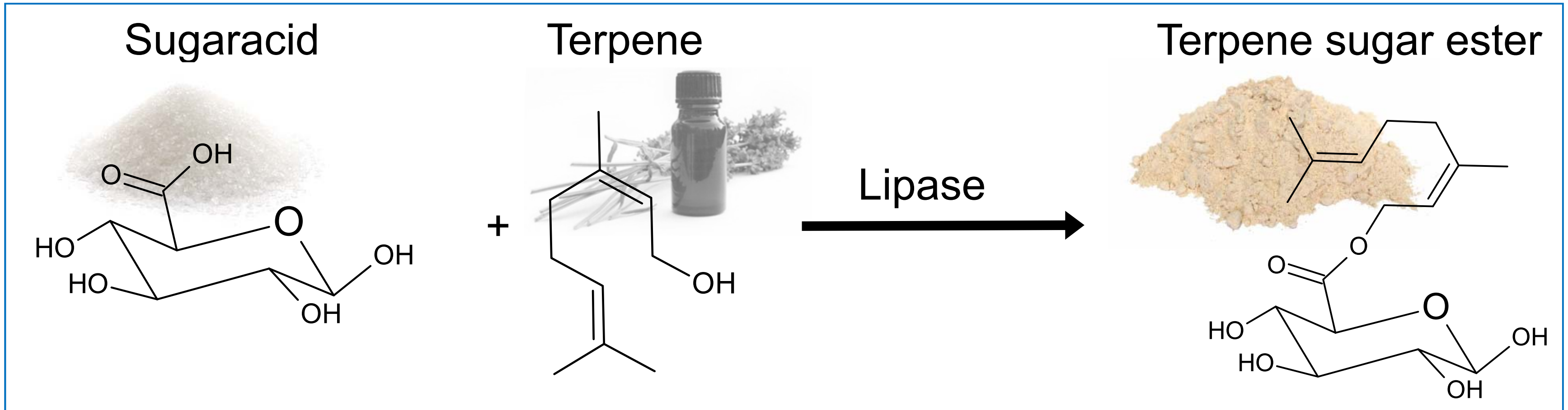
exclusive access to:

- bio-based building blocks
- enzymes
- process routes

Lubricants and Bio-based Surfactants from Renewable Resources

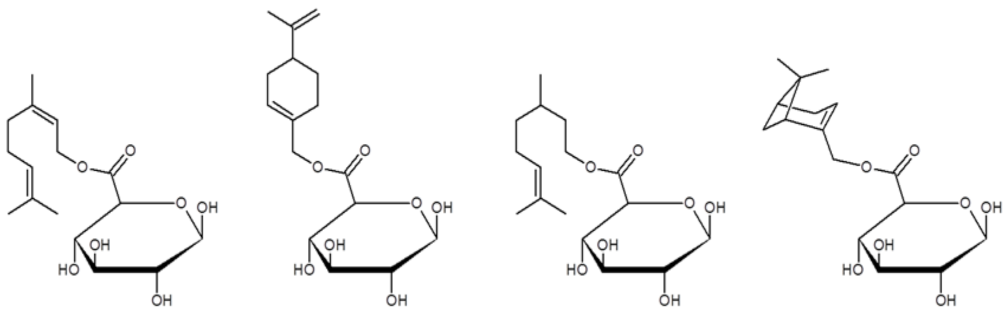
- Synthesis of new bio-surfactants from **terpenes** and sugars

– terpenes: underexploited waste stream
– suitable for lipase mediated epoxidation and follow up chemistry
– bioactivity



Lubricants and Bio-based Surfactants from Renewable Resources

- **terpene** sugar esters



1 2 3 4

Conversion of terpenols with glucuronic acid:
CalB (Novozymes 435)
40 mM equimolar substrate in *t*-butanol, 60 °C for 48 h

Terpenoid alcohol	Alcohol type	Product formation
Nerol (1)	P	+++
Geraniol	P	+++
Myrtenol (4)	P	++
Perillyl alcohol (2)	P	+++
Citronellol (3)	P	+++
Carveol	S	-
Verbenol	S	-
Linalool	S	-
Isoborneol	S	-
Terpineol	T	-

Product formation monitored by TLC
P: Primary alcohol;
S: secondary alcohol;
T: tertiary alcohol.

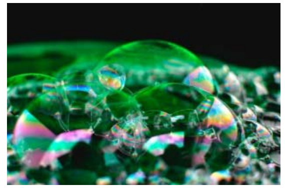
J. G. Ortiz-Tena et al., in prep.

www.biosurf.fraunhofer.de

Project acronym: BioSurf.
Project no: EIB.10.039

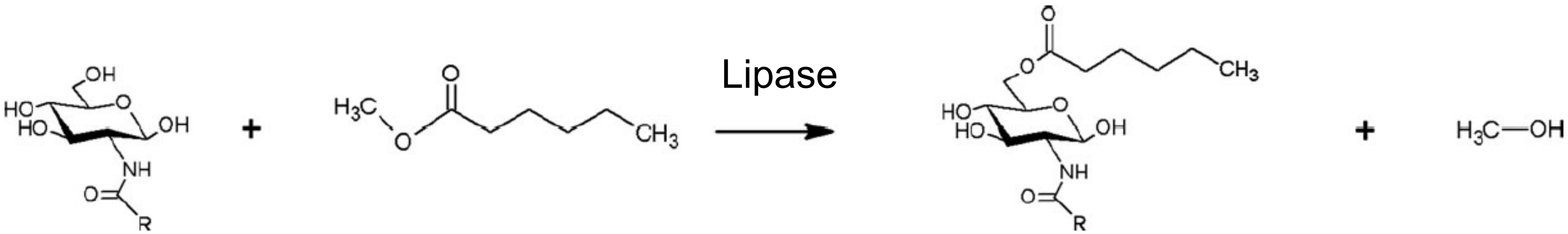
Lubricants and Bio-based Surfactants from Renewable Resources

- Synthesis of new bio-surfactants from **chitin** derived precursors



Crustacean shell waste from Europe, Asia and Africa

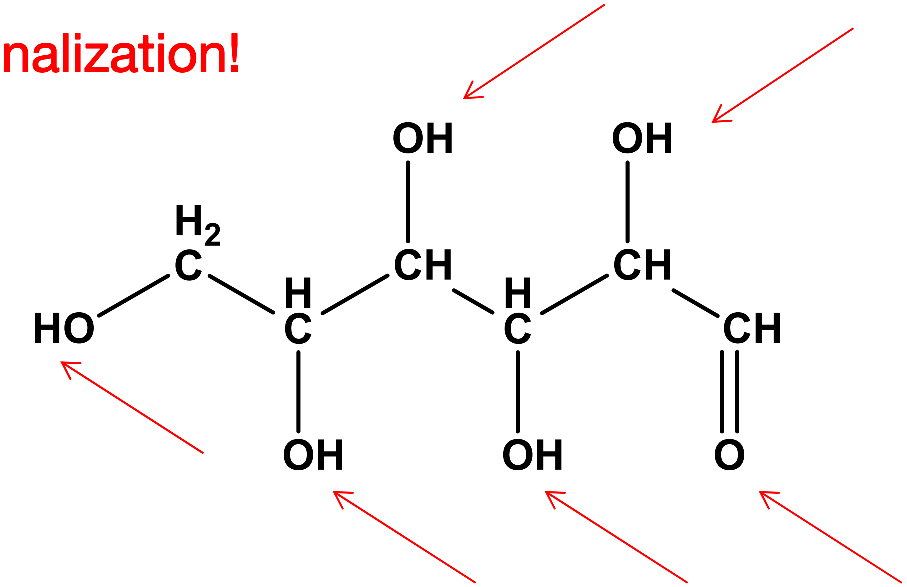
→ Chitin monomers



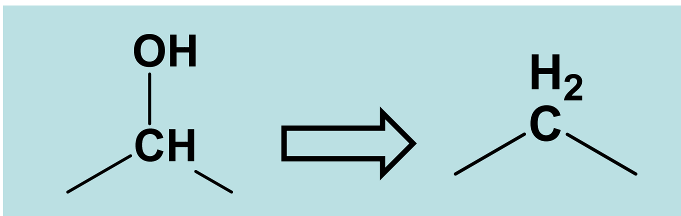
Pöhnlein, M., Slomka, C., Kukharenko, O., Gärtner, T., Wiemann, L.O., Syldatk, C., Hausmann, R.
Eur. J. Lipid Sci. Technol. **2014**, 116, 423-428.
ERA-IB BioSurf project (617 40003 0315928B).

Carbohydrates as substrates for chemistry

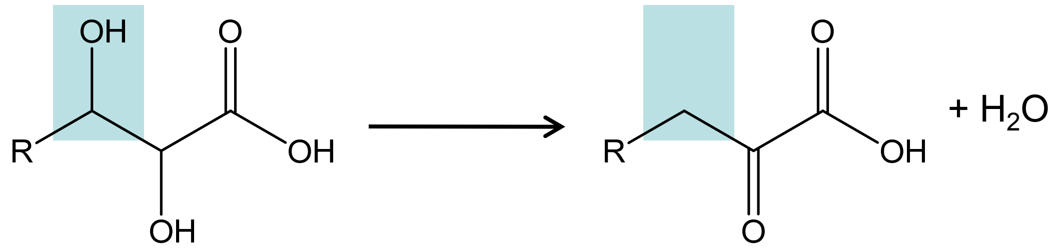
Problem of over-functionalization!



The task is defunctionalization!

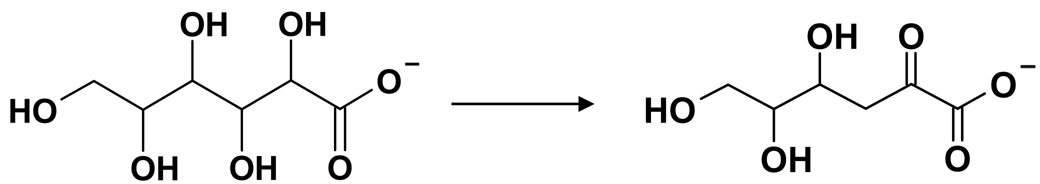


One way for dehydration: Dihydroxy Acid Dehydratase



- α–β-dehydratase (EC 4.2.1.x)
- dehydratation of 2,3-dihydroxy-isovaleric acid to α-ketoisovaleric acid
- Iron-Sulfur Cluster Enzymes

DHAD from Sulfolobus solfataricus (pH 2-3, T of 75-80 °C)



Gluconate

2-keto-3-deoxygluconate

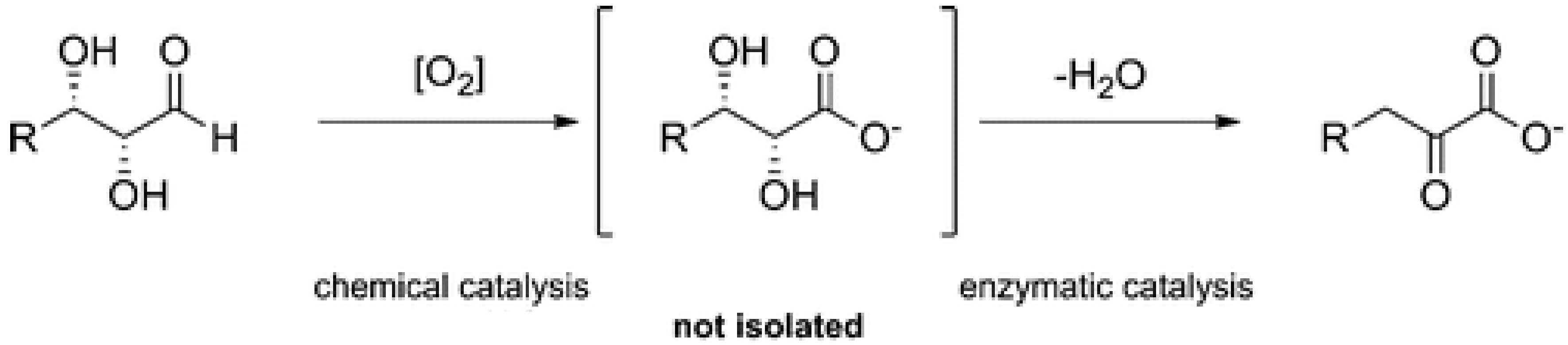
pH_{opt} of 6.2
T_{opt} = 80 °C
Activity ca. 5 u/mg
TTN: ca. 35000

Carsten et al. 2015 *J. Biotech.*

Production of β-desoxy-α-keto acids from carbohydrates

=> Represent a first partially defunctionalized intermediate towards biobased building blocks such as adipic acid, muconic acid, succinic acid, or 1,4 butanediol
=> Building block for organic synthesis because a vicinal electrophilic and nucleophilic carbon pair is obtained from chemically indifferent hydroxymethylene groups
=> Especially for carbohydrates: a generic aldol building block is formed

Combination of Heterogeneous Catalysis and Enzyme Catalysis



Sperl et al., ACS Catal., 2016, 6, pp 6329–6334

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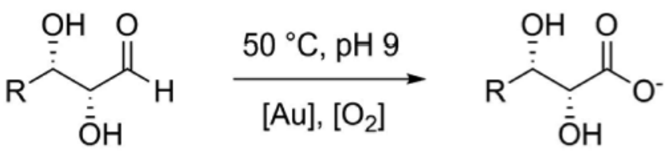

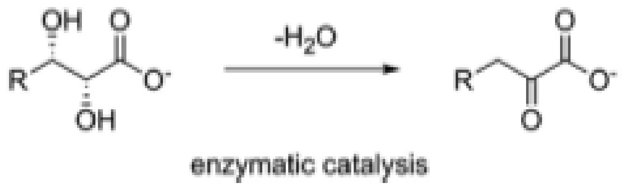

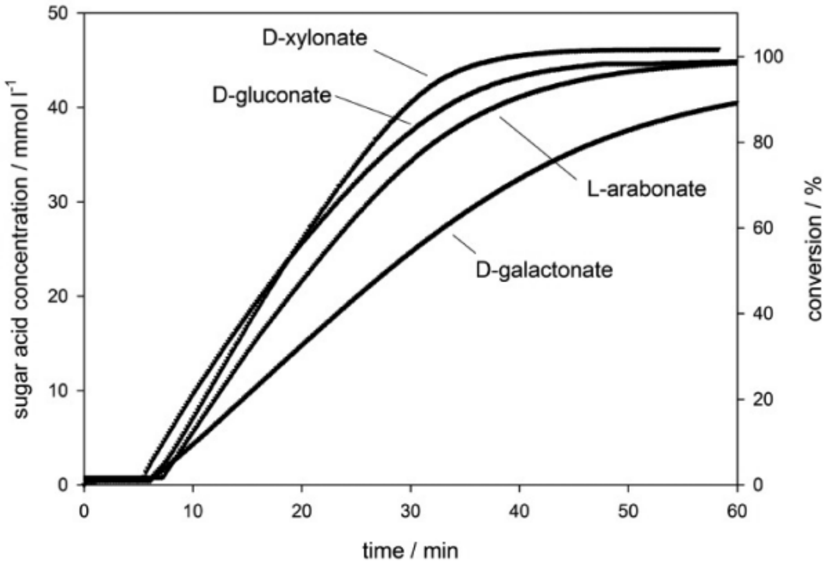

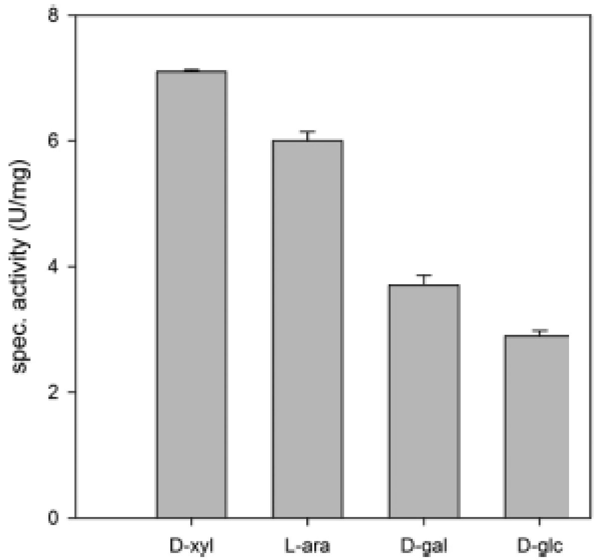

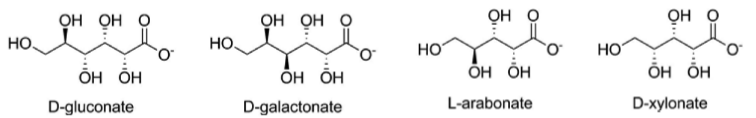

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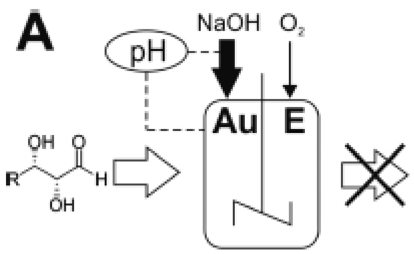
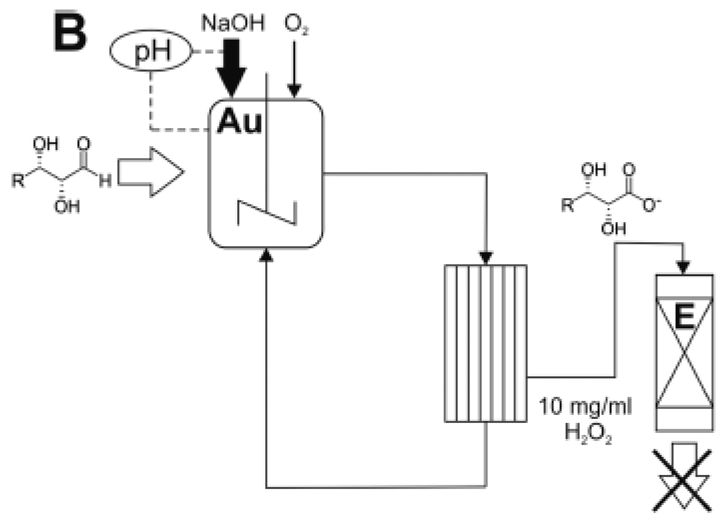
Reaction Design for the Compartmented Combination of Heterogeneous and Enzyme Catalysis



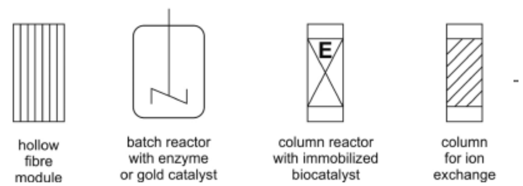


One pot

- Compromise for conditions
- Mutual inactivation
- Mechanical barriers (alginate capsules, „Tea bag“ etc.) reduced inactivation but also activity

Separation

- Continuous-flow reactor system
- Cross-flow filtration for catalyst separation



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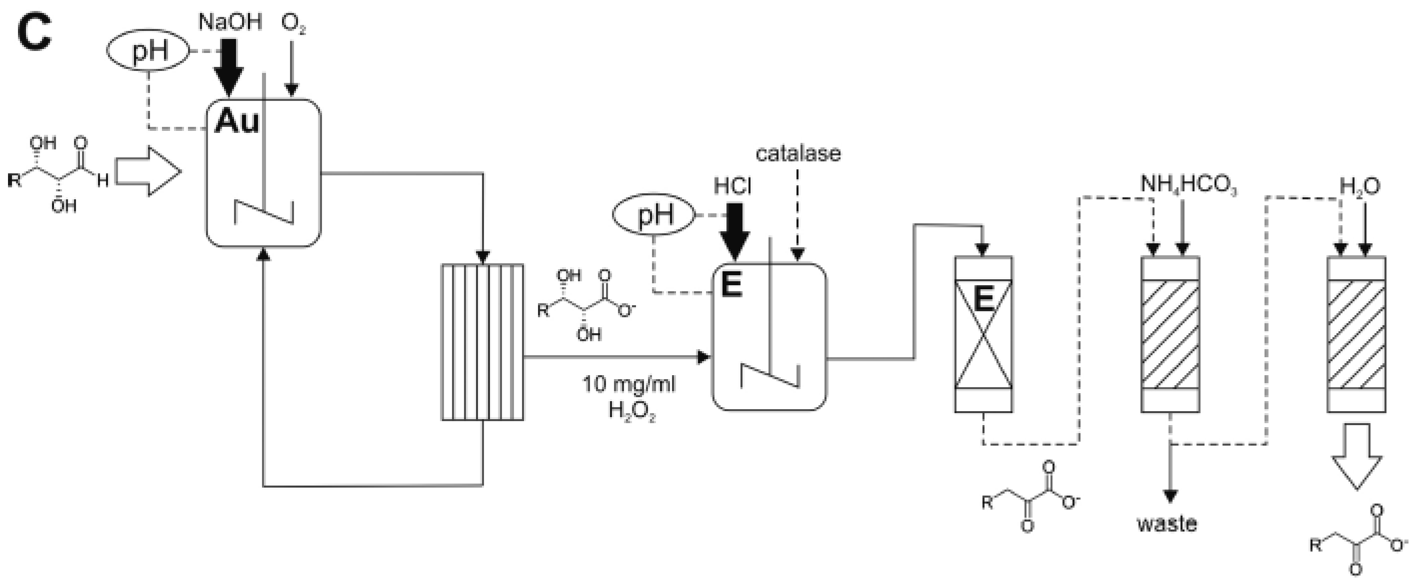

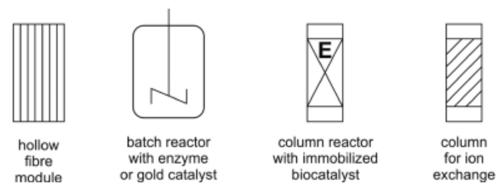

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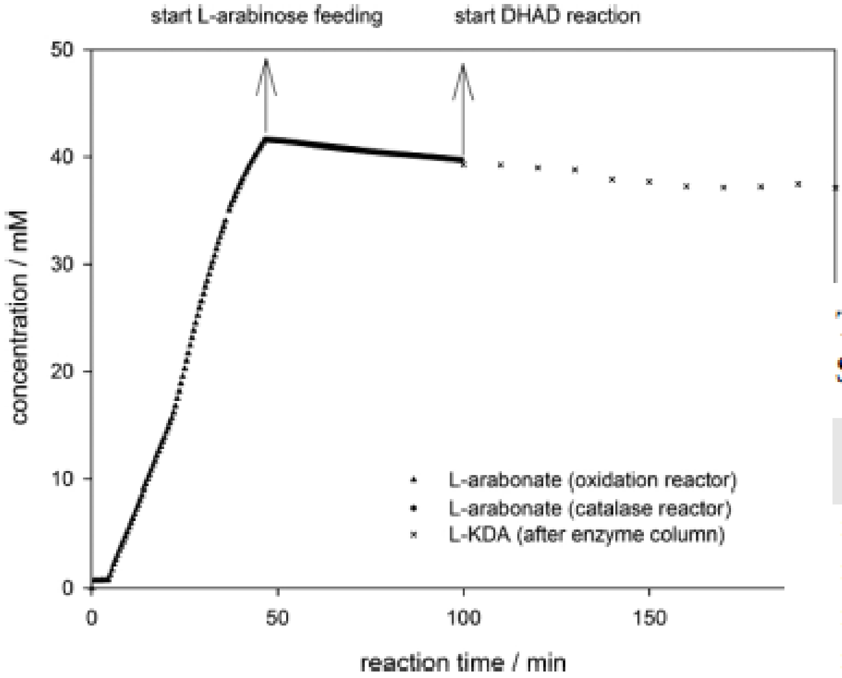

Table 1. Continuous Flow Transformation of Different Sugars to KDS

substrate	flow rate (ml min^{-1})	av concn (mM)	yield b.p. (%)[a]	yield a.p. (%)[a]
L-arabinose	1.0	38	84	58
D-xylose	1.0	31	69	n.d.[a]
D-galactose	0.5	39	87	n.d.[a]
D-glucose	0.3	41	91	86

[a] D-KDX and D-KDGal showed degradation during the ion exchange purification step. b.p. before purification, a.p. after purification.

⇒ **First step towards adipic acid, muconic acid and others**
⇒ **Increase of substrate concentration with optimized enzyme**

Sperl et al., *ACS Catal.*, 2016, 6, pp 6329–6334

Summary

In-situ peroxide production

- Semi-continuous system for high concentration of H_2O_2
- Application for Epoxide production

Bio-surfactant

- Toolbox for biocatalytic synthesis of ionic and non-ionic surfactants from biomass
- New building blocks from terpenes as hydrophobic moeity
- Development of efficient DSP strategies of the amphiphilic products

Carbohydrate based building block

- First step towards adipic acid, muconic acid and others
- Enzyme variants with higher activity and stability are at hand