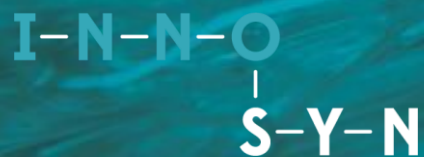


Biocatalytic Oxidations Using Oxygen From Lab to Pilot Plant

InnoSyn Webinar
21 September 2026

Dr. Martin Schürmann

Principal Scientist Biocatalysis
www.innosyn.com



Tomorrow's chemistry. Today.



InnoSyn

- An **independent process R&D service company & CMO** since 2017 (Brightlands Campus Geleen, The Netherlands)
- InnoSyn offers a team of **±75 passionate & experienced problem solvers**, applying innovative and cost-efficient technologies at any scale: **technology agnostic**
- InnoSyn has **over 30 years of experience** as a chemical process R&D group
- **Track record** of successful development and implementation of new and improved processes to chemicals based on a **strong technology base**



Technologies @ InnoSyn

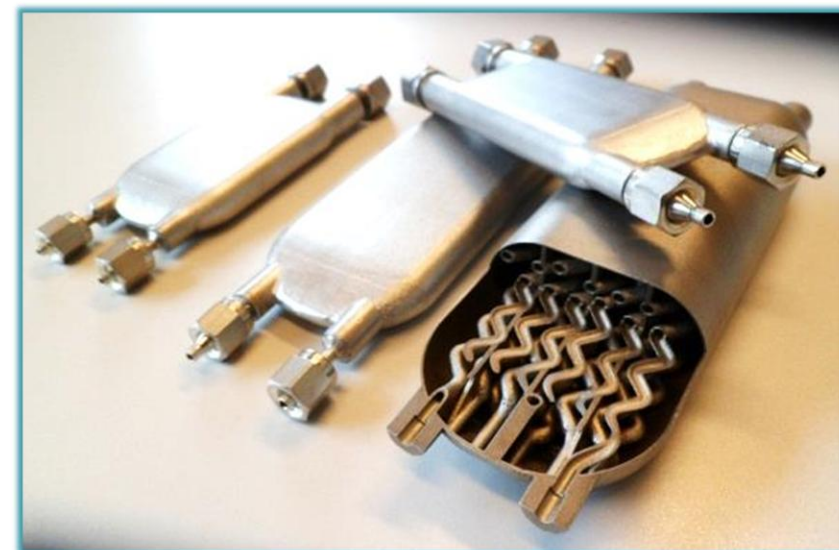
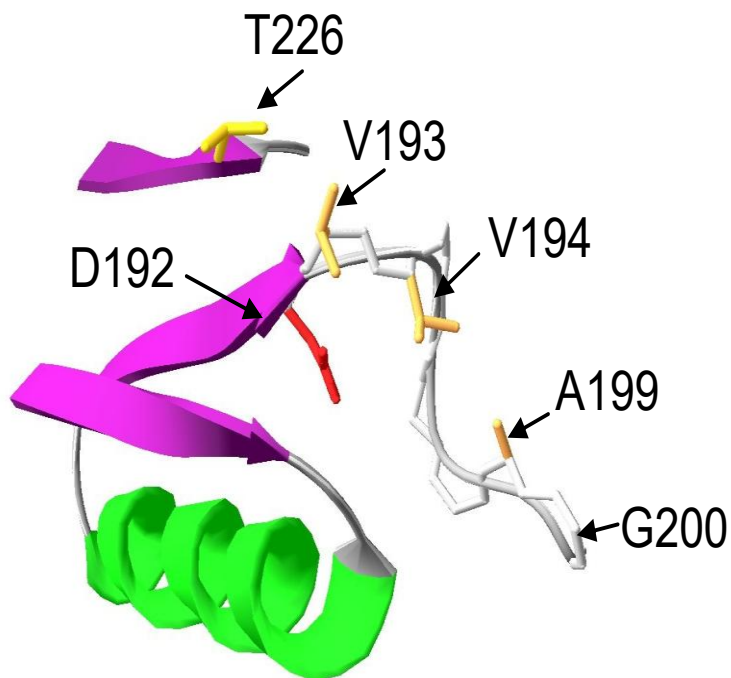
Biocatalysis

Chemocatalysis

Flow Chemistry

Photochemistry

Crystallization

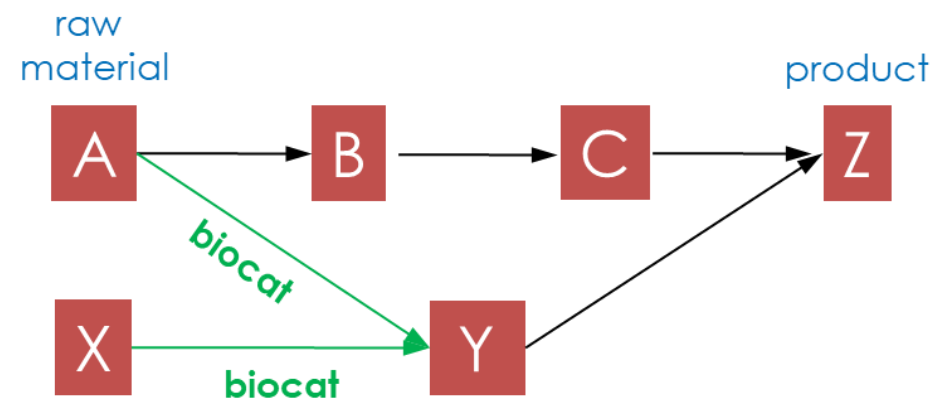


Why Biocatalysis ?

Biocatalysis is the use of **enzymes** as they are provided by nature and improved in the laboratory as catalysts **for organic chemistry**

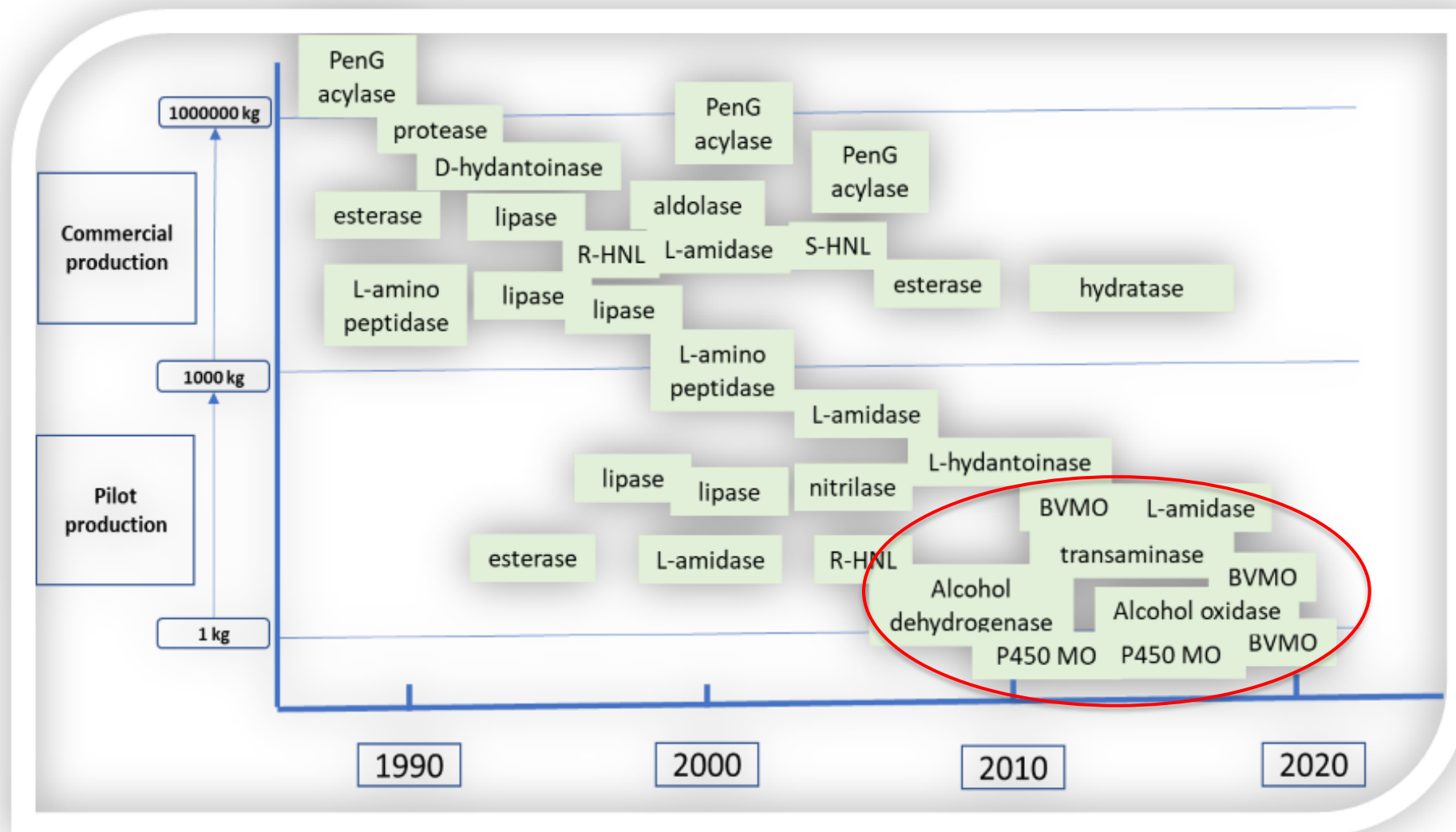
Enzymes are valuable catalysts for organic synthesis:

- generally **chemo-, regio- and often enantioselective** (quality)
- generally **mild reaction conditions: ambient temperature, pH and pressure** (process costs)
- **green chemistry**: reduced environmental burden (e.g. less organic and inorganic waste streams)
- enable **shorting of synthesis** routes
- chemical **process steps reduction**



Implementing Industrial Biocatalytic Processes

processes co-developed & implemented by InnoSyn experts



Biocatalytic Oxidations with O₂ – Concerns

- inefficient
 - low reaction rates
 - low yields
- not scalable
 - low enzyme stabilities
 - oxidative enzymes are difficult to produce
- unsafe
 - explosive mixtures of organic solvents and O₂
 - explosive mixtures of high substrates/products and O₂



Safety Aspects Working with Pure O₂

Oxygen specific safety aspects during scale-up

- operate below limiting oxygen concentration (LOC)
- keep O₂ in reactor head space below 8.6% (v/v)
- constantly measure O₂
(dissolved and in head space)
- if needed, switch off oxygen supply
- if needed, increase nitrogen sweep

solvent	T (°C)	oxygen concentration with <5% probability of ignition (vol %) ^a		
		1 bara	10 bara	20 bara
acetic acid	200	10.3	—	9.6
NMP	200	8.2 ^b	—	7.2
DMSO	200	3.9	—	—
DMSO	100	6.3	—	—
<i>tert</i> -amyl alcohol	100	9.7 ^b	—	10.0
ethyl acetate	100	9.3	—	9.8
2-MeTHF	100	9.3	—	9.1
methanol	100	7.5	—	6.9
acetonitrile	100	—	12.0	11.8
toluene	100	10.1	10.3	9.8

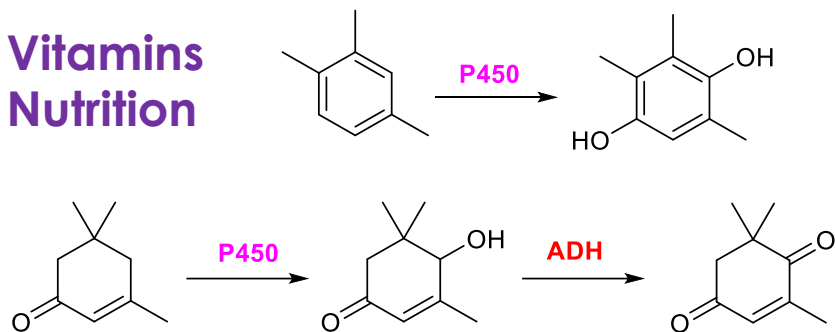


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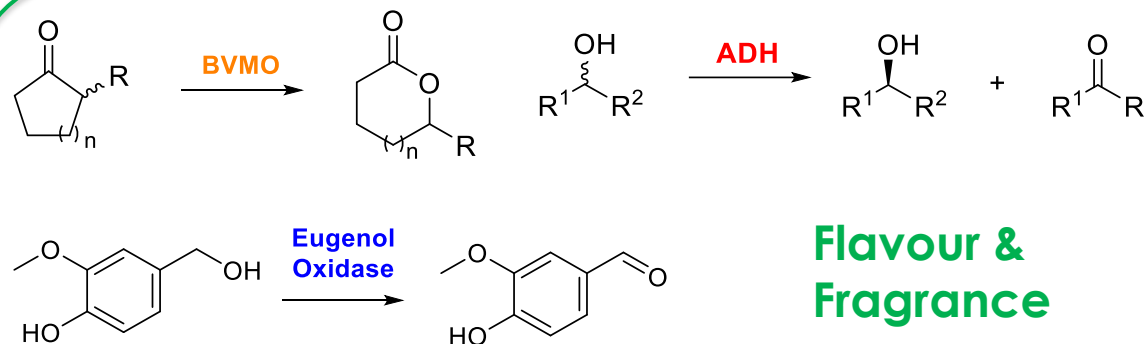
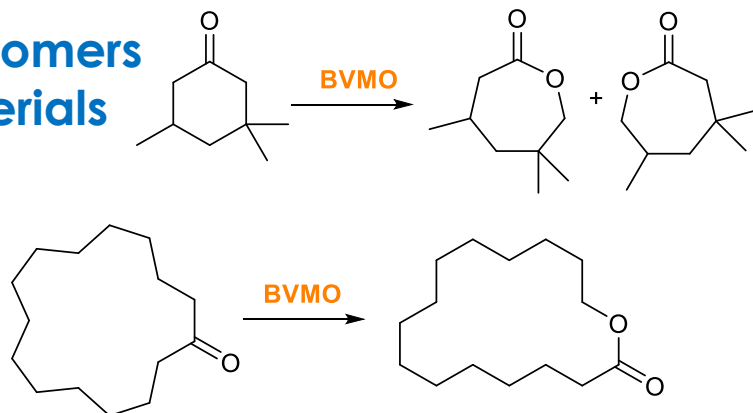
Oxidative Biocatalysis Demonstration Cases

demonstrated on 100 L pilot plant scale

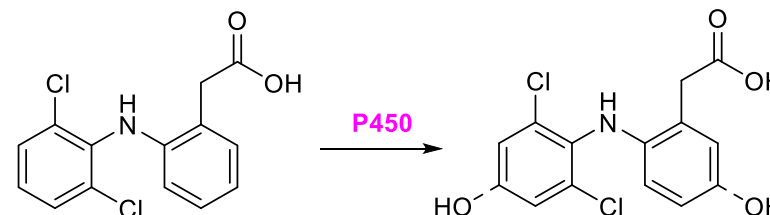
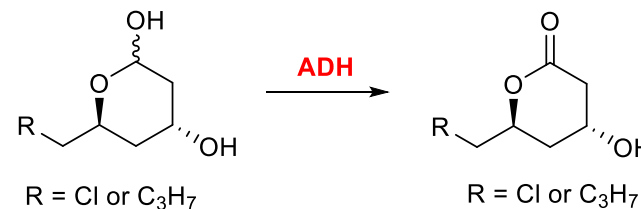
Vitamins Nutrition



Monomers Materials



Flavour & Fragrance

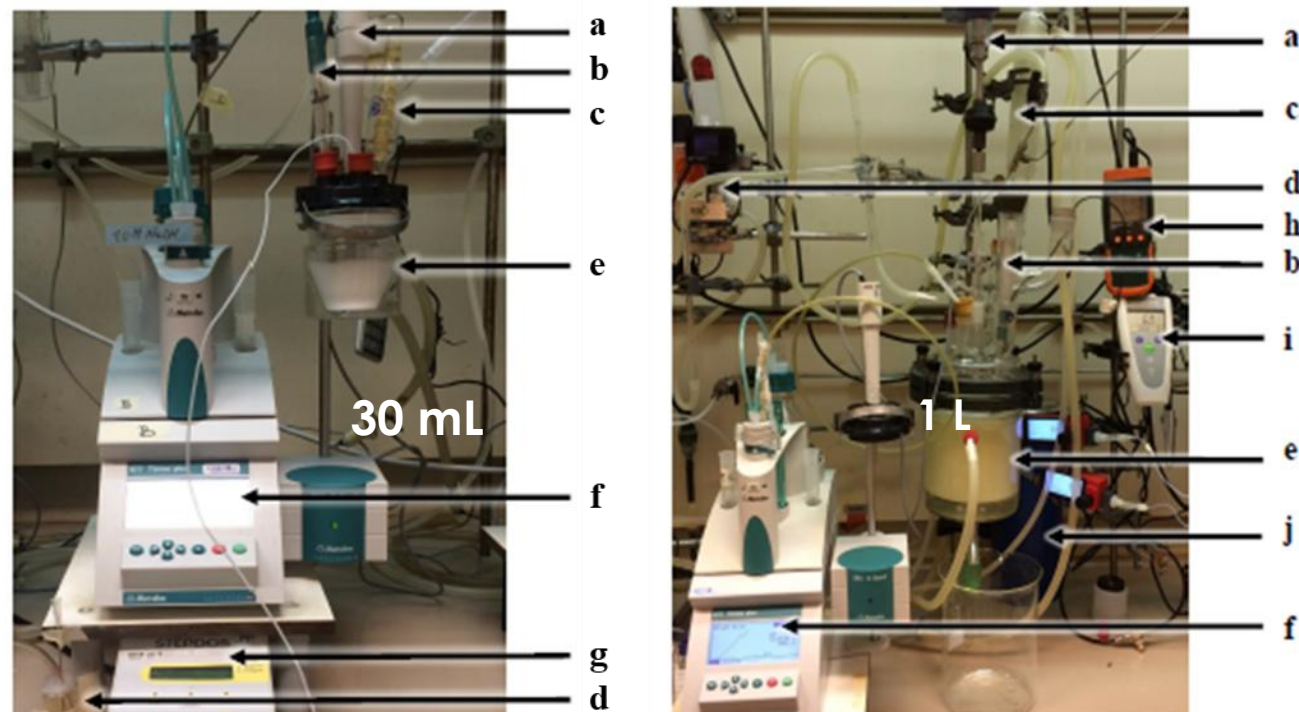


Pharma Metabolites

Biocatalysis Reaction Optimisation @ InnoSyn

e.g. for biooxidation reactions

- InnoSyn reactor setups from 30 mL to 1 L
- Reaction characterization
- Reaction optimization
 - pH, temperature,
 - solvents, emulsifiers
 - Mixing, substrate dosing
 - Biocatalyst loading
 - Biocatalyst formulation
 - Gas/oxygen supply: air or pure O₂



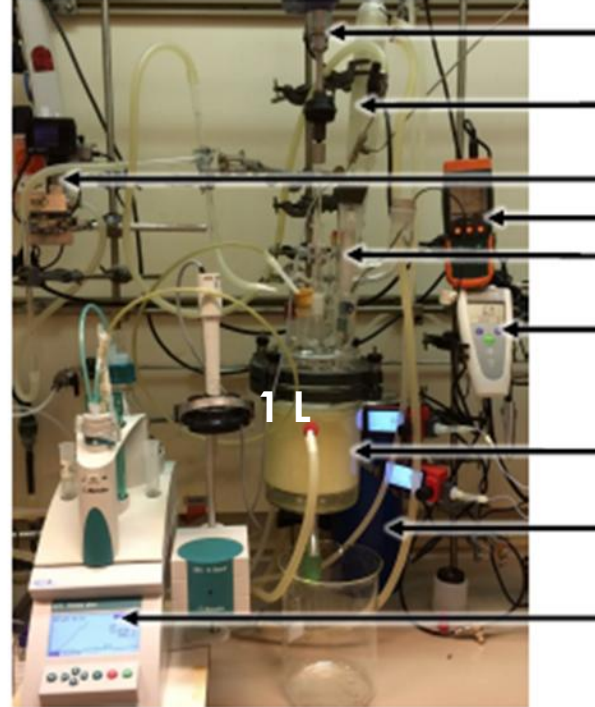
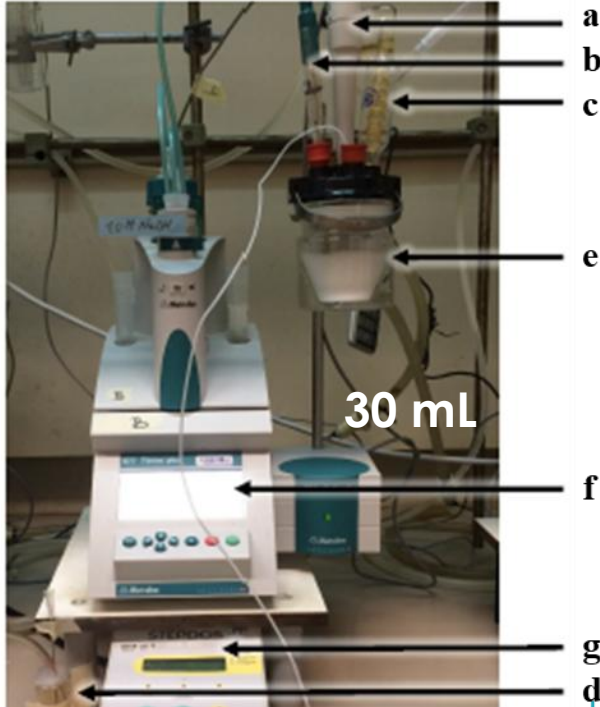
Applied reactor setup 30 mL / 1 L scale a) stirrer, b) pH electrode, c) reflux-condenser, d) controlled air supply, e) reactor, f) automatic titration device connected to pH electrode, g) air flow controller, h) oxygen sensor in gas outlet, i) oxygen sensor in reaction mixture, j) cooling trap.



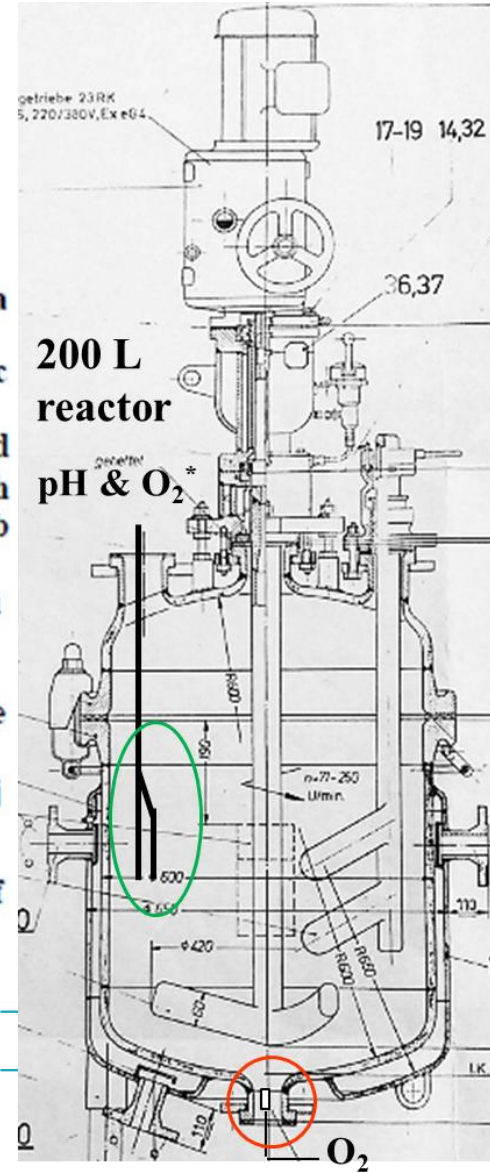
Biocatalysis Reaction Optimisation @ InnoSyn

e.g. for biooxidation reactions

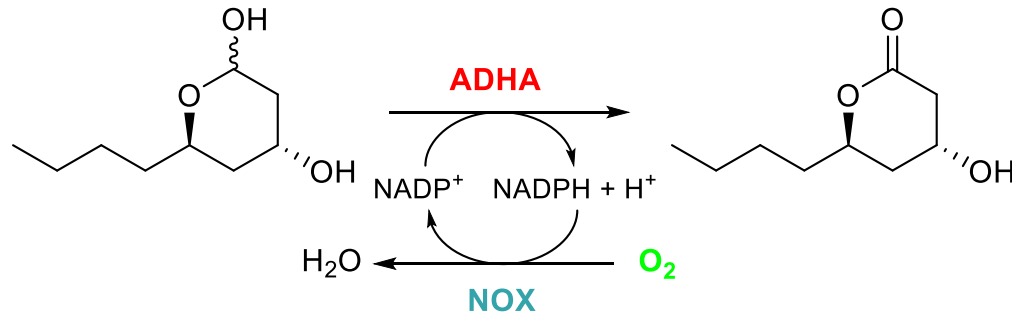
- Reactor setups at InnoSyn from 30 mL to 1 L scale
- Reaction characterization & parameter optimization
 - pH, temperature, solvents, emulsifiers, mixing etc.
 - Biocatalyst loading and formulation & oxygen supply



Applied reactor setup 30 mL / 1 L scale a) stirrer, b) pH electrode, c) reflux-condenser, d) controlled air supply, e) reactor, f) automatic titration device connected to pH electrode, g) air flow controller, h) oxygen sensor in gas outlet, i) oxygen sensor in reaction mixture, j) cooling trap.



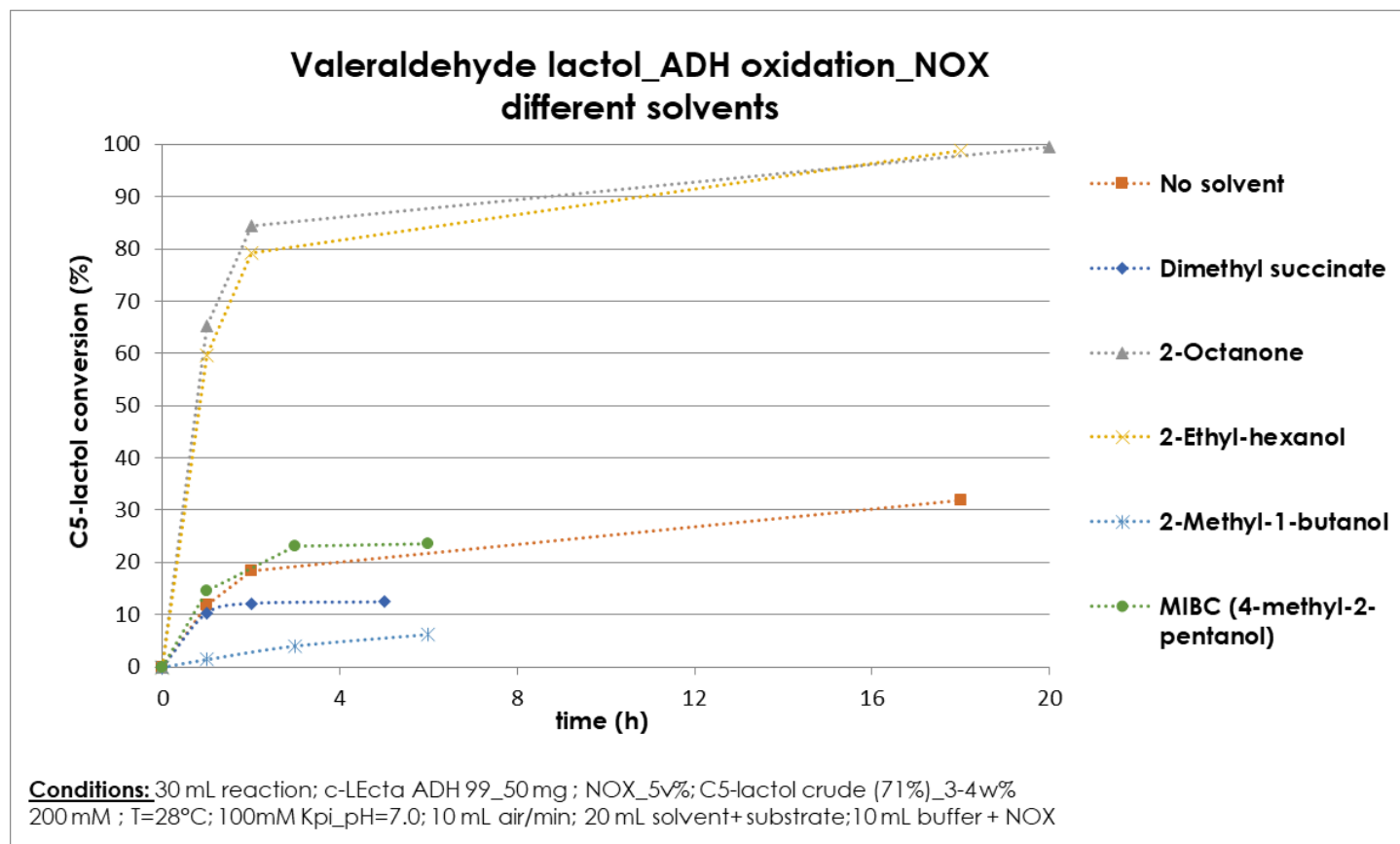
Lactol Oxidation with ADH and NAD(P)H Oxidase (NOX)



- Highly active and stable **ADHA**
- Highly active and stable **NOX** (storage stability)
- Low operational stability of **NOX** at substrate solubility limit (> 35 g/l)
- Potential **NOX** operational stability improvements:
 - Enzyme engineering of (alternative) NOX enzymes
 - Water-miscible co-solvents to solubilize lactol substrate → 1 phase **✗**
 - Immiscible organic solvents to protect NOX from substrate → bi-phasic **✓**



Solvent effect on NOX stability and productivity

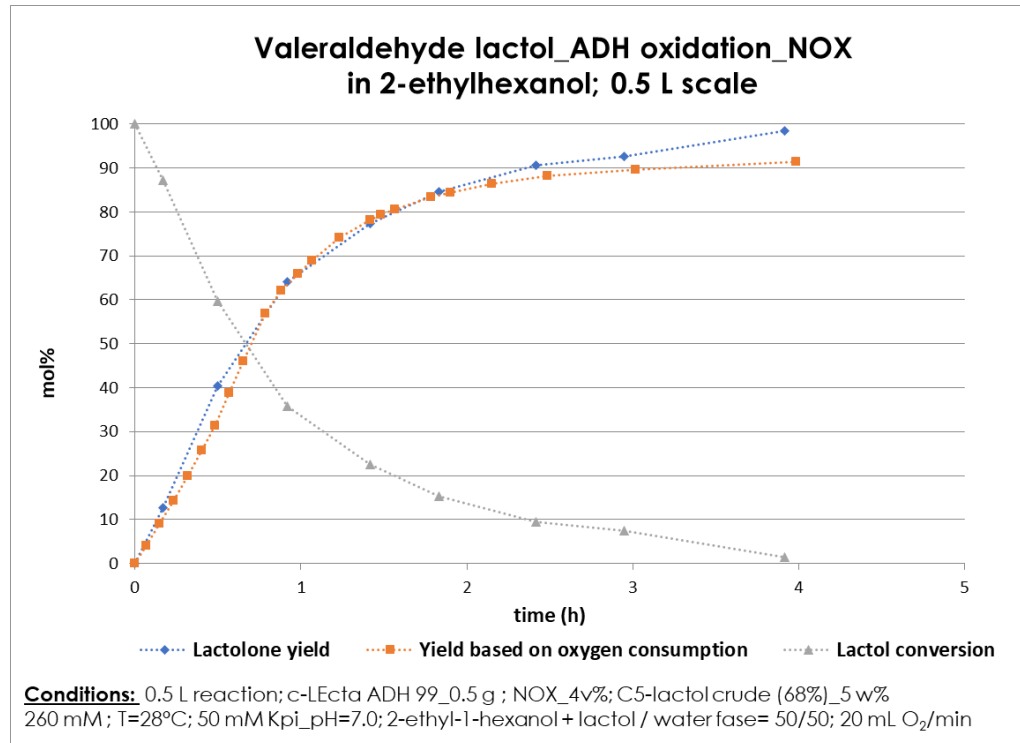


Using 2-octanone and 2-ethyl-hexanol:

- Strong increase in activity, conversion and yield
- Relatively polar solvents improved stability
- Dissolving the polar lactol substrate in second phase
- Suitable water/organic ratios range from 1:2 to 2:1

Scale-up to 1 L scale

5% (w/w) lactol in 2-ethyl-hexanol/buffer (50/50)



- 1 g/l ADHA (powder formulation)
- 4% (v/v) liquid NOX formulation
- Crude lactol from DERA aldolase reaction
- Oxygen supply using pure O₂
- product formation fits oxygen consumption almost 100%
- 50 g/l product reached
- almost quantitative assay yield

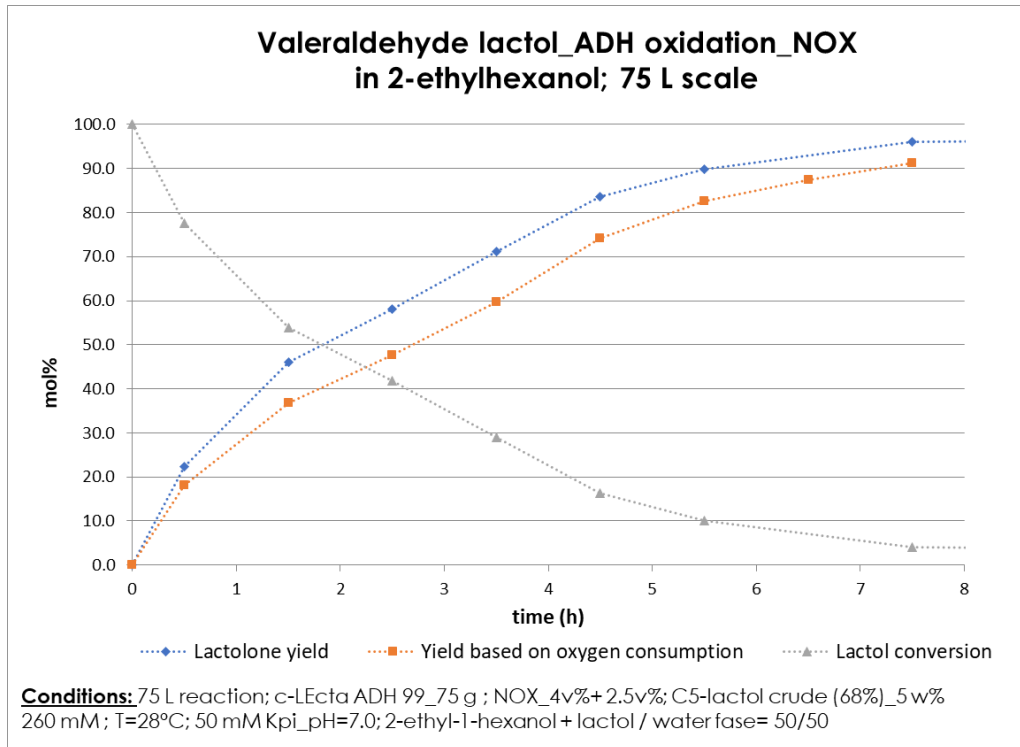


Ready for pilot plant demonstration



Pilot plant Demonstration on 75 L scale

5% (w/w) lactol in 2-ethyl-hexanol/buffer (50/50)



- 75 L reaction in 200 L reactor
- 1 g/l ADHA (powder formulation)
- 4% (v/v) liquid NOX formulation
- Crude lactol from DERA reaction
- Oxygen supply using pure O₂
- Somewhat delayed oxygen consumption
- ~50 g/l product reached
- > 95% assay yield
- > 3.5 kg lactone produced (non-isolated)
- STY: 6.3 g L⁻¹ h⁻¹

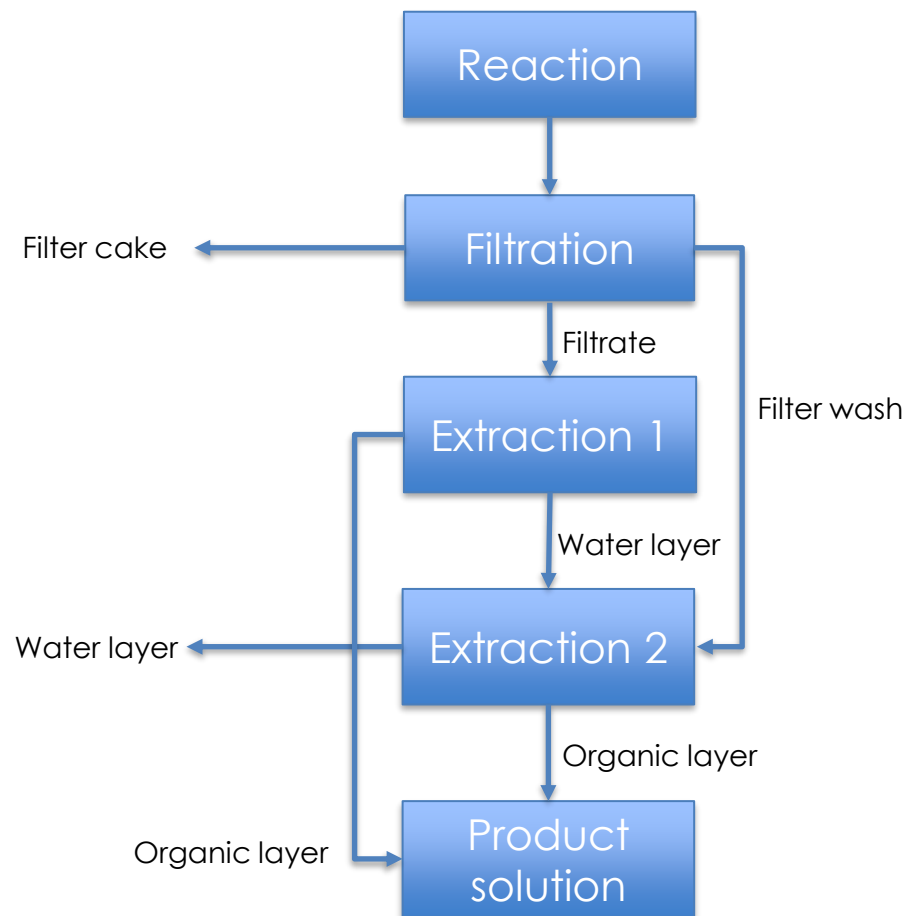


Successful pilot plant demonstration of ADH + NOX oxidation technology



Demonstration C5 lactone on 75 L scale

Work up and product isolation



Process Metrics ADHA and NOX-01

	Conversion [%]	Yield [%]	Product conc. [g L ⁻¹]	STY [g L ⁻¹ h ⁻¹]	ADHA yield [g _{prod} /g _{cww}]	NOX-01 yield [g _{prod} /g _{cww}]
0.5 L	99	99	49	12.3	4.1	3.9
75 L	96	95	47	6.3	2.9	2.0

- High product yields
- High space-time yields
- Efficiencies of ADH and NOX in similar range
- 3.5 kg of lactone product synthesized
- Technology is ready for replication in industrial environments

CWW = cell wet weight equivalents



Process Metrics ADHA and NOX-01

	Conversion [%]	Yield [%]	Product conc. [g L ⁻¹]	STY [g L ⁻¹ h ⁻¹]	ADHA yield [g _{prod} /g _{cww}]	NOX-01 yield [g _{prod} /g _{cww}]
0.5 L	99	99	49	12.3	4.1	3.9
75 L	96	95	47	6.3	2.9	2.0



Raw material
(variable) costs



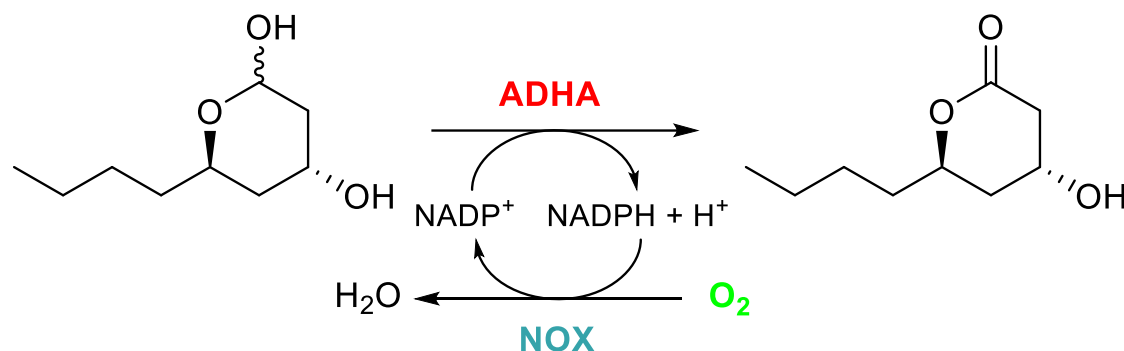
Efficient use of
installations
(fixed costs)



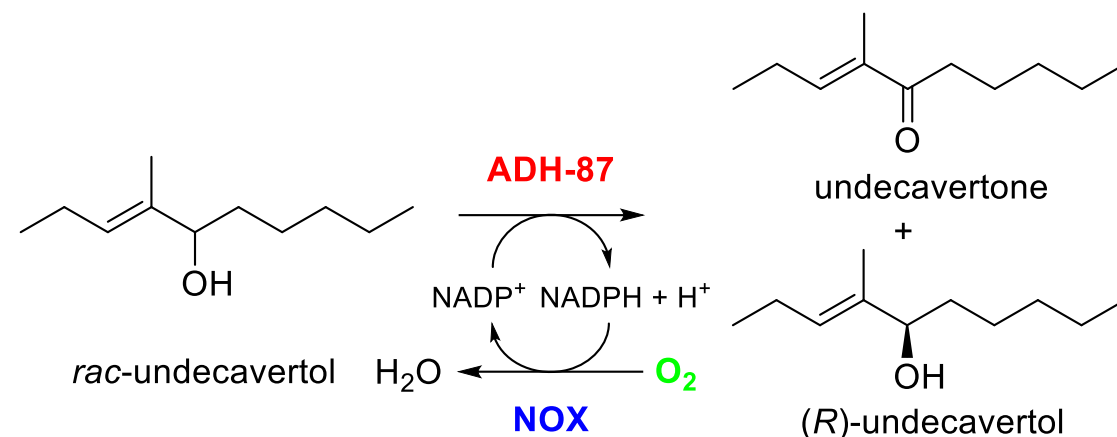
Biocatalyst cost
price contribution
(variable costs)



Lactol & Alcohol Oxidation by ADH/NOX system



Metric	Value
Product concentration	47 g L ⁻¹
Product yield	95%
Reaction volume	75 L
Volumetric productivity	6.3 g L ⁻¹ h ⁻¹
Product isolated	3.5 kg

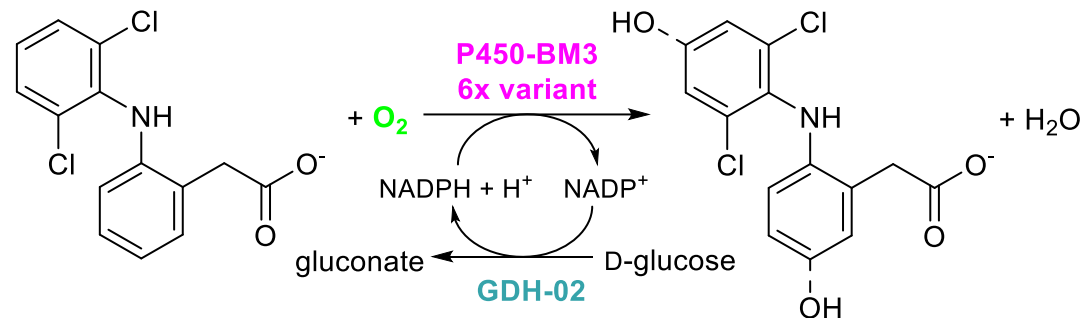
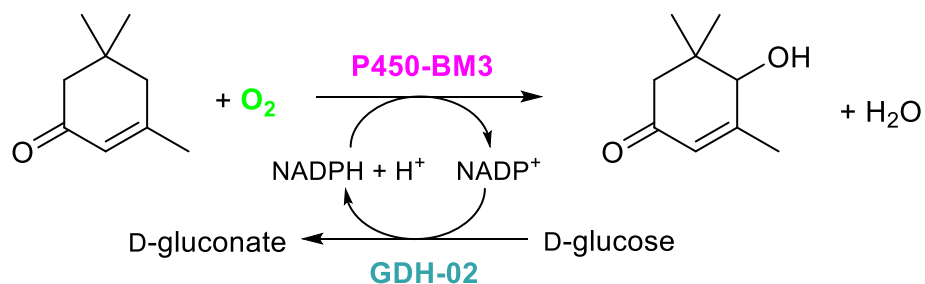


Metric	Value
Product concentration	210-224 g L ⁻¹
Product yield	49% (99% theor. max. yield)
Reaction volume	3 x 125 L
Volumetric productivity	14 g L ⁻¹ h ⁻¹
Product isolated	85 kg (3 batches)

Givaudan[®]

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Hydroxylations by InnoSyn's P450/GDH systems

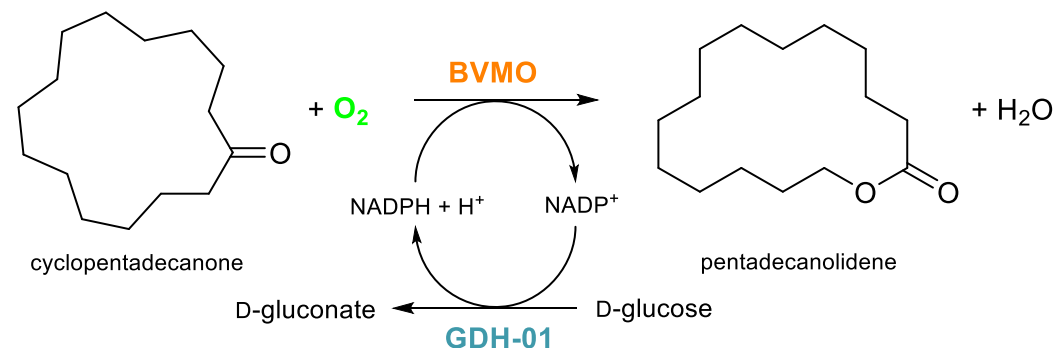
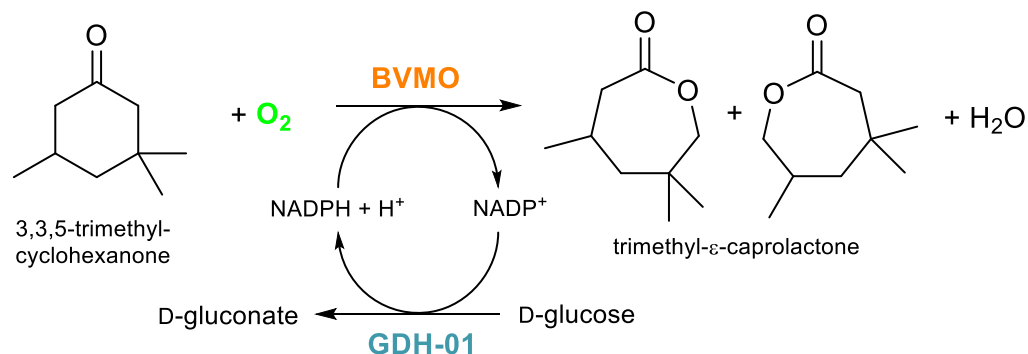


Metric	Value
Product concentration	6-10 g L $^{-1}$
Product yield	83%
Reaction volume	2 x 100 L
Volumetric productivity	1.3 g L $^{-1}$ h $^{-1}$
Product isolated	1.05 kg

Metric	Value
Product concentration	3 g L $^{-1}$
Product yield	92%
Reaction volume	2 x 100 L
Volumetric productivity	0.6 g L $^{-1}$ h $^{-1}$
Product isolated	0.6 kg



Baeyer-Villiger Oxidations by InnoSyn BVMO/GDH



Metric	Value
Product concentration	24 g L ⁻¹
Product yield	85%
Reaction volume	100 L
Volumetric productivity	2.7 g L ⁻¹ h ⁻¹
Product isolated	2.2 kg

Metric	Value
Product concentration	40 g L ⁻¹
Product yield	92%
Reaction volume	100 L
Volumetric productivity	4.0 g L ⁻¹ h ⁻¹
Product isolated	3.8 kg

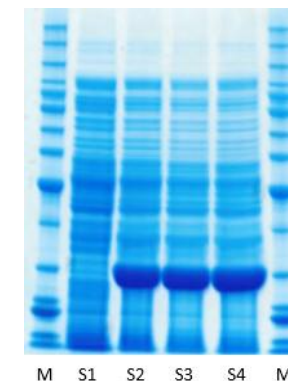
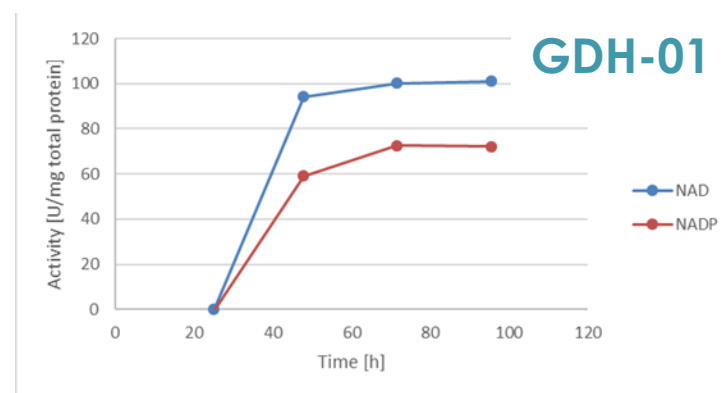
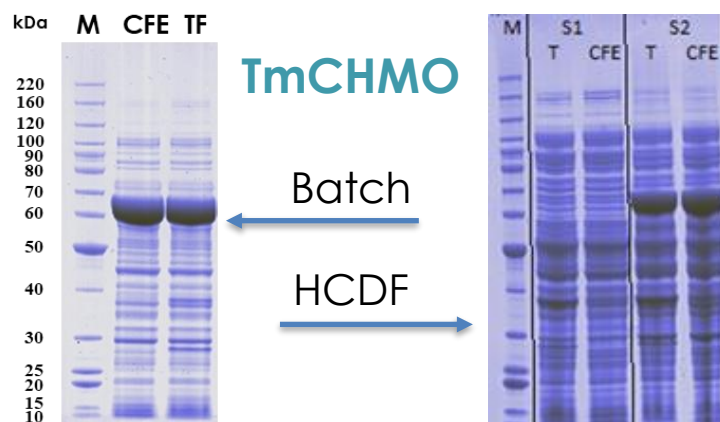


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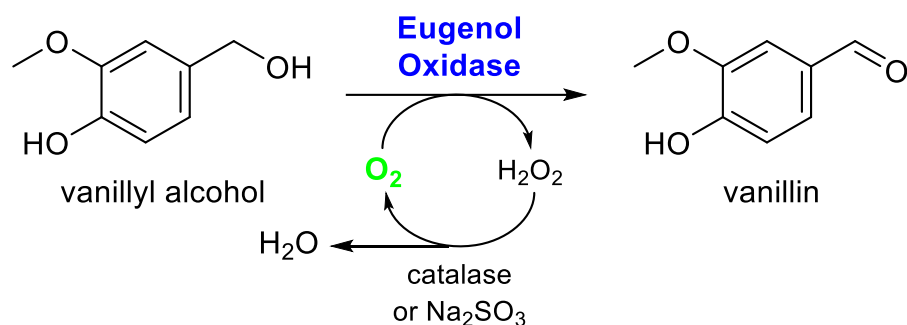
Fermentative Production GDH-01 and TmCHMO

From batch to high cell-density fermentations (HCDF)

Mode	Enzyme	Fermentation time	Biomass production	Enzyme Activity
Batch	TmCHMO	24 h	35 g/kg (cell wet weight/broth)	0.16 U/mg
HCDF	TmCHMO	100 h	375 g/kg (cell wet weight/broth)	0.12 U/mg
HCDF	GDH-01	96 h	310 g/kg (cell wet weight/broth)	70-100 U/mg NAD(P) ⁺



Alcohol Oxidation by InnoSyn's AOX system



Metric	Value
Product concentration	38 g L ⁻¹
Product yield	86%
Reaction volume	125 L
Volumetric productivity	4.2 g L ⁻¹ h ⁻¹
Product isolated	4.0 kg

Sustainability evaluation

(Including enzyme or chemocatalyst production)

Total Process Metrics per kg product	Biocatalyst	Chemocatalyst
PMI	69	94
Mass Net (kg)	86	108
Energy (MJ)	3060	4303
GWP (kg CO ₂ equiv.)	114	147
Acidification (kg SO ₂ equiv.)	0,47	0,58
Eutrophication (kg phosphate equiv.)	0,20	0,15
Water (kg)	680	416

EUGO Oxidase

Breakdown of PMI by Class



→ Water and solvent contribute similarly

Cu-ligand catalyst

Breakdown of PMI by Class



→ Solvents are the biggest contributors

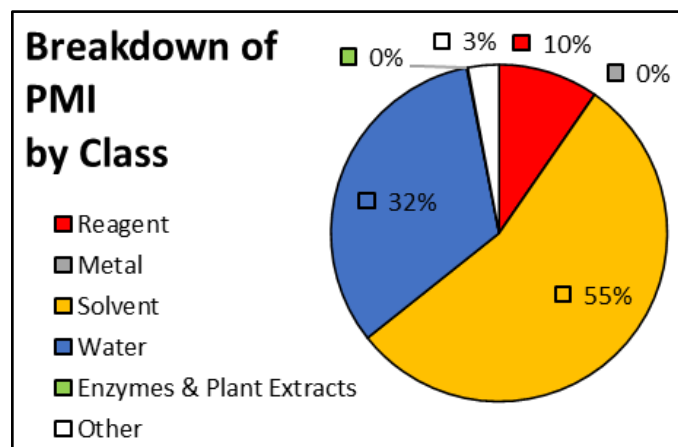
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PMI Comparison AOX and ADH/NOX Processes

using the ACS GCI PRT PMI-LCA tool*

- Applying the PMI-LCA tool based on the pilot plant recipes:

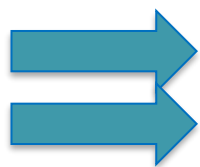


Total Process Metrics per kg product	EUGO Vanillin	ADH/NOX C5-lactol
PMI	69	43
Mass Net (kg)	86	66
Energy (MJ)	3060	2531
GWP (kg CO2 equiv.)	114	114
Acidification (kg SO2 equiv.)	0,47	0,35
Eutrophication (kg posphate equiv.)	0,20	0,10
Water (kg)	680	289

- Good PMI values for both processes
- Extraction solvent is the main contributor to PMI and other LCA values
- Little PMI contribution by fermentative enzyme production

Oxygen Supply, Reactors and Productivities

Demonstration case	Reactor type	Oxygen supply	STY [g L ⁻¹ h ⁻¹]
1. P450 Aromatics	Stirred tank reactor (STR)	Oxygen	0.15
2. P450 API	STR	Air	0.4
3. P450 Alkenes	STR	Oxygen	1.5
4. Cyclohexanone BVMO	STR	Oxygen	2.7
5a. Macrocyclic BVMO	STR	Oxygen	4.0
6. Alcohol AOX	STR	Oxygen	4.2
7a. Lactol ADH	STR	Oxygen	6.3
7b. Alcohol ADH	STR	Oxygen	14.0



Sufficient oxygen for an efficient biocatalytic reaction can be supplied

Conventional stirred tank reactors are suited for a safe operation with O₂





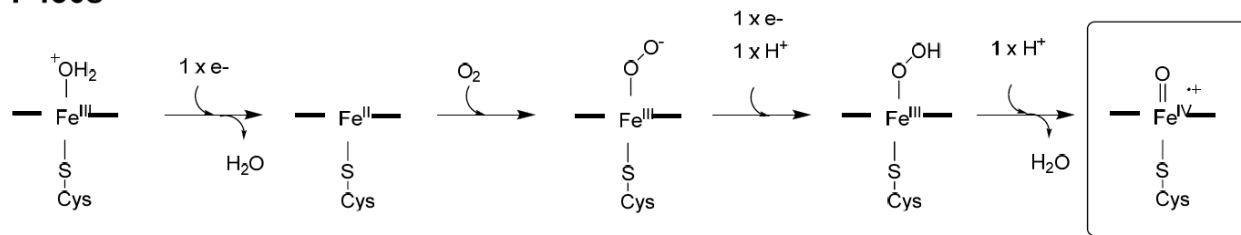
What's next in Biooxidations Process Development?

Bayesian Optimization for
Unspecific Peroxygenase (UPO)
Reaction Optimization

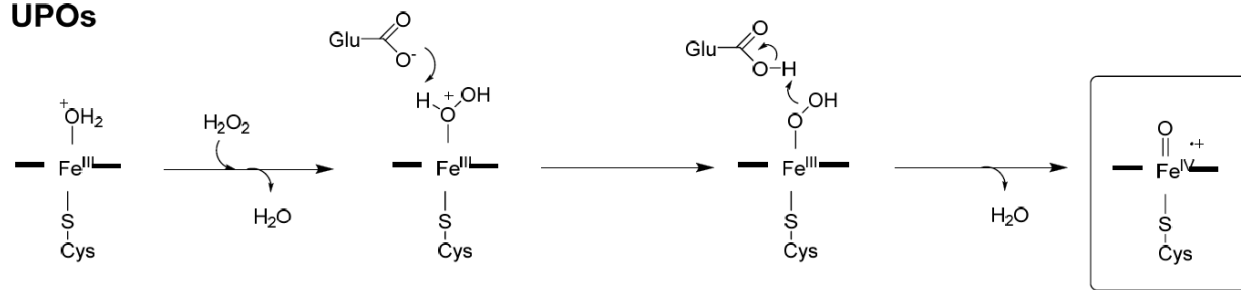
UPO vs. P450 Enzymes

- Different strategies to arrive at reactive Compound IV
- Much simpler in case of UPOs

P450s

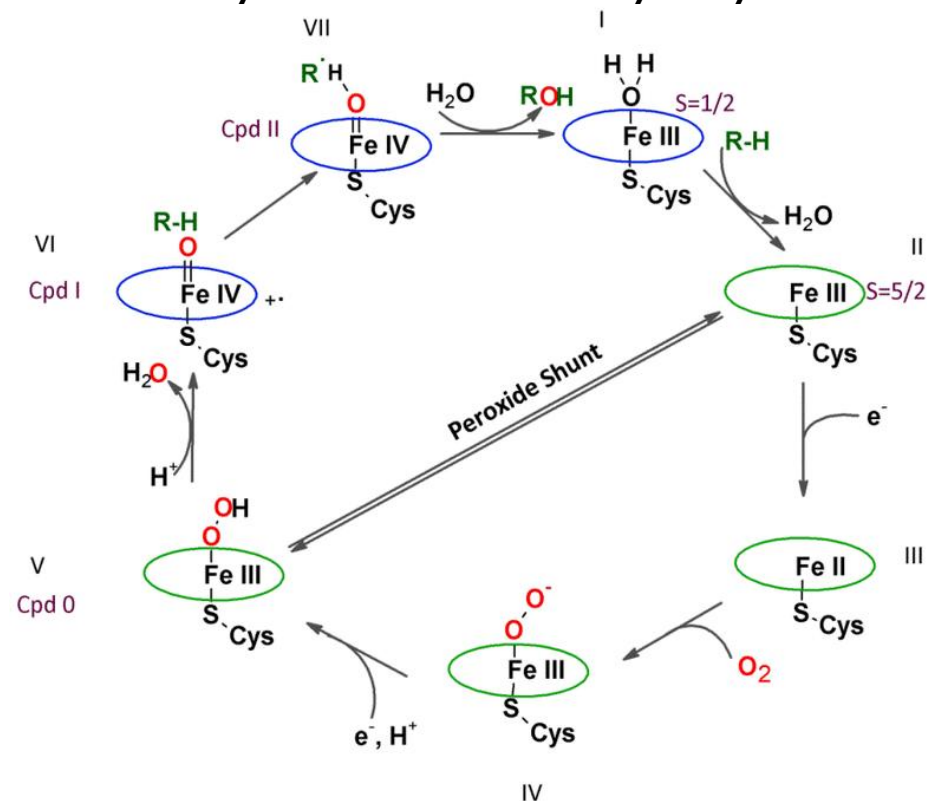


UPOs

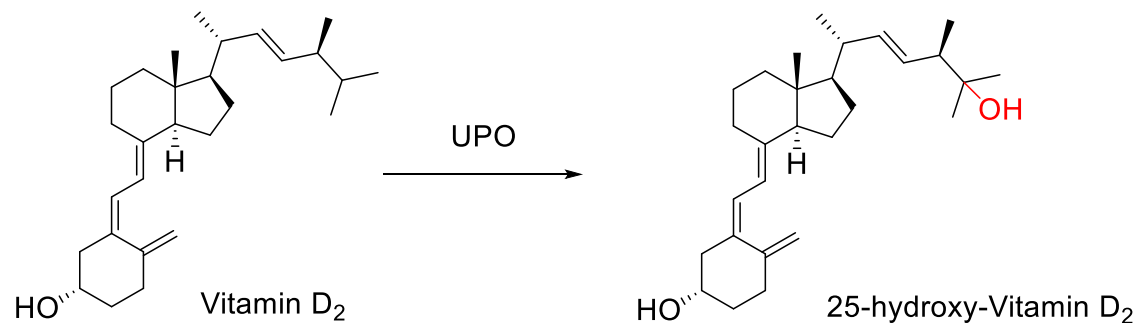


G. Grogan, *JACS Au* **2021**, 1, 1312–1329

Cytochrome P450 catalytic cycle



UPO Hydroxylation/Oxidation of Vitamin D₂



- 25-hydroxy-vitamin D: biol. active form of VitD
- UPO enzymes provided by Bisy (Graz, Austria)
- Screening & preliminary optimization at IRNAS (Sevilla, Spain)
- InnoSyn tasks:
 - Reaction intensification
 - Scale-up
 - Pilot plant demonstration

Results by project partners IRNAS & Bisy¹:

- *Hypoxylon* species UPOs most active
 - 20 mM vitamin D₂ (~8 g/l)
 - In 1% isopropanol and 200 g/l cyclodextrin
 - 100 mM H₂O₂
- 88% yield in 24 h
- Essentially full regioselectivity
- Alternatives to hydrogen peroxide:
 - Ascorbate plus O₂
 - Pyrogallol plus O₂
 - Both leading to 80-90% yield, respectively

¹ *Biotechnol. Biofuels Bioprod.* (2026). <https://doi.org/10.1186/s13068-026-02792-0>

Optimization of 25-OH-VitD₂ Productivity

Boundary conditions

- Limited enzyme amounts at this stage of the project
- Efficient use of lab equipment and human resources

Bayesian Optimization using INNOptimizer

- Developed by **InnoSyn affiliate SynSilico** to enable every scientist & engineer to use Bayesian Optimization

INNOptimizer®



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Revolutionize your research with intelligent experimental design that delivers breakthrough results **up to 70% faster**.

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📈 Data-Driven

💡 Intelligent

🎯 Precise

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- Home
- Optimizations
- Examples
- Learning Resources
- Change Log

OptiBot **NEW**

Experiments

10 remaining
0 used of 10 total



Test



Welcome back!



Updated 2m ago

Ready to optimize
your next
experiment?

+ New Optimization →

Total Experiments

13

Across all optimizations



Active Optimizations

2

Currently running



Average Improvement

28.8%

Across all optimizations



Recent Experiments

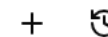
0

Last 7 days



Recent Optimizations

OptiBot **NEW**



Try one of these to get started:

What is Agentic INNOptimizer[®] and what can it do?

How do I start my first optimization?

Explain Bayesian optimization in 3 sentences.

How many AI messages do I have left this month?

INNOptimizer[®]



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Ask OptiBot...



Enter to send, Shift+Enter for a new line.

Optimization of 25-OH-VitD₂ Pproductivity

Boundary conditions:

- Limited enzyme amounts at this stage of the project
- Efficient use of lab equipment and human resources

Bayesian Optimization using INNOptimizer

- Developed by **InnoSyn affiliate SynSilico** to enable every scientist & engineer to use Bayesian Optimization

INNOptimizer set-up:

- Objective type:** Multi-Target (Desirability)
- Acquisition Function:** Expected Improvement (EI, balances exploration and exploitation)
- Discrete Exclude **Constraint:** Oxidant type (One oxidant per reaction, no combinations)

DoE comparison: 27 rxns. for 3-level design

Optimisation target	Direction	Importance
25-OH-VitD ₂ yield 5 h	maximise 	40%
25-OH-VitD ₂ yield 24 h	maximise 	40%
Overoxidation 24 h	minimise 	20%

Rxn. parameter	Parameter type	Parameter range
Temperature	Discrete	20, 25, 30, 35, 40°C
pH value	Discrete	6.0, 6.5, 7.0, 7.5, 8.0
Oxidant	Categorical, H ₂ O ₂	100, 150, 200, 250 mM
	Or pyrogallol	20, 30, 40, 50, 100 mM
	Or ascorbic acid	20, 30, 40, 50 mM



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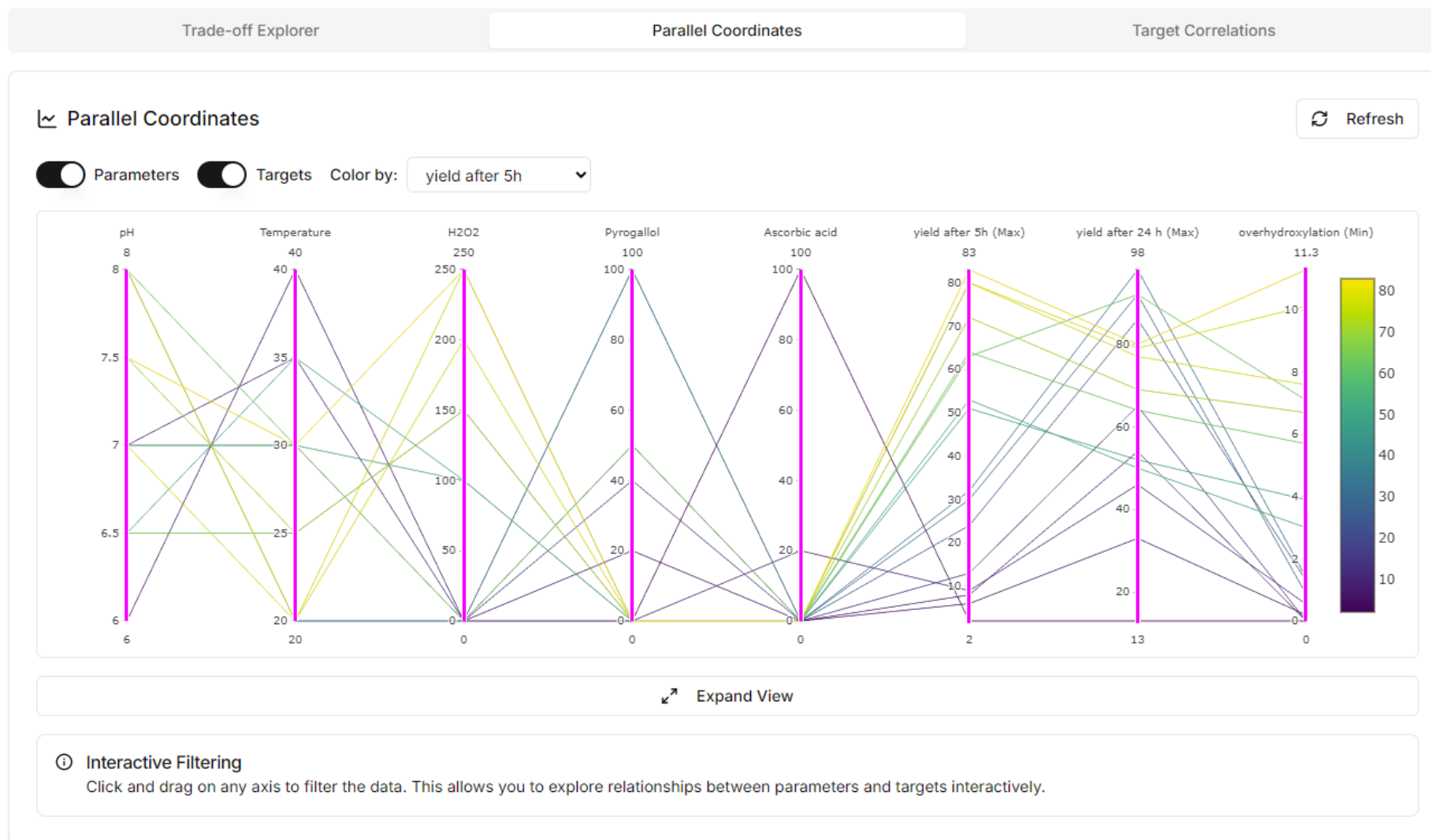
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INNOptimizer Results Visualization & Evaluation

Multi-Target Analysis

Understand trade-offs between multiple objectives



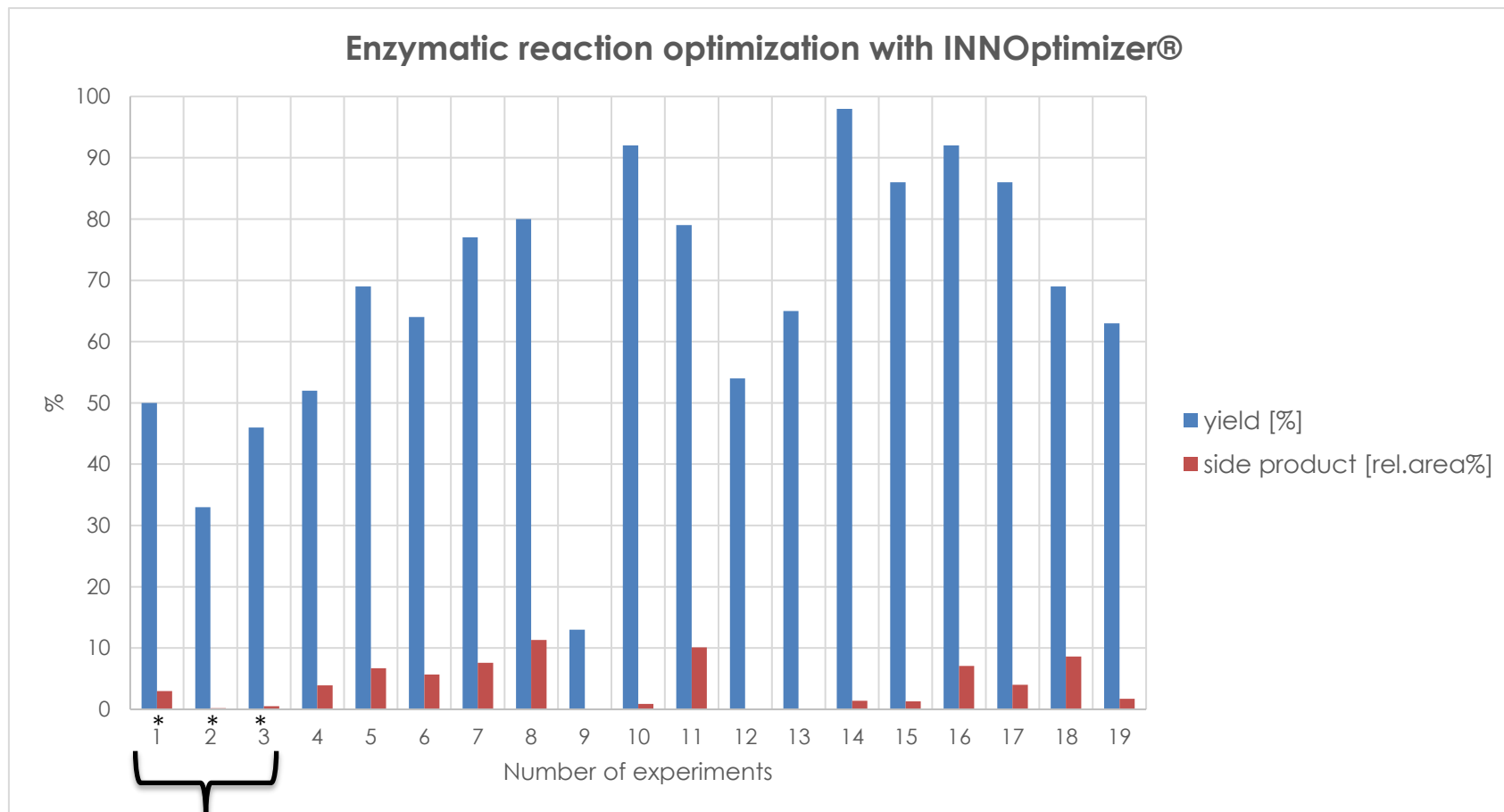
Parallel Coordinates

- All rxn. parameters
- All targets
- 1 interactive graph

Best results

- High yield
- Low byproduct
- At pH 8.0
- With pyrogallol

Conventional/Manual Result Graph



Data from initial seeding experiments, not BO suggested

- From yields below 50%
- To multiple above 80%
- Within only 14 reactions
- 1 enzyme
- 3 targets
- 3 reaction parameters
- 1 parameter constraint
- opt 14 results
 - 91% yield
 - $\leq 1\%$ overhydroxylation

Conditions opt14			
pH	oxidant	mM	temp.
8	pyrogallol	100	40°C



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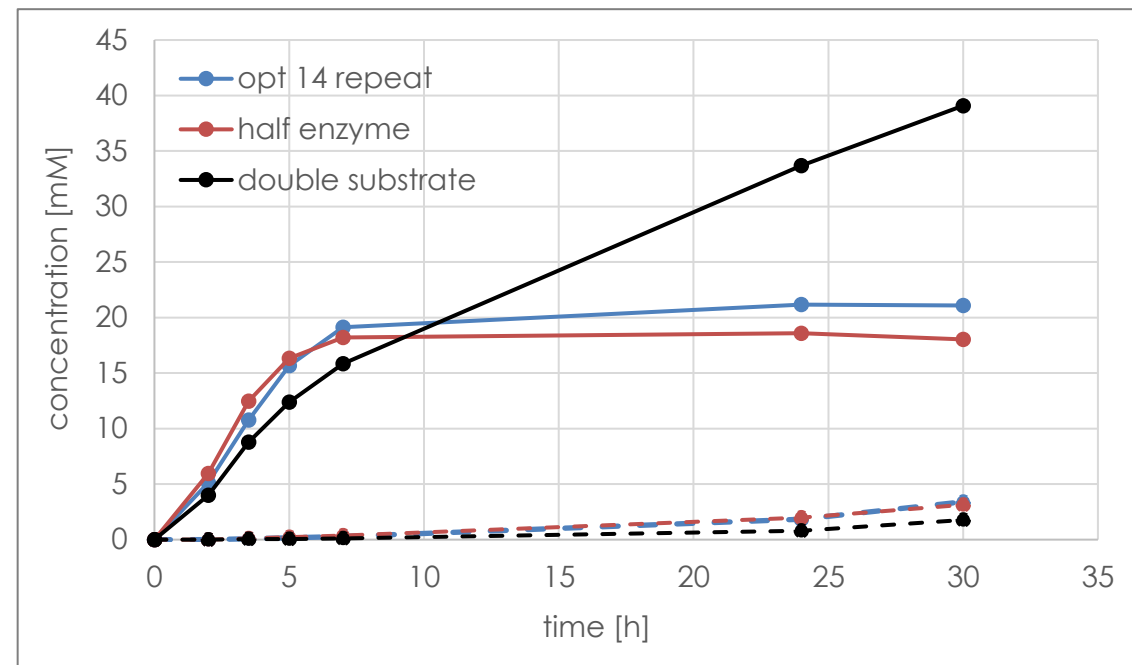
I-N-N-O
|
S-Y-N
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Manual Optimzation of VitD₂ and UPO Loading

- Repeating opt 14 conditions on larger scale
 - Plus opt 14 conditions with
 - 50% enzyme loading OR
 - Doubled substrate concentration
- Almost identical rate and yields with ½ UPO
- ≥ 94% yield at 40 mM VitD₂ (~16 g/L) after 30 h
- Less overoxidation at double VitD₂ loading

	Yield 30 h [%]	Activity [$\mu\text{mol min}^{-1} \text{mg}^{-1}$]
opt 14 (best cond.)	100	3.8
Half enzyme	90	8.0
Double substrate	94	3.0

mg = mg enzyme powder



Scale-up is ongoing...



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I-N-N-O
|
S-Y-N
Tomorrow's chemistry. Today.

Conclusions

- **Biocatalytic oxidations with pure oxygen** can be
 - Scalable & safe
 - Efficient & sustainable
- **Biocatalytic processes** are not by definition sustainable
 - They need to resource and cost efficient
- **Unspecific Peroxygenases (UPOs)**
 - Are emerging alternatives for Cytochrome P450s and Epoxidases
 - Can be applied with hydrogen peroxide or alternative oxidants plus oxygen



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Thank you for your attention !

Any questions ?