Department of Chemistry
Technical Chemistry

Biocatalysis

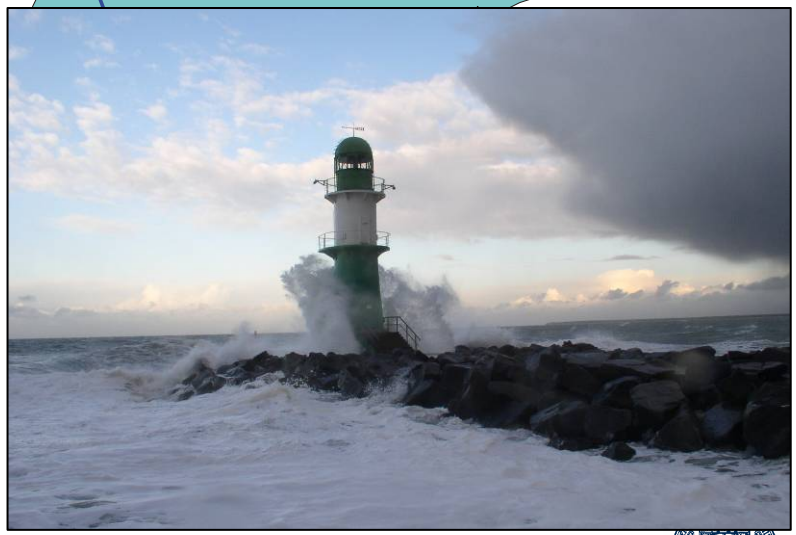
Udo Kragl

Universität Rostock, Institut für Chemie,
Lehrstuhl für Technische Chemie
Albert-Einstein-Str. 3A, 18059 Rostock, Germany
Tel. +49-381-498-6450, Fax +49-381-498-6452
Email udo.kragl@uni-rostock.de
www.chemie.uni-rostock.de/kragl

University of Rostock - f

Ge

Bonn



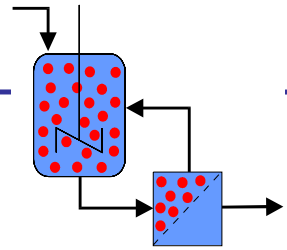
Technical Chemistry - Research Topics

- **Rostock University, Department of Chemistry**
  - Biocatalysis, ionic liquids
  - Downstream processing of low molecular weight componds
  - Renewable resources
  - Trace analyis
  - Optimisation of reaction conditions, modelling

- **Leibniz Institute for Catalysis**
  - Multi phase catalysis
  - Polymerisation of propene oxide
  - Functionalisation of renewable resources...
  - ...

in total 22 coworker

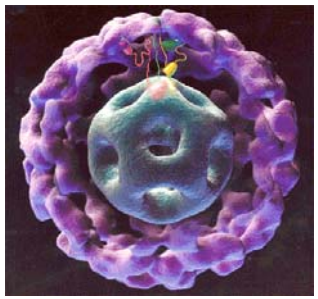
Technical Chemistry – Research Topics



- **Biocatalysis**
  - (enantio)-selective oxidation, C-C-bonding
  - biocatalysis in ionic liquids
- **Downstream processing of low molecular weight compounds**
  - membrane processes for desalting, concentration
  - combination of extraction, chromatography, $scCO_2$ extraction, membrane processes (product orientated)
- **Use of renewable resources („Biogene Rohstoffe“)**
  - fermentation (rare sugars, biomaterials)
  - plant compounds
  - chemo- and biocatalytic functionalisation
- **Trace analysis (environment, processes)**
  - Equipment: GC, LC, GC-MS, LC-MS, ion chromatography
- **Optimisation of reaction conditions**
  - model based or „black box“ (genetic algorithm)

Outline

- **introduction**
- **examples**
  - **industry**
  - **academia**
- **take home message**



´Enzymes are proteins,
things of beauty and joy forever`
<R. N. Perham, 1976>

Opinions & Prejudices - hopefully overcome

Biocatalysts

- are sensible and not stable

⇒ operational stability for several months has been achieved

- work only in diluted aqueous solutions

⇒ molar solutions, organic solvents possible

- give only low space-time yields

⇒ several kg/(L×d) possible

Facts

Biotransformations are used for synthesis of bulk and fine chemicals, such as:

high-fructose corn sirup	Glc-Fru isomerase	> $8x10^6$ t/a
acrylamide	nitrile hydratase (wc)	> 30,000 t/a
7-aminocephalosporinic acid	acylase	> 200 t/a
chiral amines	lipase	> 100 t/a
chiral alcohols	oxidoreductase (wc)	1 t/a (?)

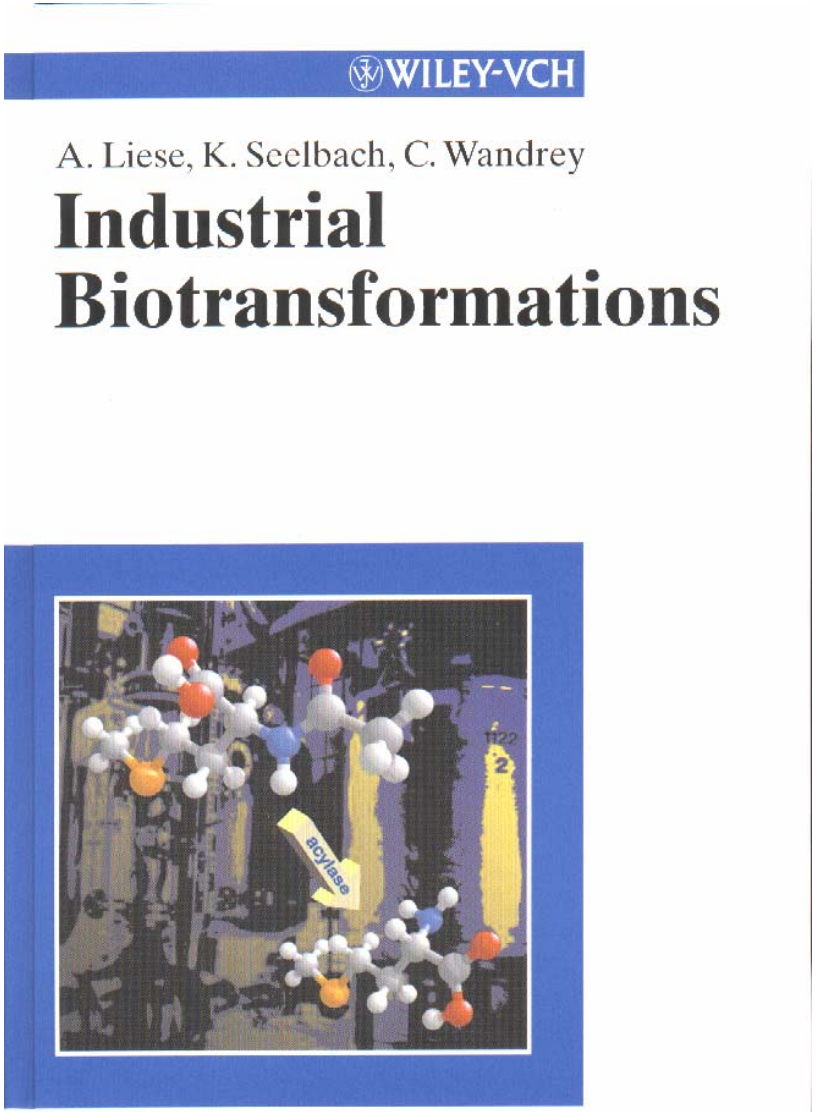
... today more than 100 biotransformations are applied in industry...

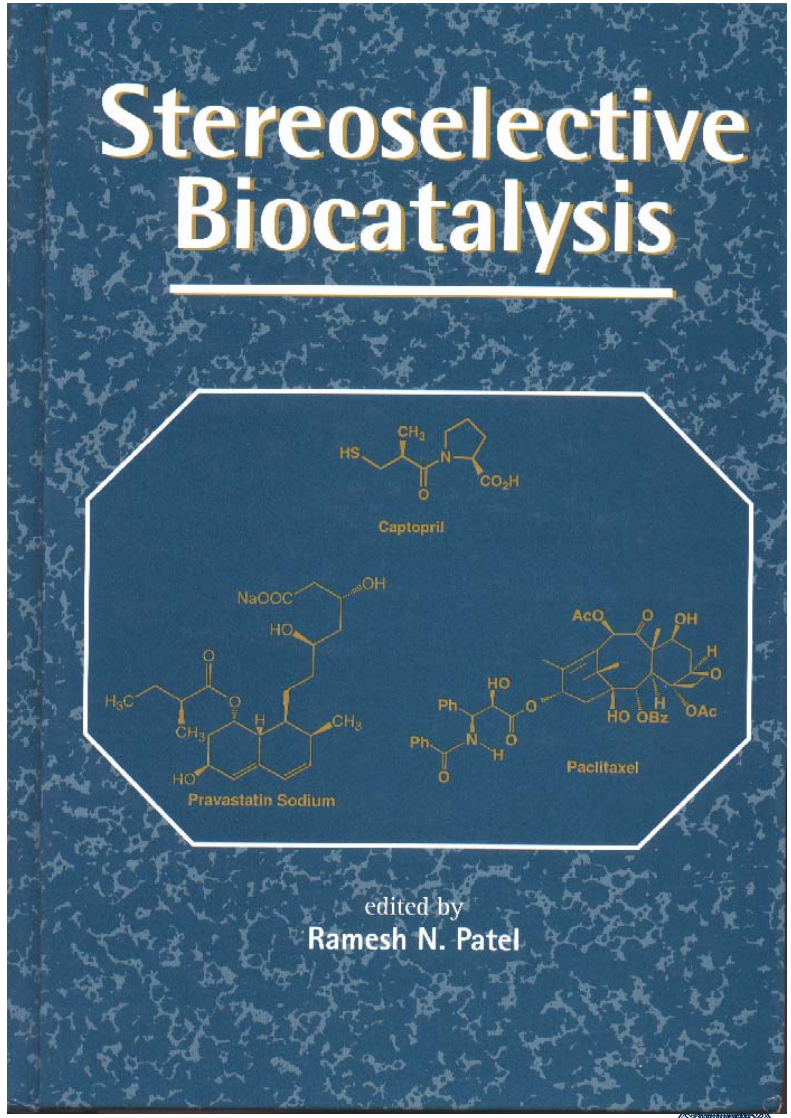
Productivities of Biotechnological Processes

Process/Product	Productivity ($g\ l^{-1}\ hr^{-1}$)	Price ($\$\ kg^{-1}$)
Fermentations		
Citric acid	7.0	1.5
L-Lactic acid	4.0	2.5
Glutamic acid	3.0	2.0
Ethanol	1.5	0.70
Lysine	0.6	13
Penicillin G	0.17	20
Vitamin B_{12}	0.001	8000
Enzymatic		
Glucose —> fructose	200	2.0
Fumaric acid —> aspartic acid	20	3.0
Cinnamic acid —> phenylalanine	15	10
Chemical		
Acetic acid (MeOH + CO)	500	0.75
Ethanol from ethylene	80	0.70
DL-Methionine	15	3.0

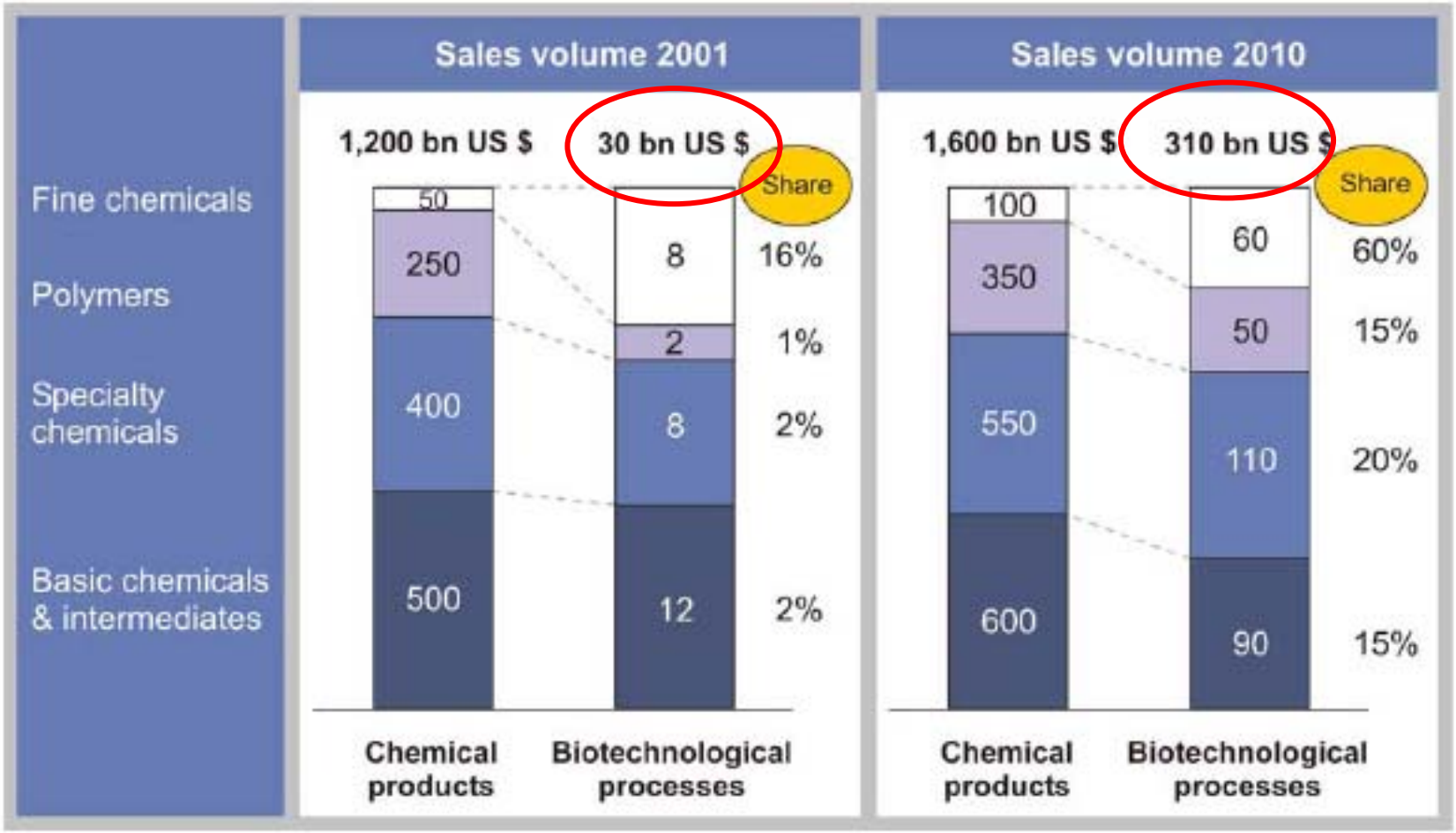
Roger Sheldon, 1993

Recent Books on Biocatalysis & Biotransformations





Biocatalysis – Established with a Great Future



Growth of sales volume for Biotechnology until 2010

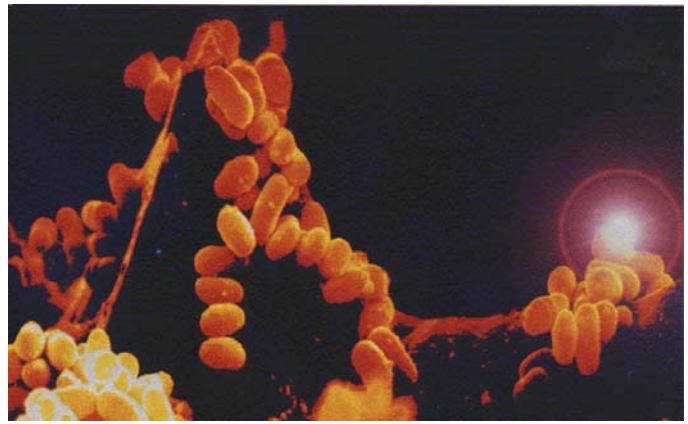
Festel Capital, 2004

Biotechnology

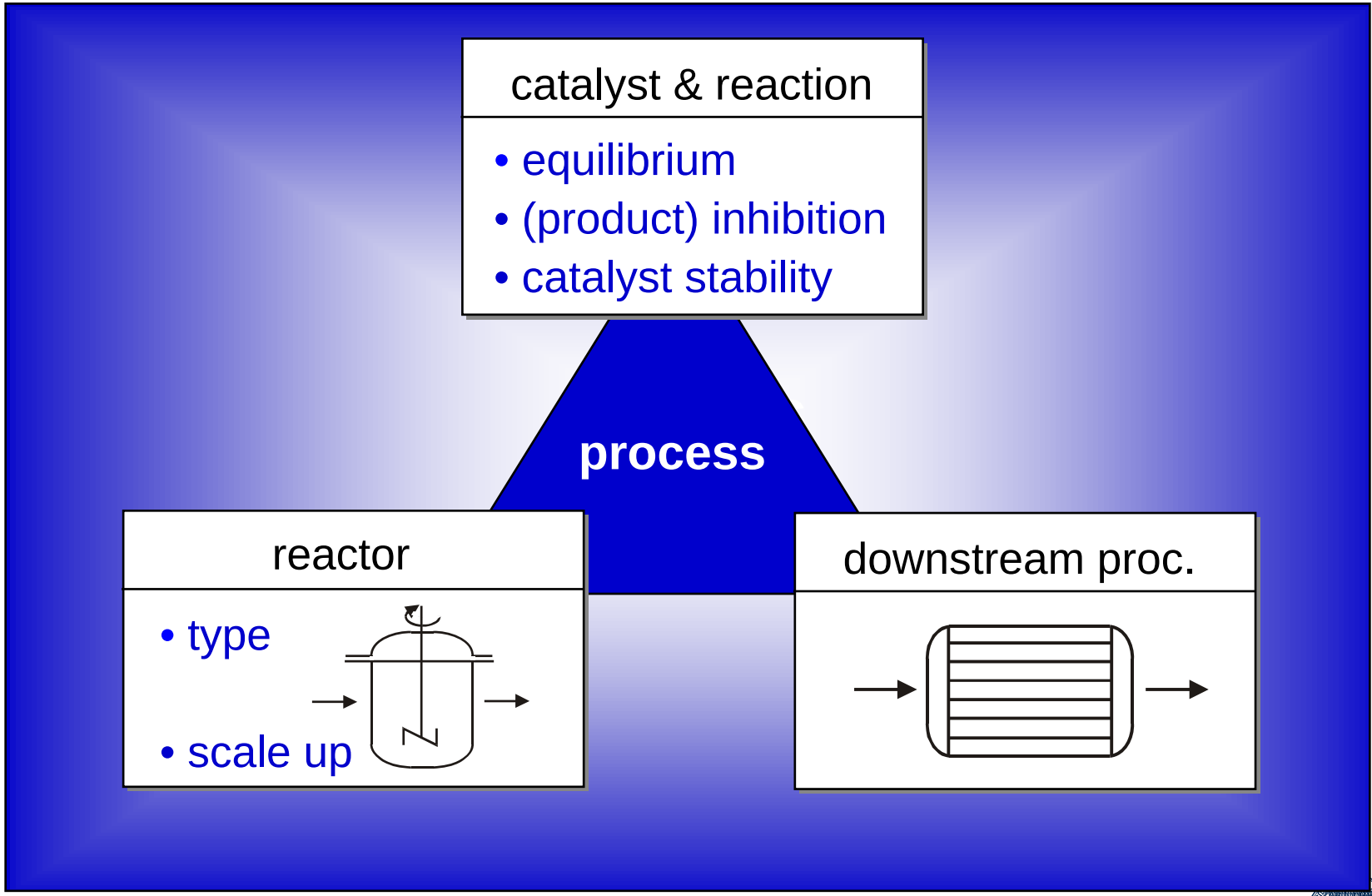
- **biotransformation**
  - whole cell
  - enzyme

educt —(**biocatalyst**)→ **product**

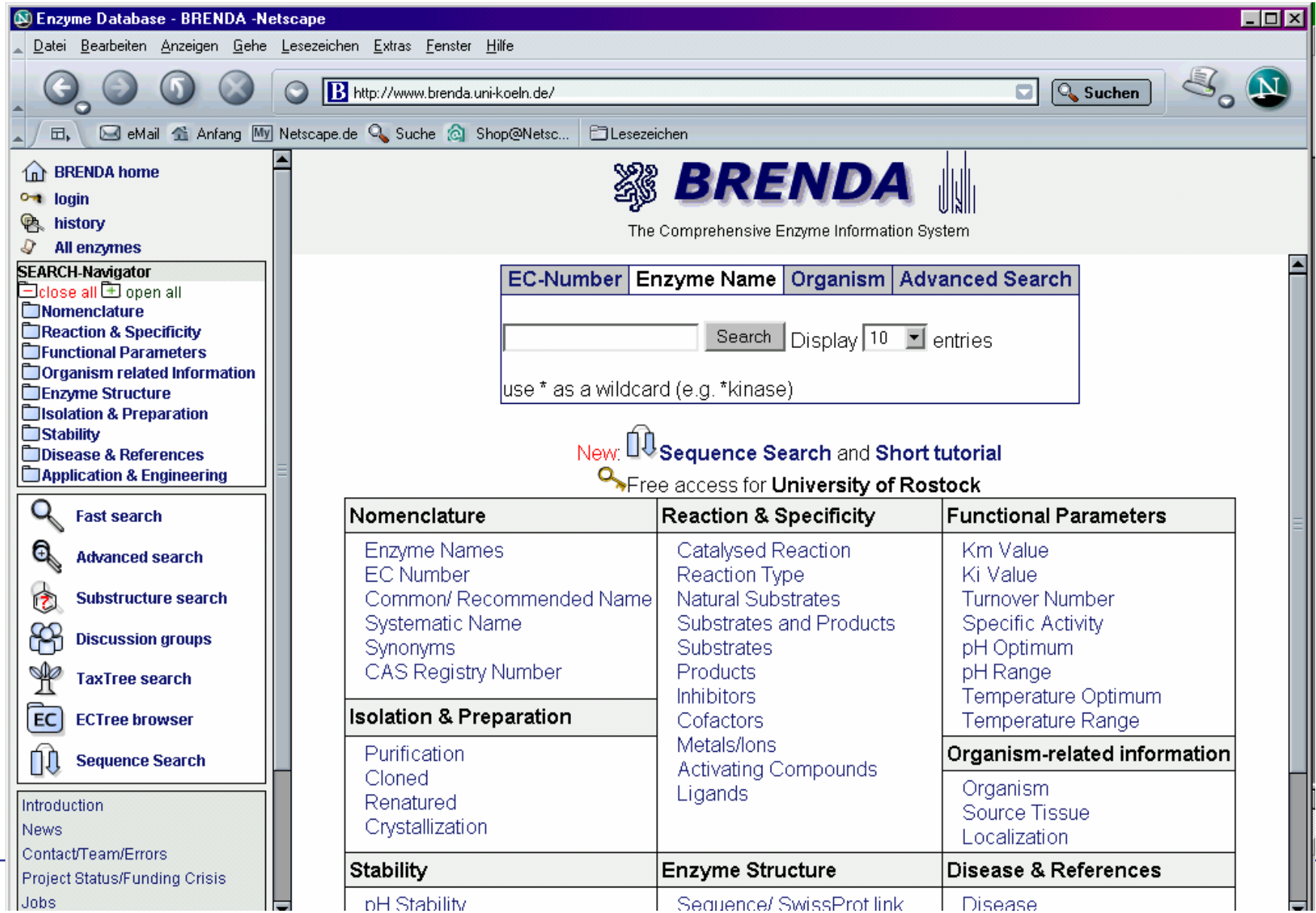
- **fermentation**
  - de novo synthesis from nutrients



Process Development – an Integrated View



www.brenda.uni-koeln.de



Suppliers of Enzymes

- Fluka, Sigma, Merck...
- Novo Nordisk
- Amano
- Toyobo
- Unitika
- Biocatalysts
- Codexis
  www.codexis.com

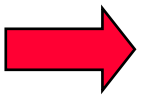
- University groups working in Biochemistry, Mikrobiology

Enzyme Kinetics

- pH, temperature
- activity: 1 U = 1 μmol/min; 1 kat = 1 mol/s
- Michaelis-Menten type saturation kinetics
- for >1 substrates order of binding to the enzyme has to be considered
  - ordered
  - random
  - ping-pong
- substrate-surplus & product inhibition may occur
- references:
  - Cornish-Bowden
  - Segel
  - Biselli, Kragl, Wandrey in Drauz, Waldmann (eds)

Recovery of Enzymes or Cells

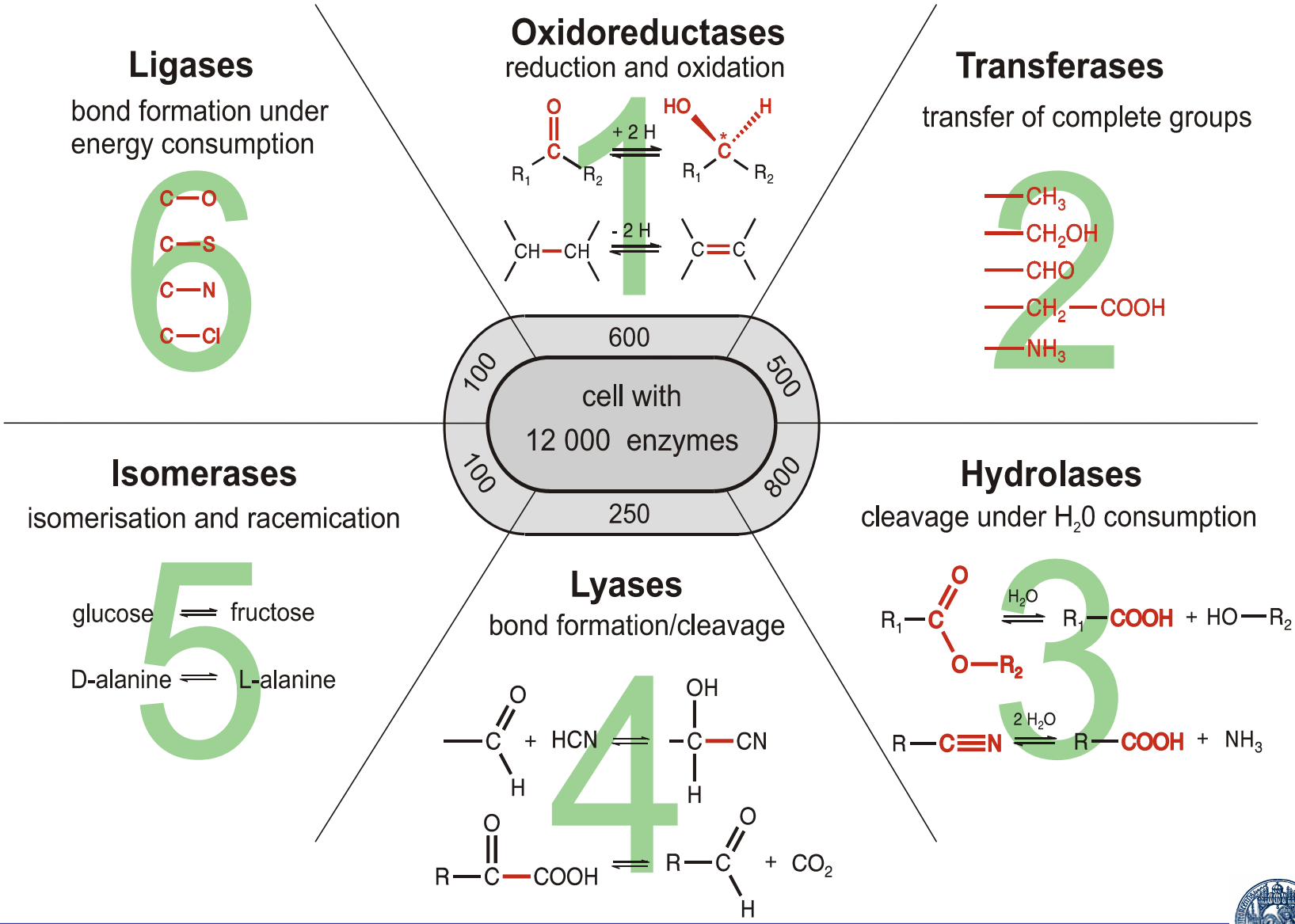
- immobilisation on a heterogeneous support by
  - adsorption
  - covalent attachment (eg. Eupergit)
- cross-linking
- entrapment in hydrogels (alginate...)



covalent attachment often increases thermal stability, but method has to be developed individually, immobilisation yield <100% (acitivity!)

- ultrafiltration (enzymes)
- microfiltration, centrifugation

Classification of Enzymes



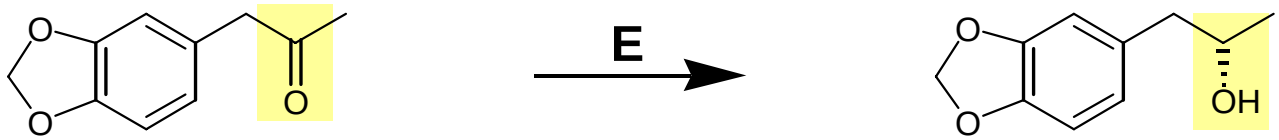
Enzyme Classification & Enzyme Source

- enzymes with the same EC-number and CAS-number but from different origin may react totally different!

- lipase EC 3.1.1.3
  - *Burkholderia plantarii* (BASF-prozess)
  - *Pseudomonas cepacia*
  - *Mucor miehei*
  - *Pseudomonas fluorescens*
  - *Candida antarctica*
  - *Serratia marescens*

Alcohol Dehydrogenase EC 1.1.1.1

Zygosaccharomyces rouxii



(*S*)-**2**

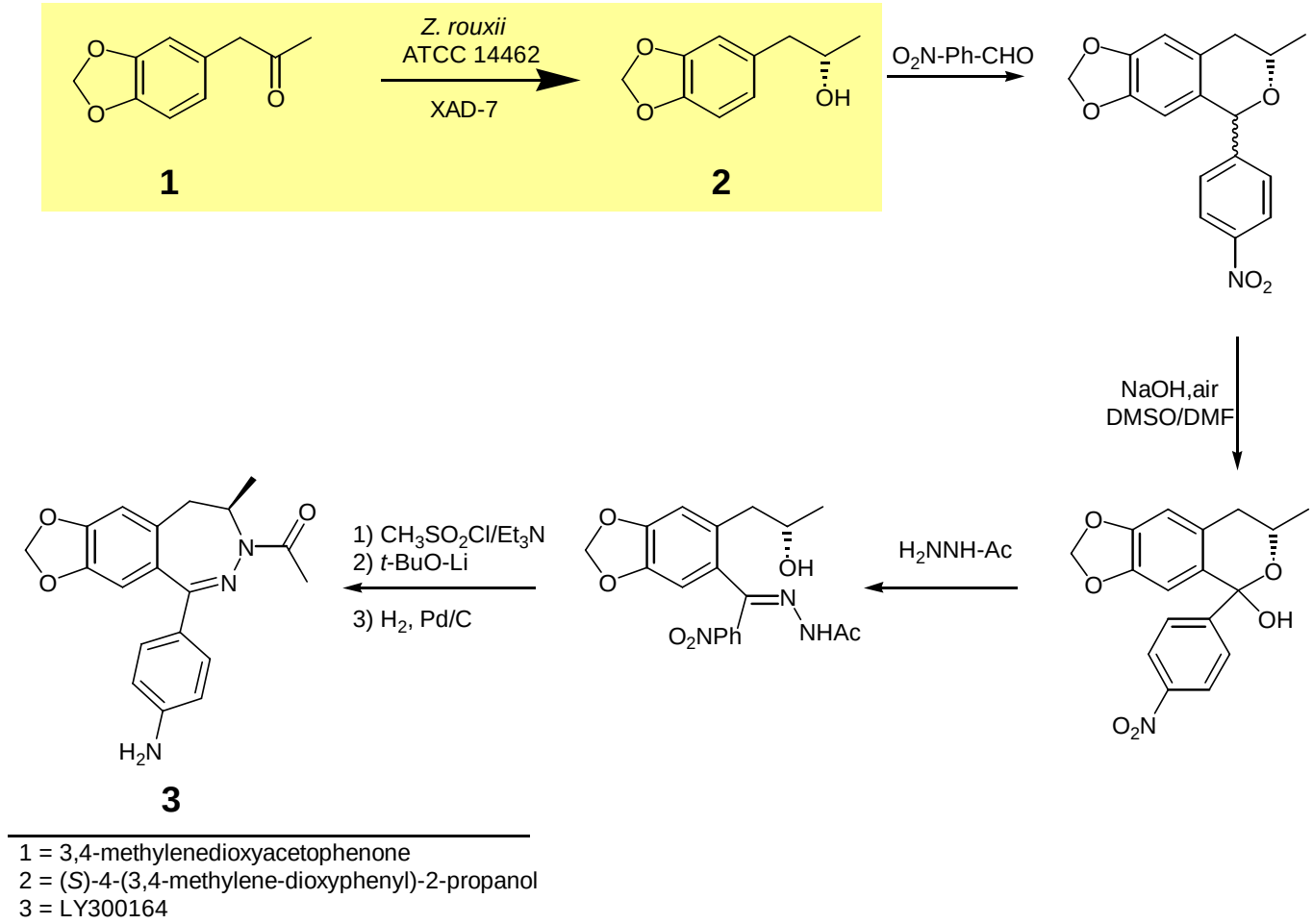
1 = 3,4-methylenedioxyacetophenone
2 = 4-(3,4-methylenedioxyphenyl)-2-propanol

Eli Lilly

reaction conditions:		***process parameters:***	
[1]:	**< 0.011 M, 2 g L^{-1}**	**yield:**	96 %
pH:	7.0	**ee:**	> 99.9 %
T:	33 - 35 °C	**reactor type:**	batch
		reactor volume:	300 L
		capacity:	kg scale
		space-time-yield:	75 g $L^{-1}d^{-1}$

Anderson, B.A. et al. (1995) J. Am. Chem. Soc. **117**, 12358-12359

Integration in Chemical Synthesis



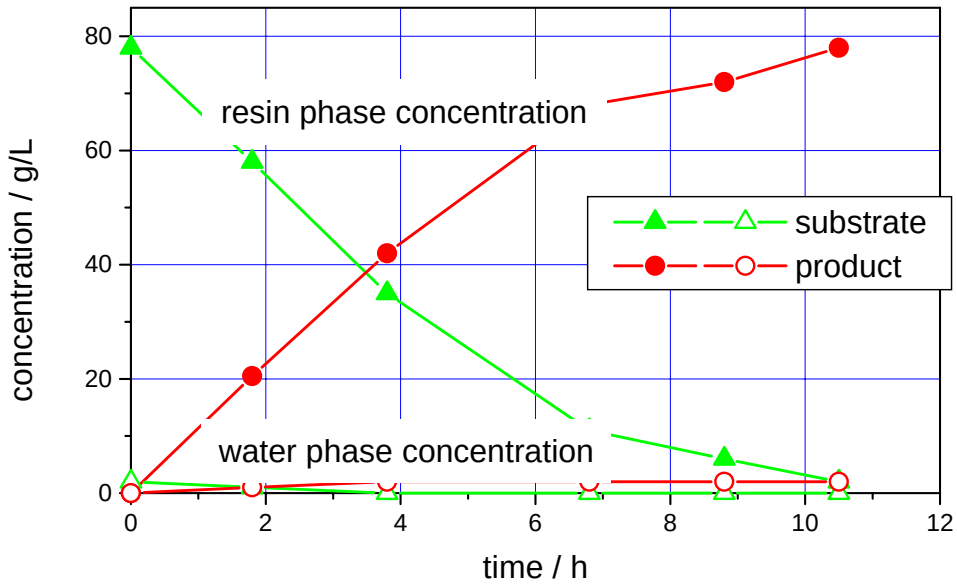
1 = 3,4-methylenedioxyacetophenone
2 = (*S*)-4-(3,4-methylene-dioxyphenyl)-2-propanol
3 = LY300164

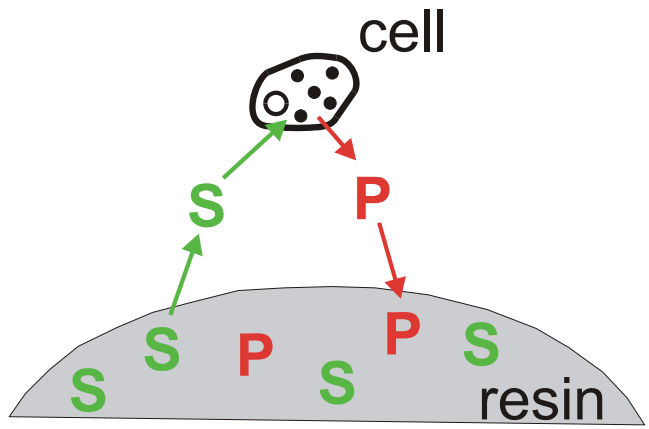
The product is tested for treatment of amylotropic lateral scleposis.

Ketone Reduction using Whole Cells

- whole cells of *Zygosaccharomyces rouxii*
- substrate and product are **toxic** for the cells

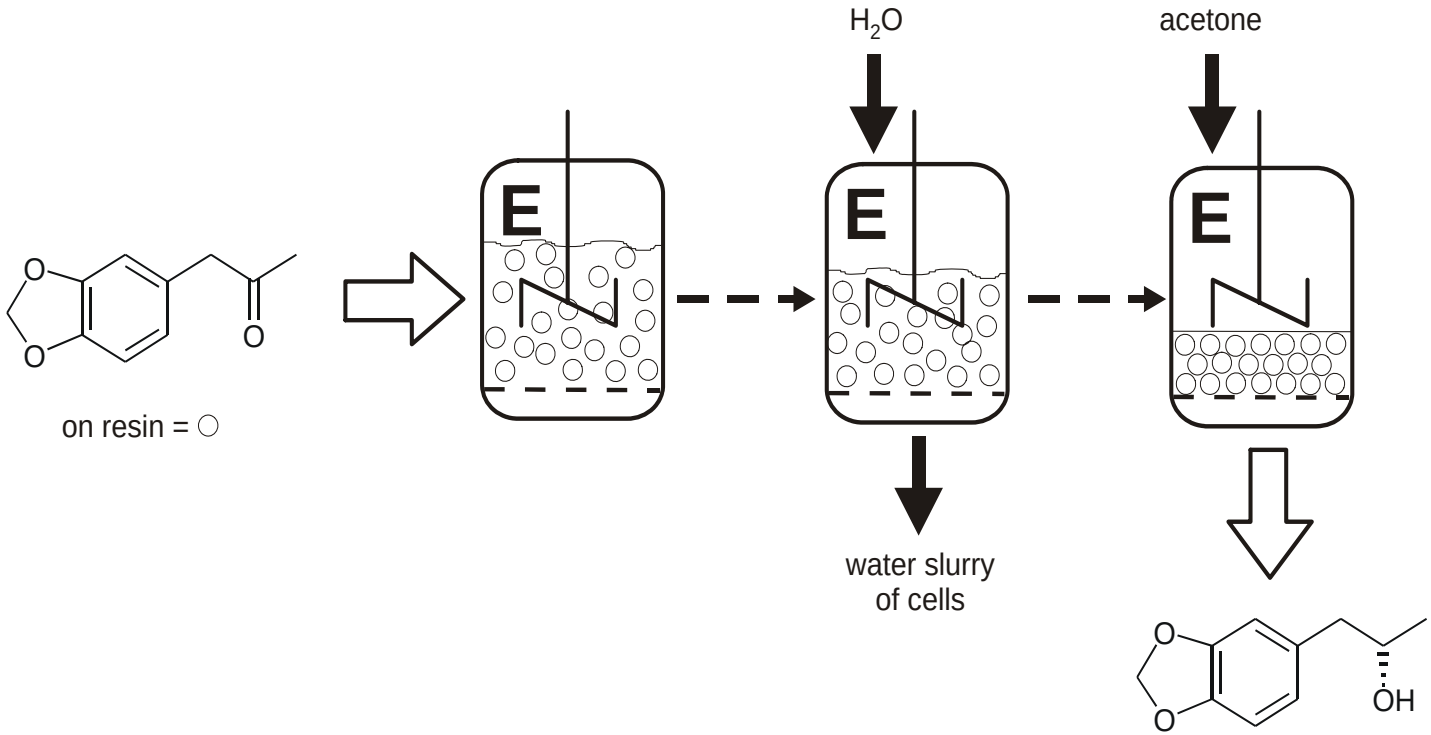
  ⇒ XAD-resin as substrate reservoir and for product extraction





Vicenzi et al., Enz. Microbial. Technol. **20**, 494 (1997)

Flow Scheme

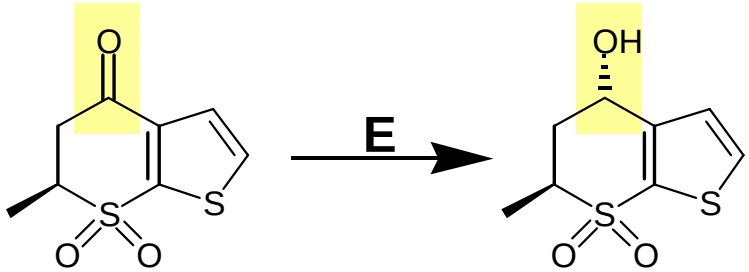


- **XAD-7 resin is retained by 150 µm filter**
- **yeast cells are in filtrate**
- **product is liberated by washing resin with acetone**

Vicenzi, J.T., Zmijewski, M.J., Reinhard, M.R., Landen, B.E., Muth, W.L., Marler, P.G. (1997) Enzyme Microb. Technol. **20**, 494-499

Alcohol Dehydrogenase EC 1.1.1.1

Neurospora crassa



(6S)-**1** *(4S,6S)*-**2**

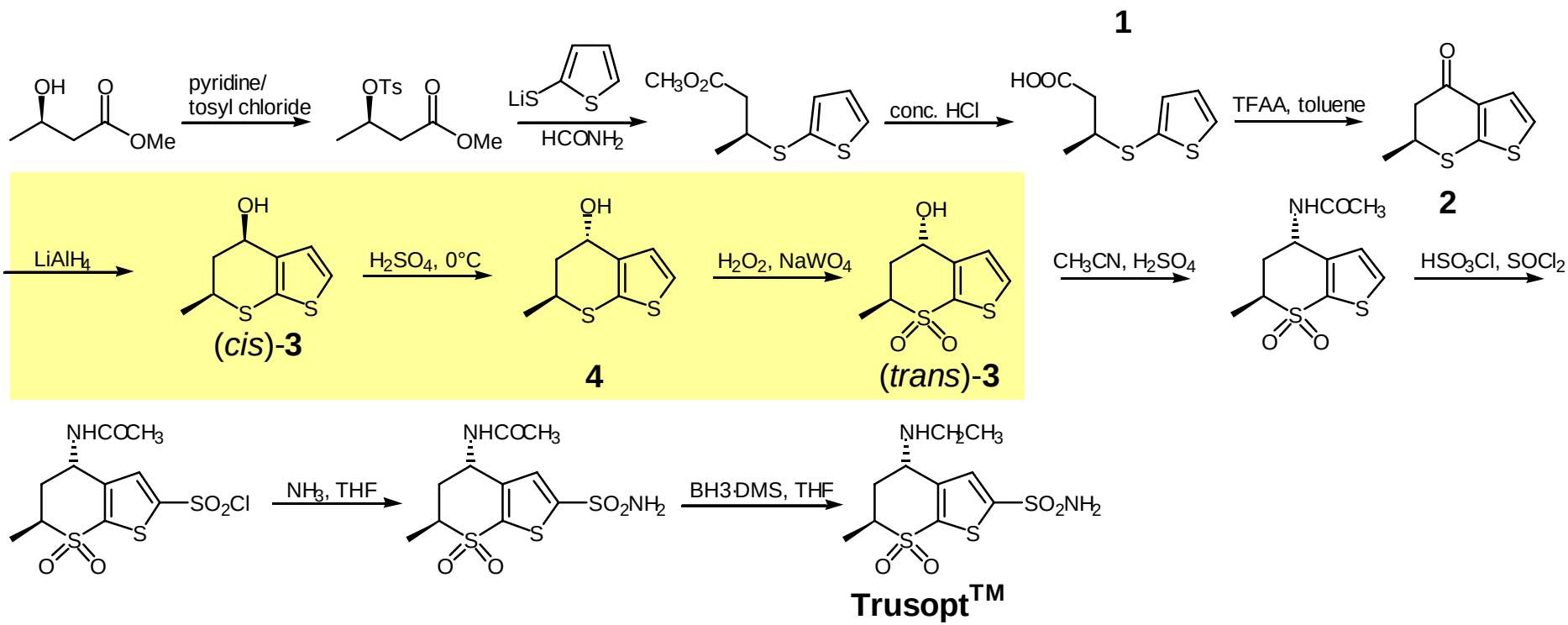
1 = 5,6-dihydro-6-methyl-4H-thieno[2,3b]thiopyran-4-one-7,7-dioxide
2 = 5,6-dihydro-4-hydroxy-6-methyl-4H-thieno[2,3b]thiopyran-7,7-dioxide

Zeneca Life Science Molecules

reaction conditions:		***process parameters:***	
pH:	**3.8-4.3**	**yield:**	> 85
T:	33 °C	**ee:**	**> 98 %**
medium:	aqueous	**reactor type:**	batch
catalyst:	suspended whole cells	**capacity:**	multi t
		start-up date:	1994

Holt, R. A. and Rigby, S. R. (1996) Zeneca Limited, US 5580764

Substitution of Chemical Steps

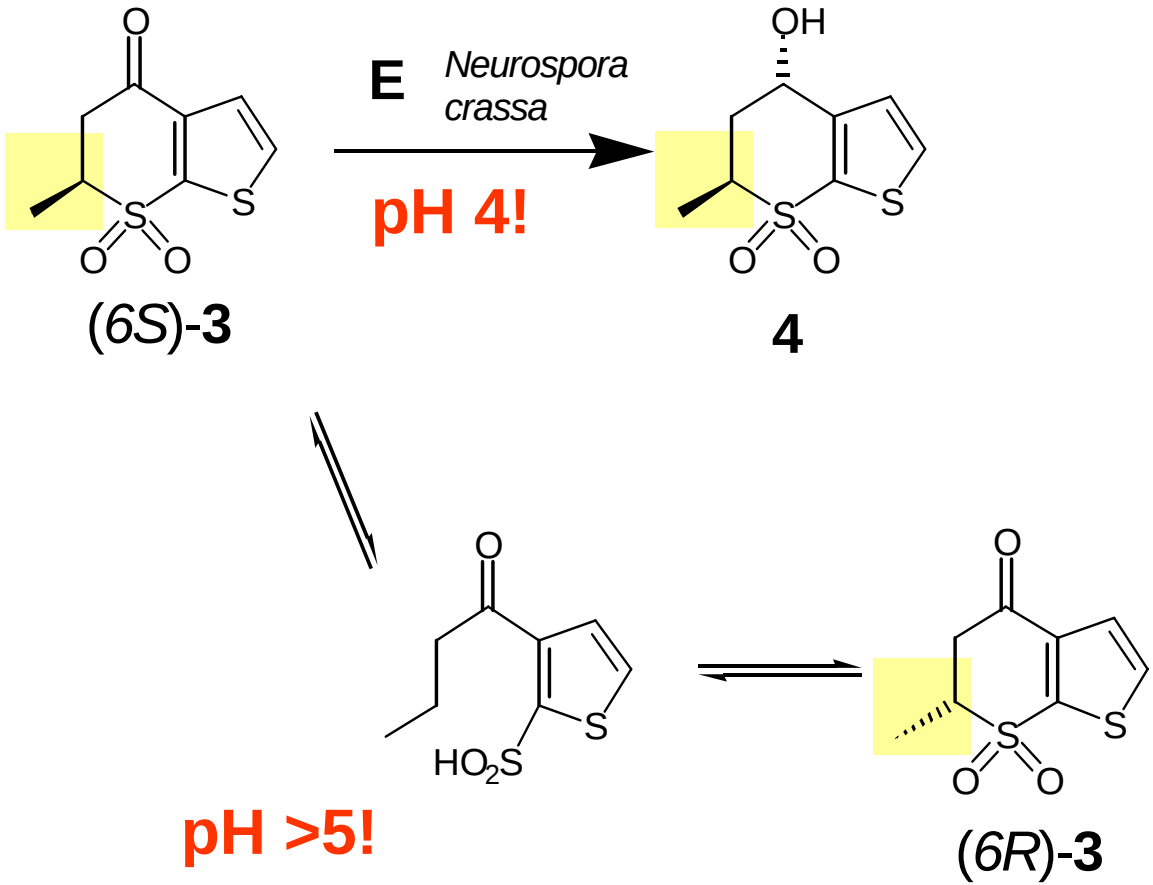


- Trusopt™ (Merck) is a novel, topically active treatment for glaucoma
- inversion of *cis* alcohol is **incomplete in chemical synthesis**

Blacker, A.J., Holt, R.A. (1997) in: Chirality In Industry II
pp. 246-261, John Wiley & Sons, New York

Why Biotransformation at pH 3.8 - 4.3 ?

to prevent epimerization that takes place above pH 5!



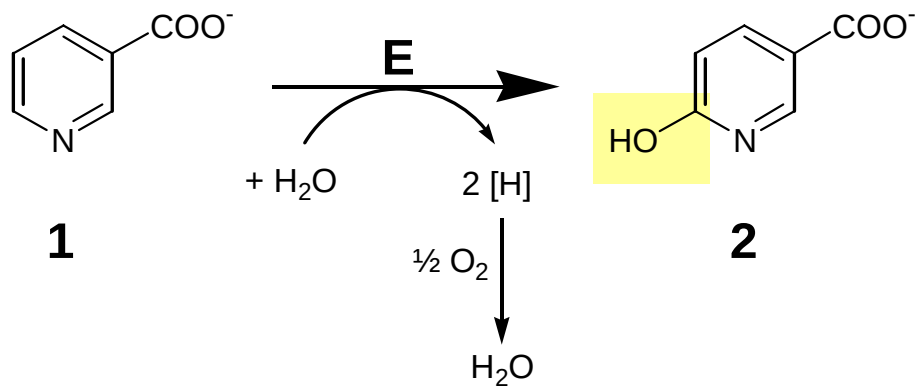
Enzymatic Oxidation Reactions

- oxidation with enzymes of EC class 1:
  - **oxidoreductases**
  $\rightarrow$ oxygenation with O_2 or H_2O_2
  ($\rightarrow$ dehydrogenation)

- oxidation with enzymes of EC class 4:
  - **lyases**
  $\rightarrow$ addition of water

Nicotinic Acid Hydroxylase EC 1.5.1.13

Achromobacter xylosoxidans



1 **2**

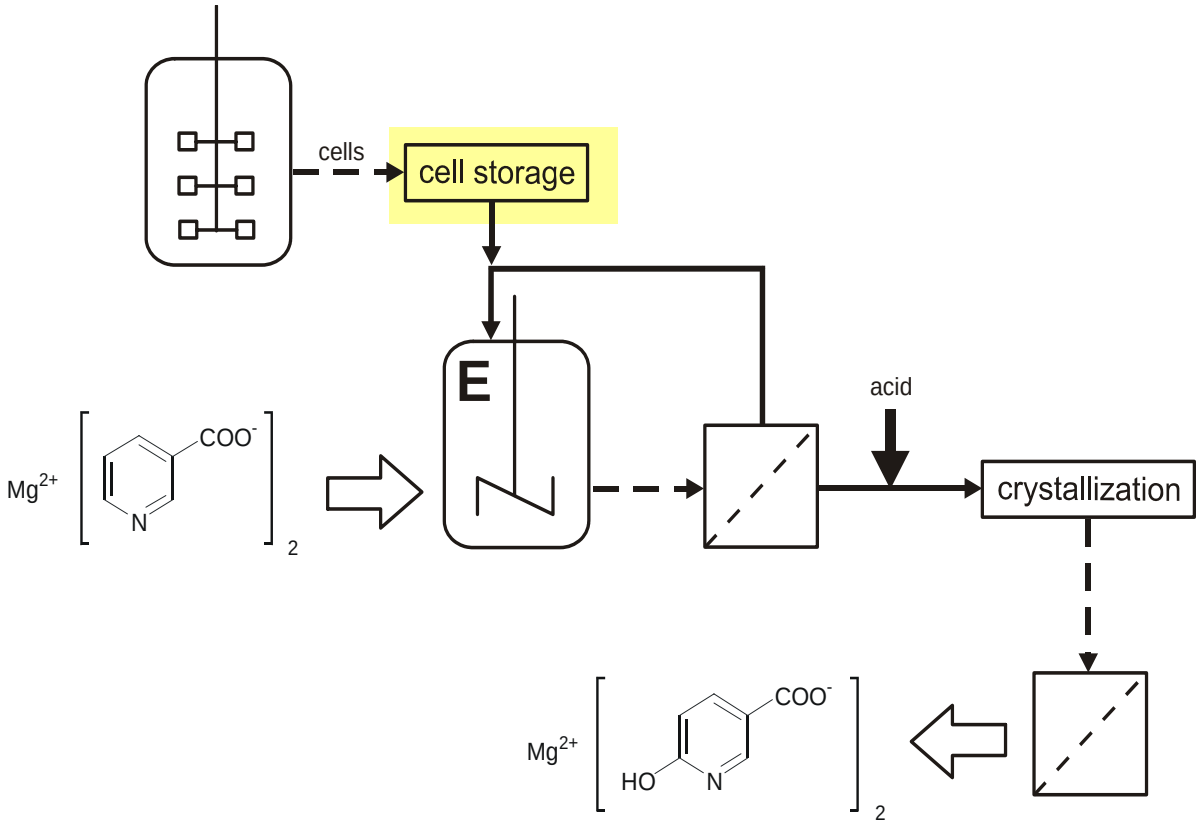
1 = niacin = nicotinic acid = pyridine-3-carboxylate
2 = 6-hydroxynicotinate = 6-hydroxy-pyridine-3-carboxylate

Lonza AG

reaction conditions:		***process parameters:***	
[1]:	0.533 M, 65 g L^{-1}	**conversion:**	>90 %
pH:	7.0	**yield:**	> 90 % (overall)
T:	30 °C	**reactor type:**	batch
catalyst:	suspended whole cells	**reactor volume:**	12 000 L
		capacity:	several tons
		residence time:	12 h

Lehky, P., Kulla, H., Mischler, S. (1995), Lonza AG, EP 0152948 A2
Kulla, H.G. (1991), Chimia **45**, 81-85

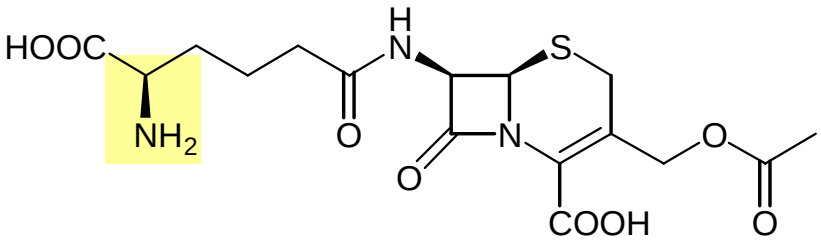
Flow Scheme



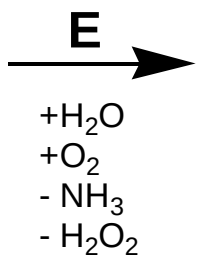
In contrast to the biotransformation the chemical synthesis of 6-substituted nicotinic acids is difficult and expensive due to the separation of by-products.

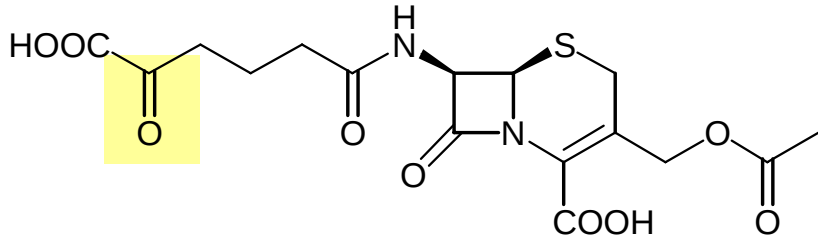
D-Amino Acid Oxidase EC 1.4.3.3

Trigonopsis variabilis



1





2

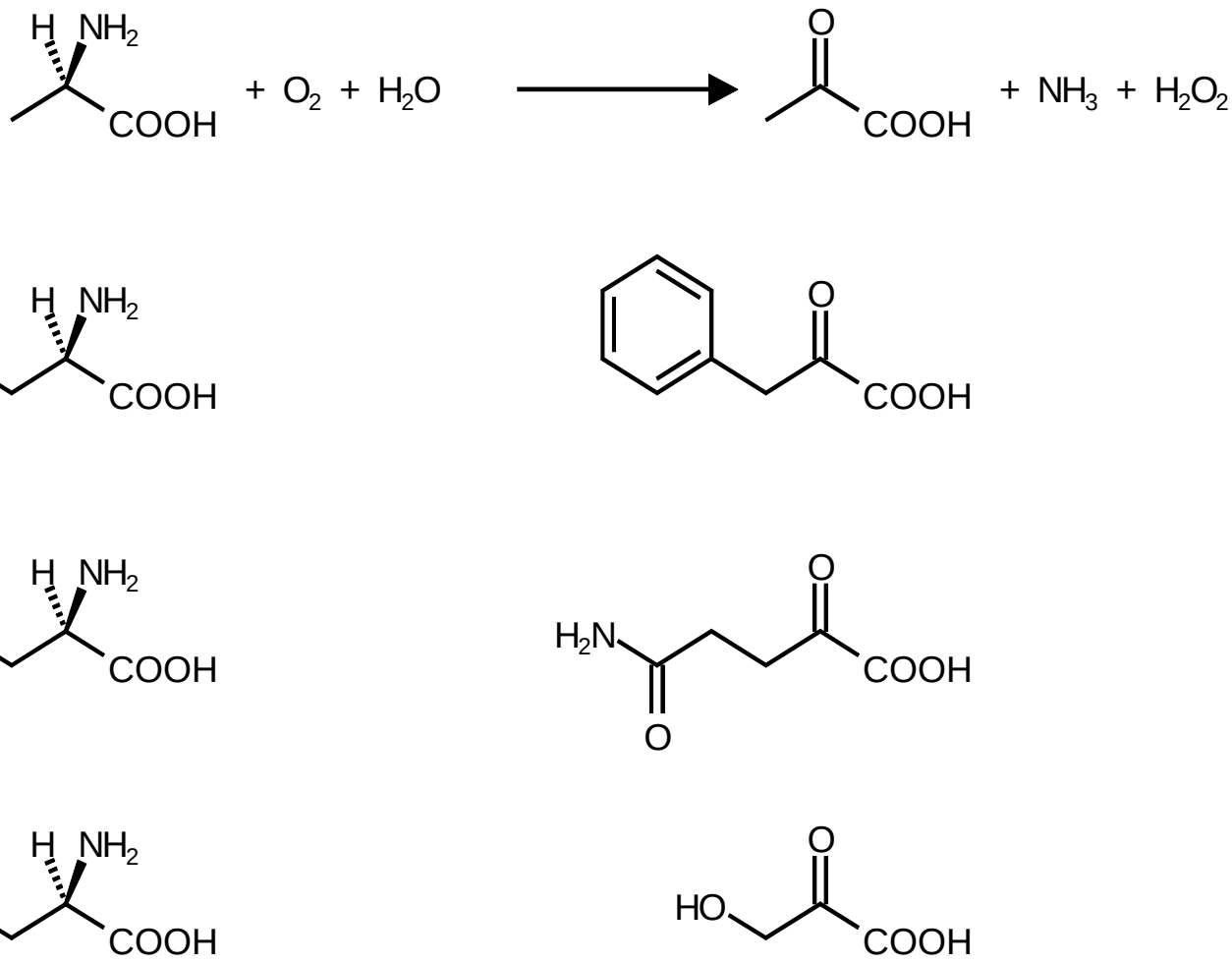
1 = Cephalosporin C
2 = α-ketoadipinyl-7-aminocephalosporanic acid

Hoechst Marion Roussel

reaction conditions:		***process parameters:***	
[1]:	0.02 M, 7.47 g L^{-1}	**reactor type:**	**batch**
pH:	7.3	**reactor volume:**	10 000 L
T:	25 °C	**capacity:**	200 $t \cdot a^{-1}$
catalyst:	**immob. enzyme**	**residence time:**	1.5 h
		dsp:	direct transfer to 7-ACA production
		enzyme consumption:	1.1 $U \cdot g^{-1}$

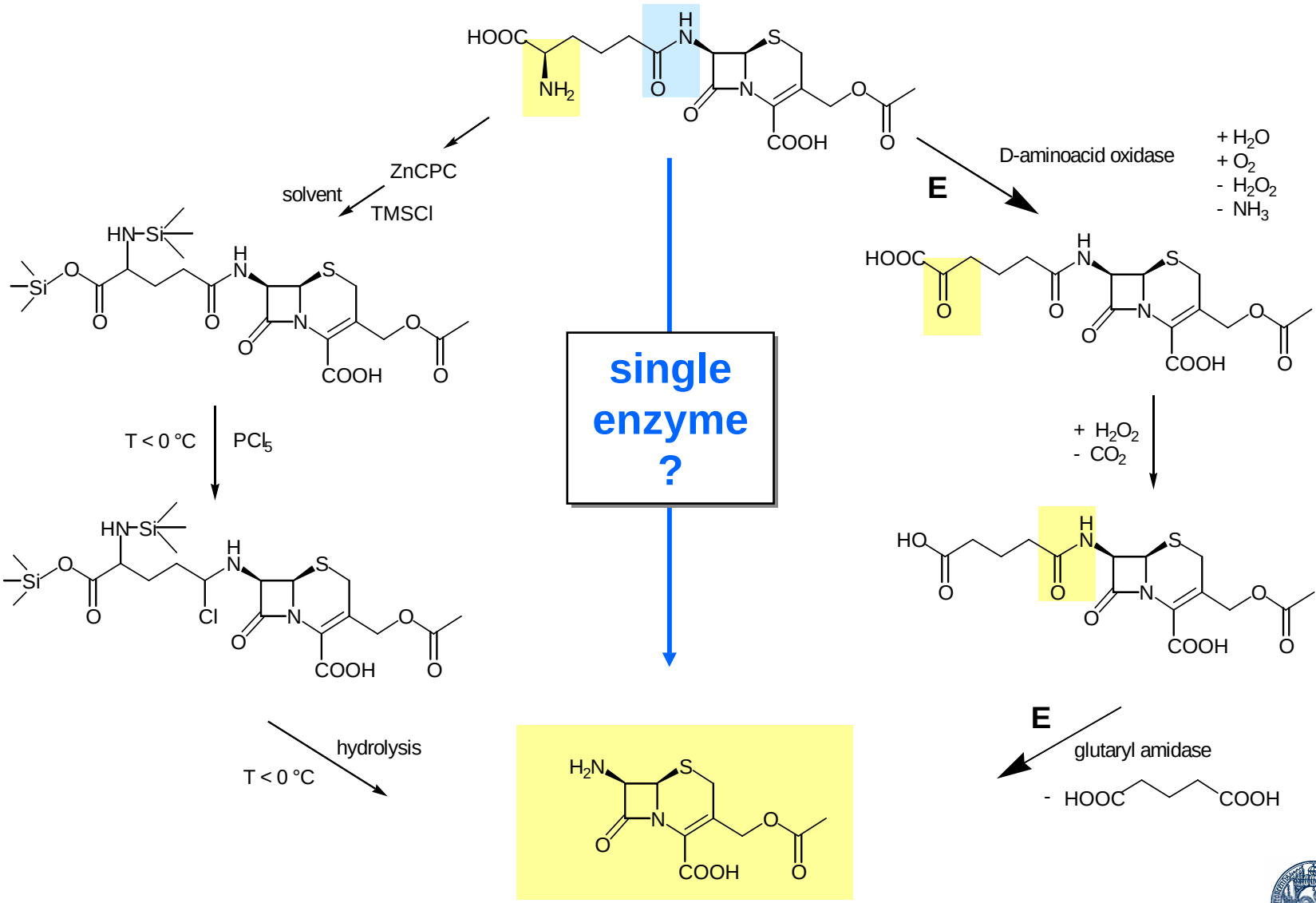
Aretz, W. (1998) Hoechst Marion Roussel, personal information

D-Amino Acid Oxidase

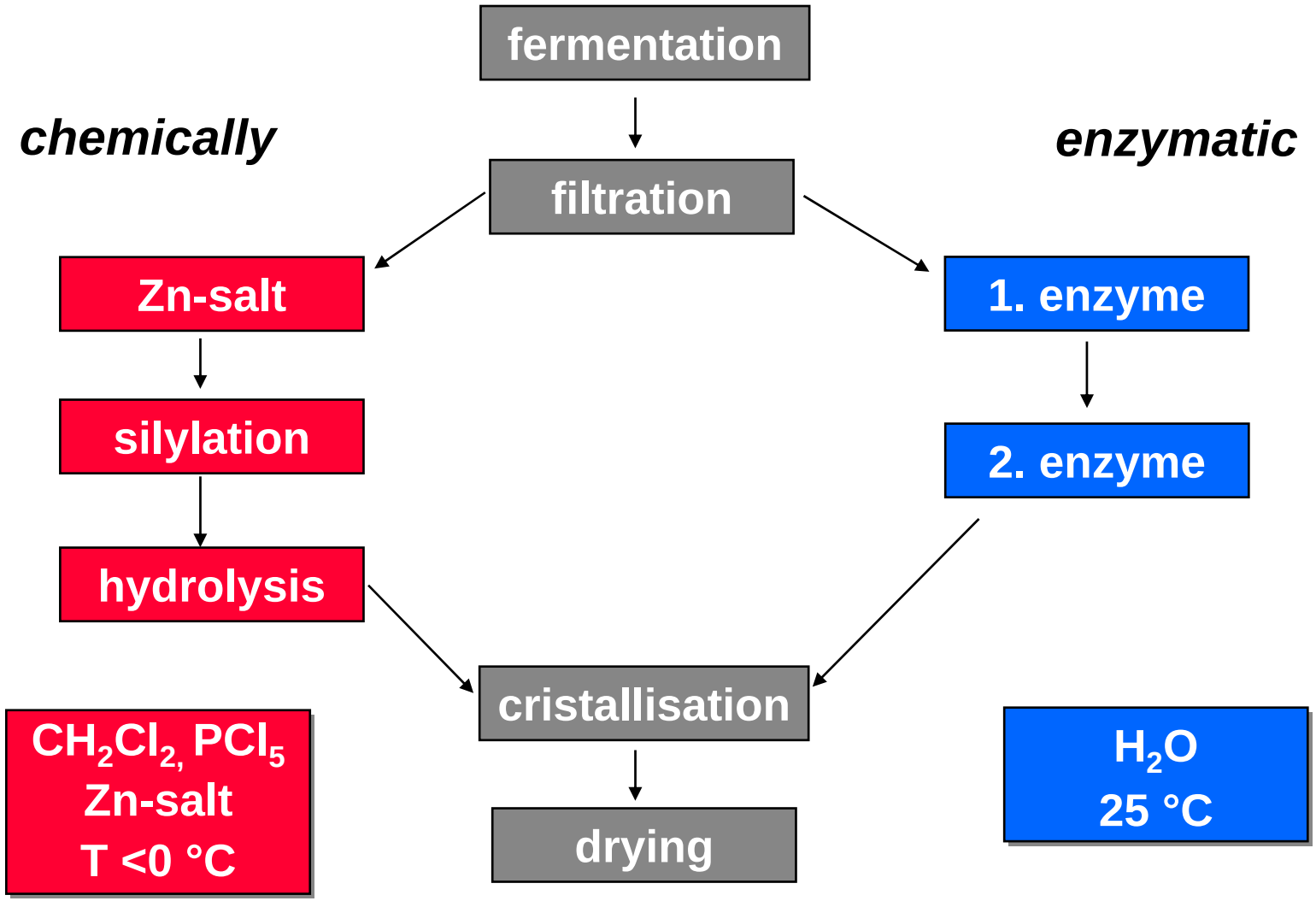


E.C. 1.4.3.3, *Trigonopsis variabilis*, 25 U/mg

Substitution of Chemical Synthesis



Synthesis of 7-ACS - Comparision of Processes



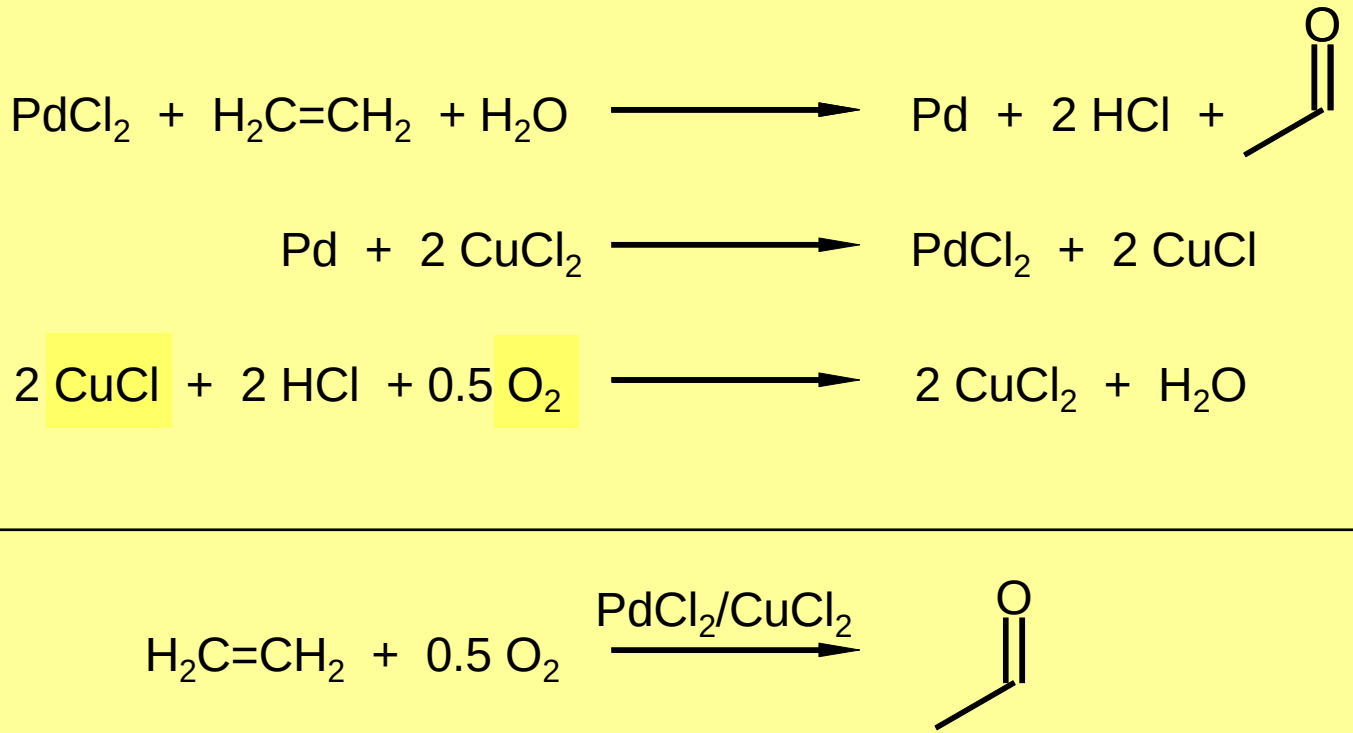
Advantages of the Enzymatic Process

- **no toxic compounds**
- waste water can be easily degraded biologically
- no dangerous chemicals
- reduction of the amount of **waste**:
  - **0.3 t** compared to **31 t** (for 1 t 7-ACA)

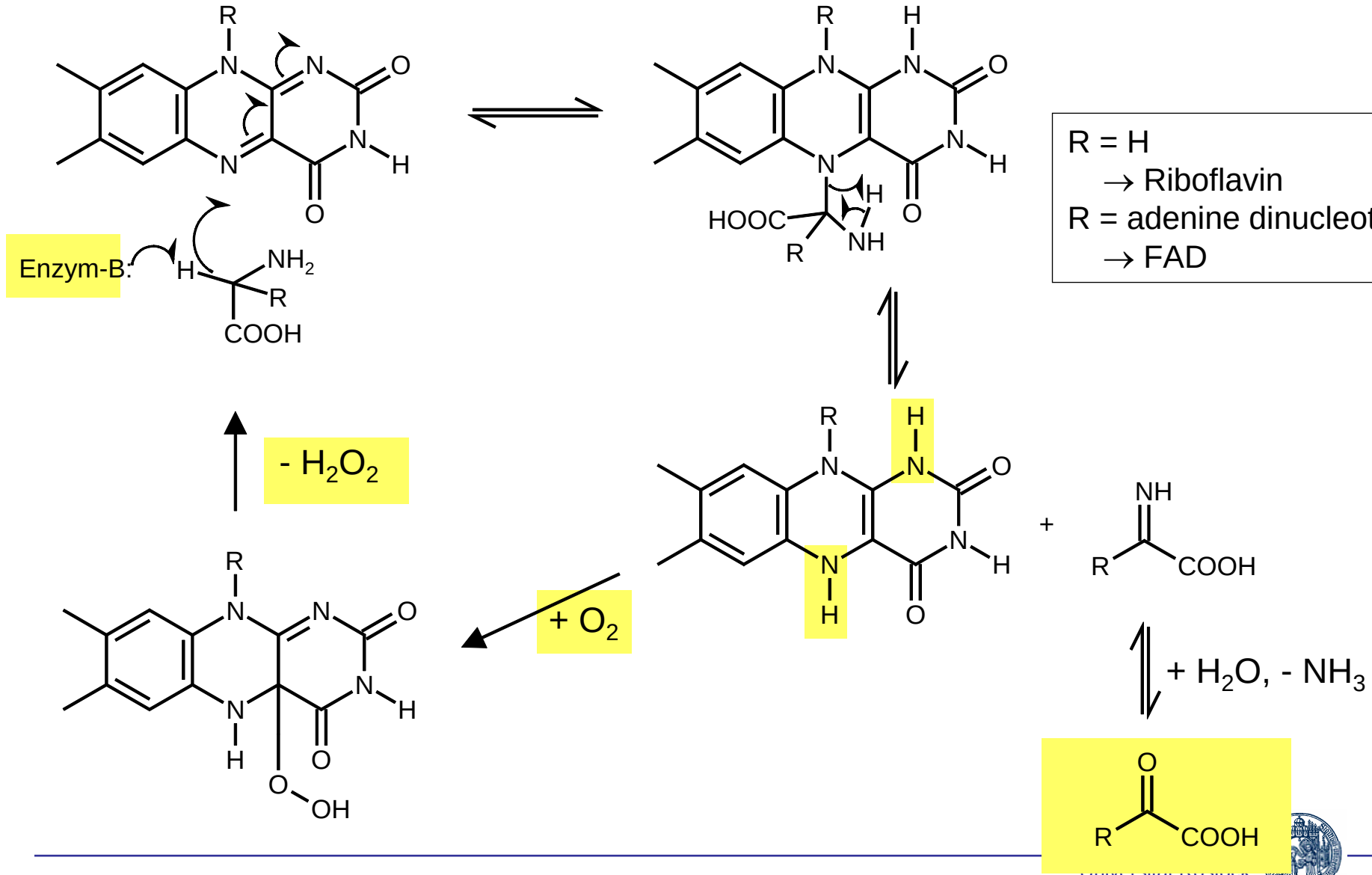
→ **reduction of the cost contribution for waste treatment and disposal from 21% to 1% of the overall process costs**

Oxygen Activation: FAD

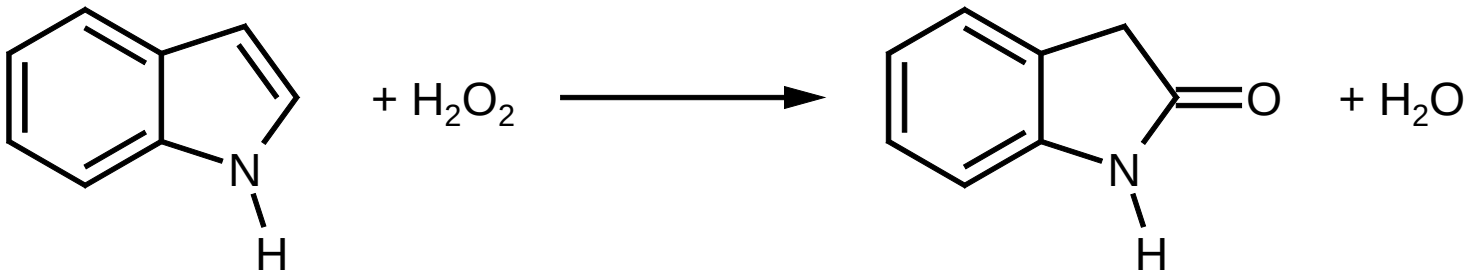
- alkene oxidation, coordinated to Pd (Wacker-process → acetaldehyde

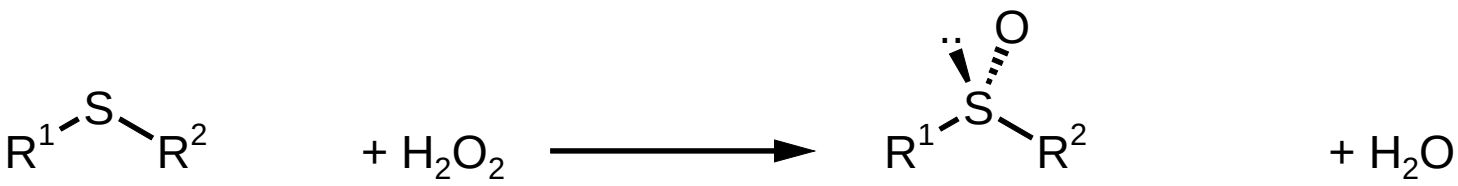


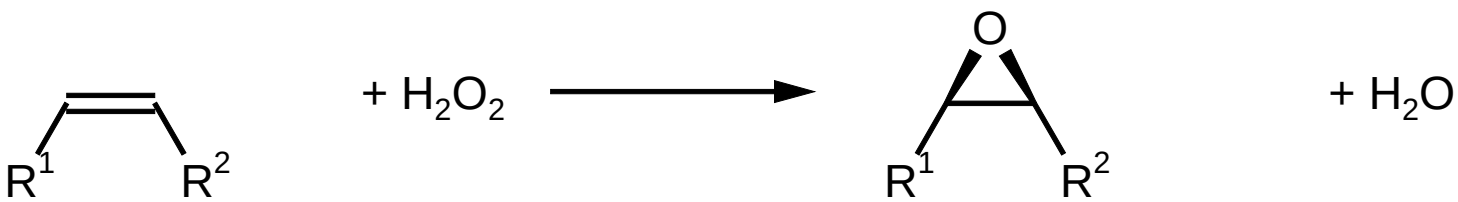
Oxygen Activation: FAD



Chloroperoxidase Catalyzed Oxidation EC 1.11.1.10

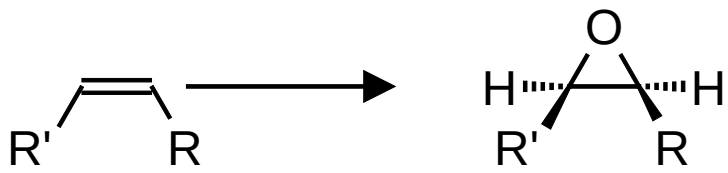


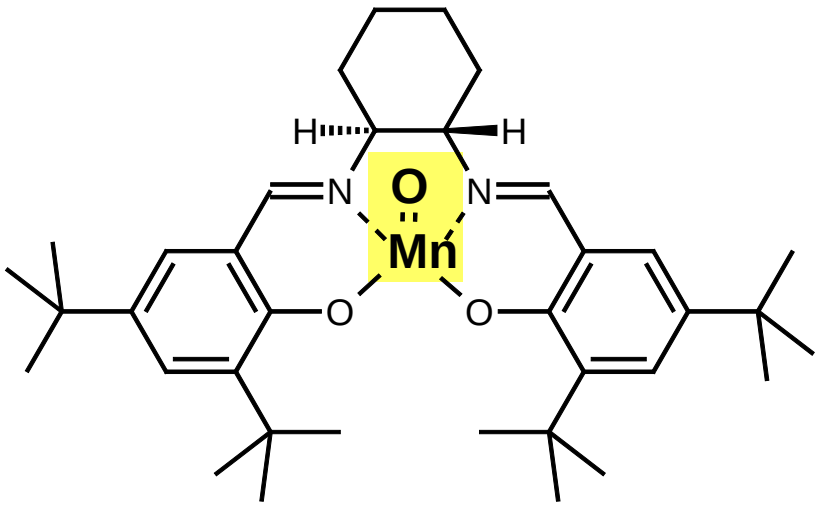




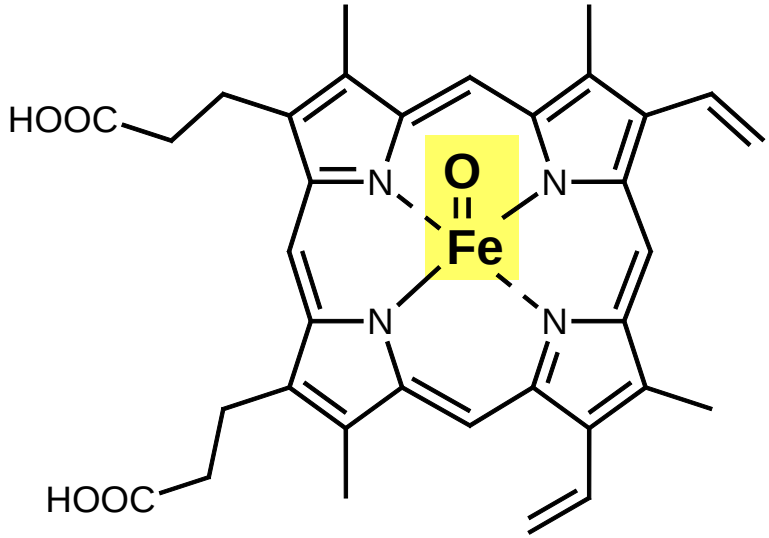
Oxygen Activation: Chemo- ↔ Biocatalyst

- oxygen transfer





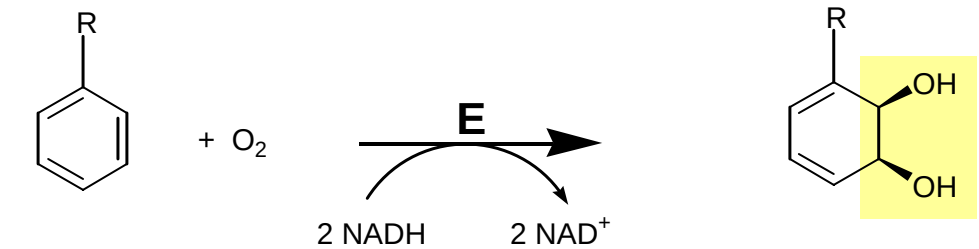
Mn-salen complex
NaOCl
0.75 min^{-1}



peroxidase (hem)
H_2O_2
75 min^{-1}

Benzoate Dioxygenase EC 1.14.12.10

Pseudomonas putida



1 (*R,S*)-**2**

R = H, F, Me, CF_3

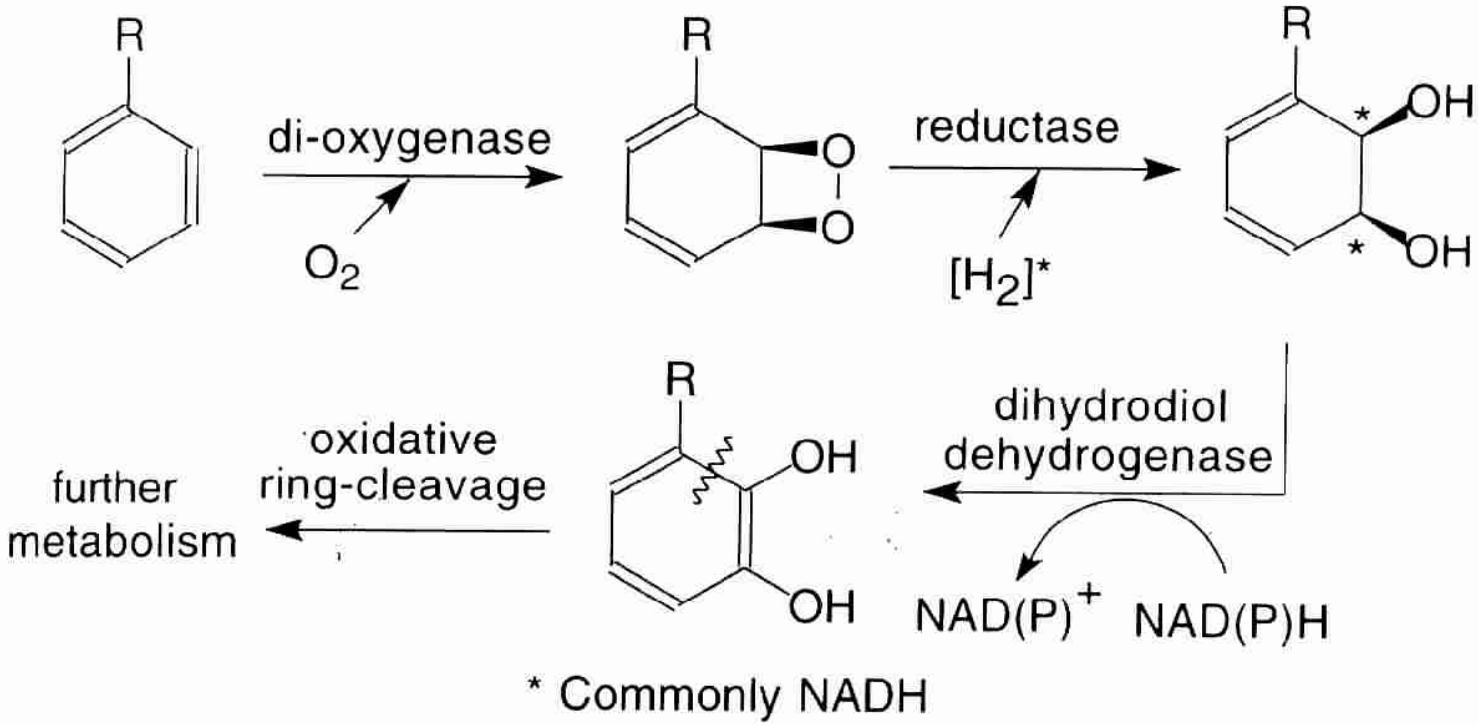
1 = benzene
2 = 1,2-dihydroxycatechol

ICI

reaction conditions:		***process parameters:***	
catalyst:	suspended whole cells	**selectivity:**	> 99.5 %
		capacity:	several t a^{-1}

Evans, C., Ribbons, D., Thomas, S., Roberts, S. (1990), Enzymatix Ltd., WO 9012798 A2

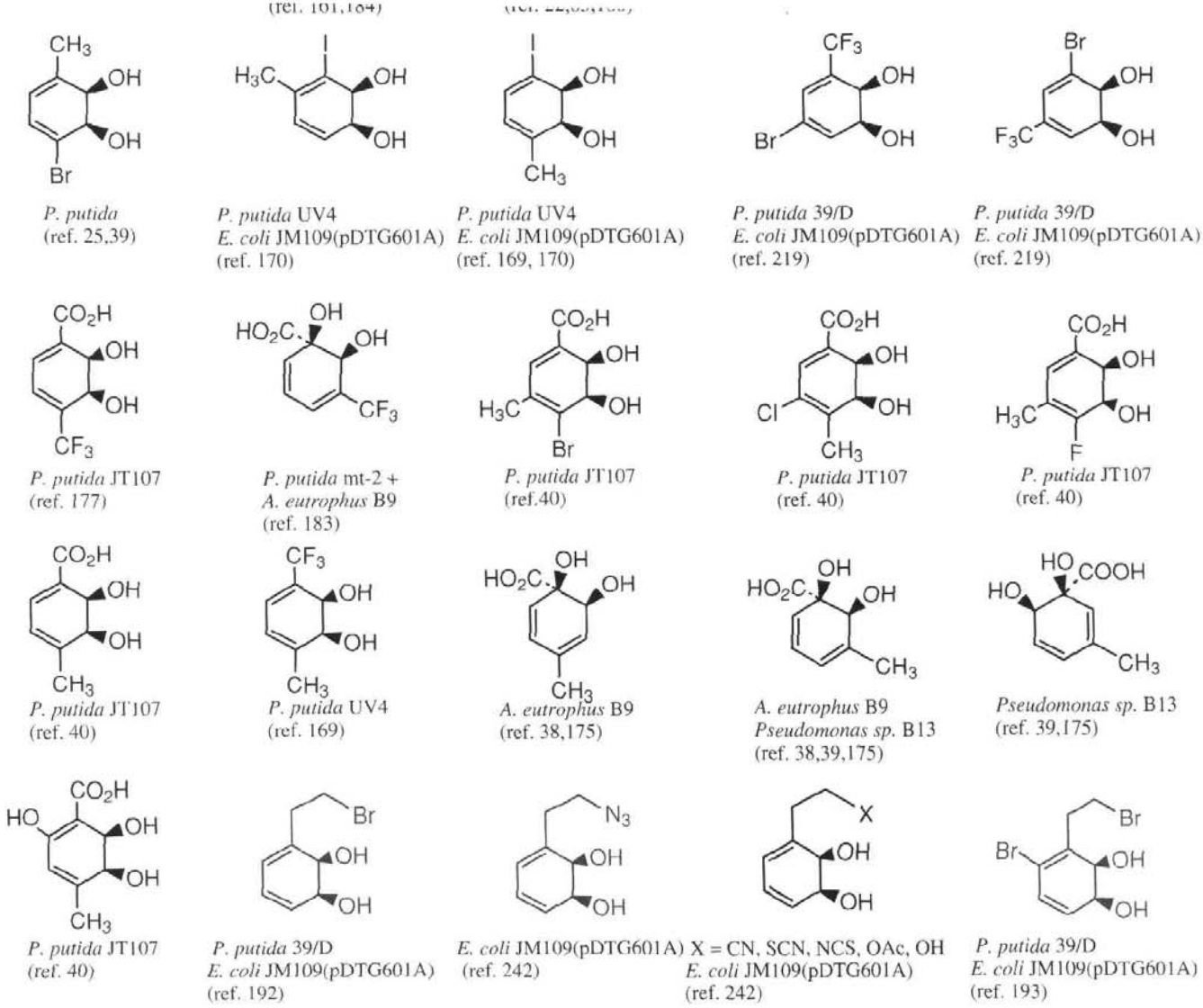
Degradation of aromatics by microbial di-oxygenases



- 1968: mutant strain from *P. putida* was lacking the activity of the dihydrodiol dehydrogenase.
- *Pseudomas putida* exhibits a high tolerance to the aromatic substrates that are normally toxic to microorganisms.

taken from K. Faber: Biotransformations in Organic Chemistry

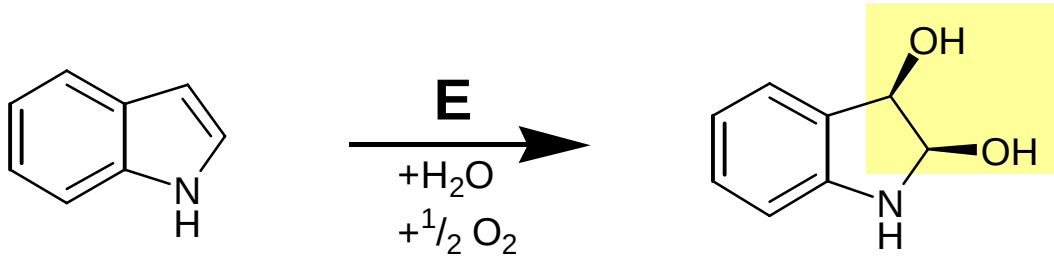
Product Range of Benzoate Dioxygenase



Hudlicky et al., Aldrichimica Acta **32**, 35-62 (1999)

Naphthalene Dioxygenase EC 1.13.11.11

Pseudomonas putida



1 **2**

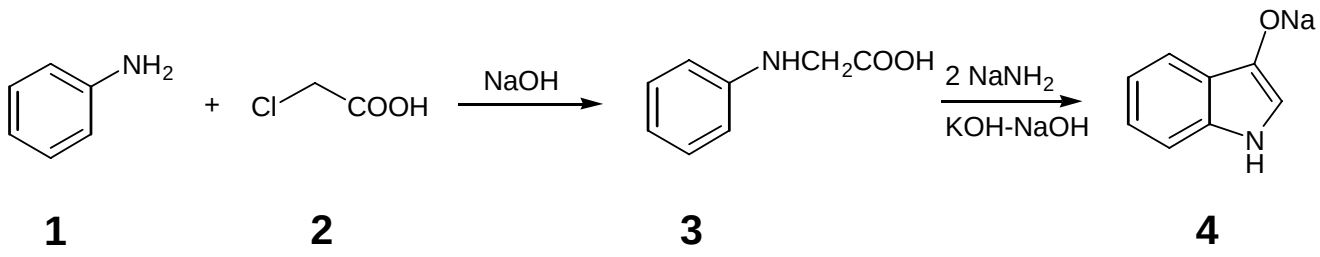
1 = 1*H*-indole
2 = 2,3-dihydro-1*H*-indole-2,3-diol

Genencor

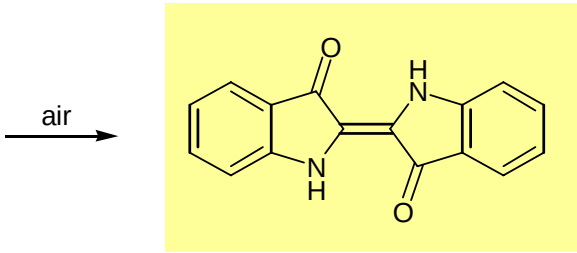
reaction conditions:		***process parameters:***	
catalyst:	whole cell	**reactor type:**	batch

Serdar, C. M., Murdock, D. C., Ensley, B. D. (1992), Amgen Inc., US 5173425

Classical Route to Indigo



1 **2** **3** **4**

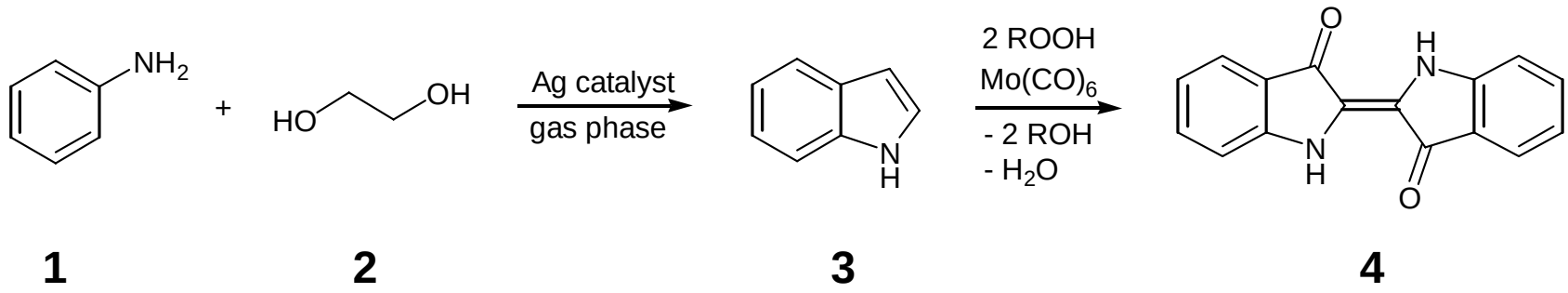


5

1 = phenylamine
2 = chloro-acetic acid
3 = phenylamino-acetic acid
4 = sodium salt of 1*H*-indole-3-ol
5 = indigo

three steps are needed starting from aniline

New Catalytic Route (Mitsui Toatsu Chemicals)

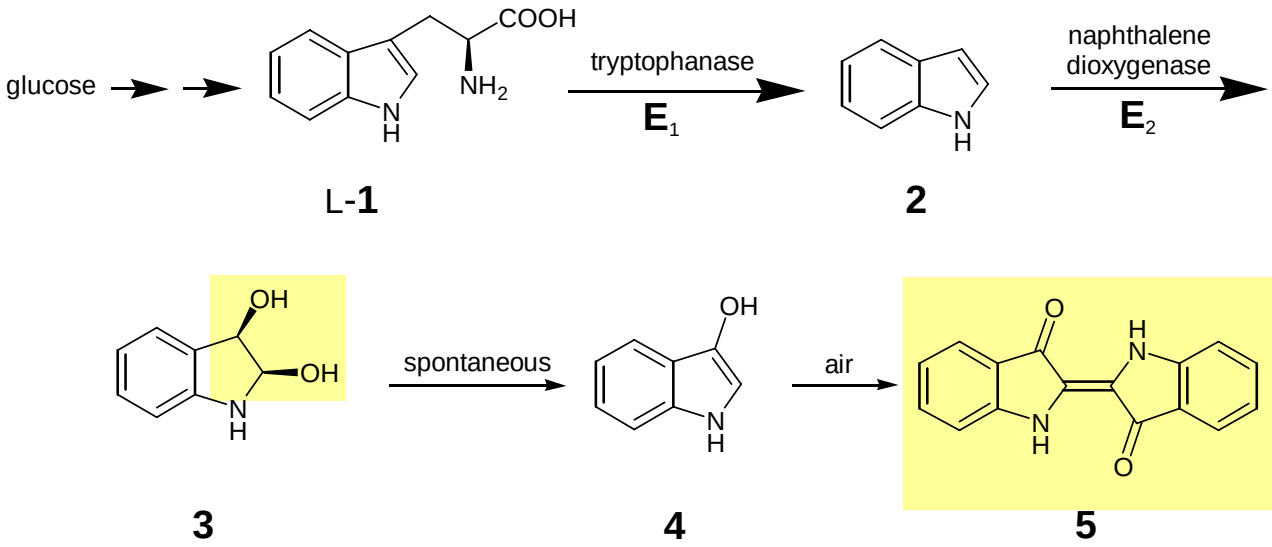


1 **2** **3** **4**

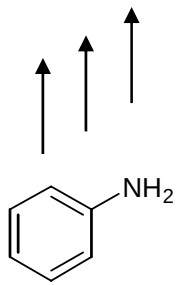
1 = phenylamine
2 = ethane-1,2-diol
3 = 1*H*-indole
4 = indigo

⇒ produces less organic salts than the classical route

Biosynthesis of Indigo



1 = L-tryptophan
2 = 1*H*-indole
3 = 2,3-dihydro-1*H*-indole-2,3-diol
4 = 1*H*-indole-3-ol
5 = indigo

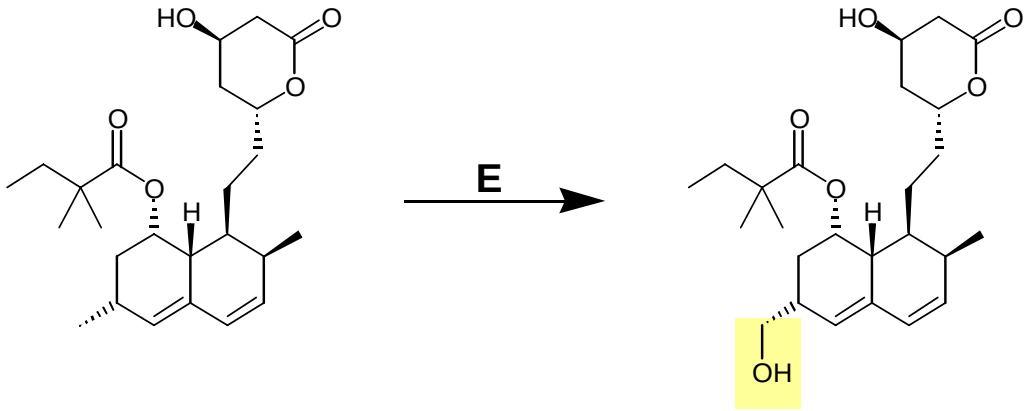


classical chemical route

starts from cheap glucose with tryptophan as intermediate

Murdock, D., Ensley, B. D., Serdar, C., Thalen, M. (1993), Bio/Technology, **11**, 381-385

Nocardia autotropica



1 **2**

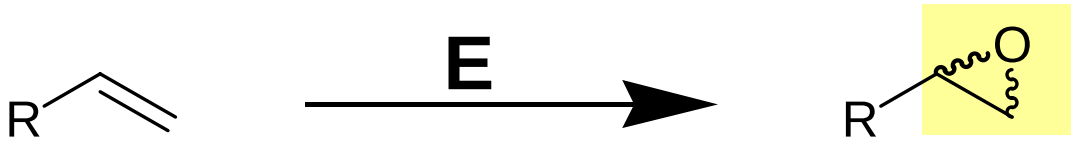
1 = Simvastatin
2 = 6-β-hydroxy-methyl-simvastatin (major product)

Merck Sharp & Dohme

reaction conditions:		***process parameters:***	
[1]:	< 0.05 10^{-3} M, < 0.02 g L^{-1}	**yield:**	24 %
[2]:	1.85 10^{-3} M, 0.8 g L^{-1}	**selectivity:**	70 %
pH:	6.8	**reactor type:**	fed batch
T:	27 °C	**reactor volume:**	19,000 L
catalyst:	suspended whole cells		

Gbewonyo, K., Buckland, B.C., Lilly, M.D. (1991), Biotech. Bioeng. **37**, 1101-1107

Nocardia corallina

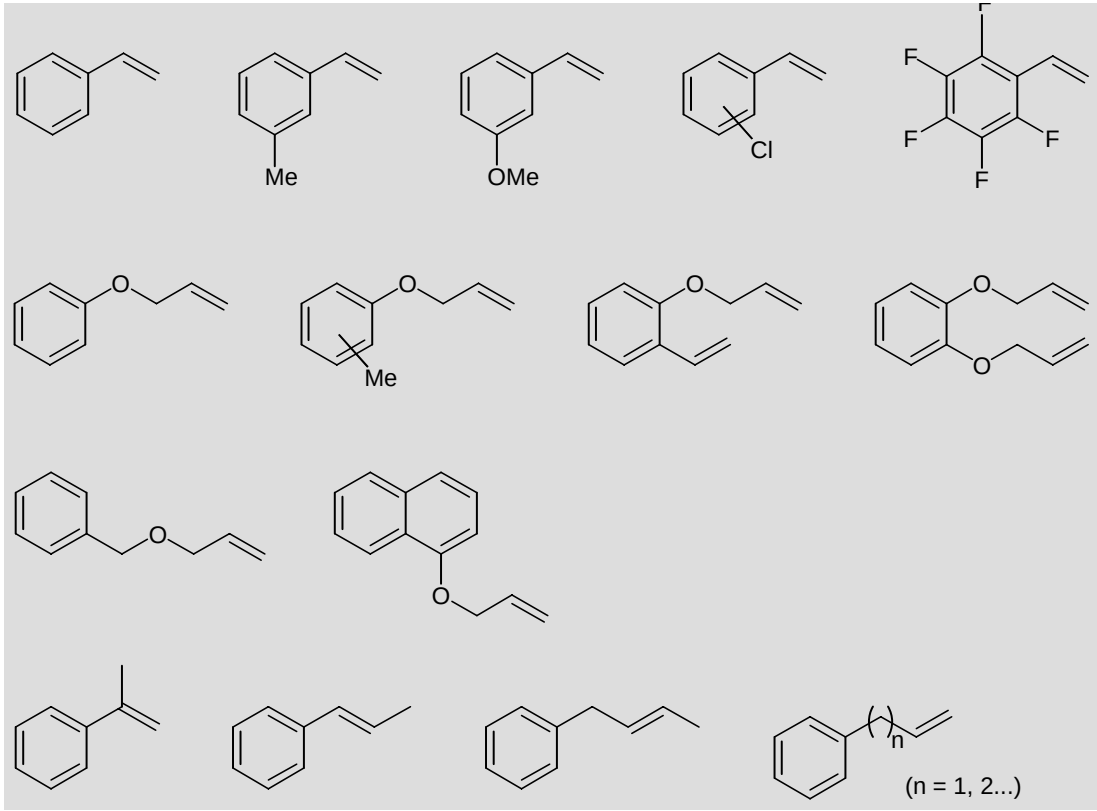


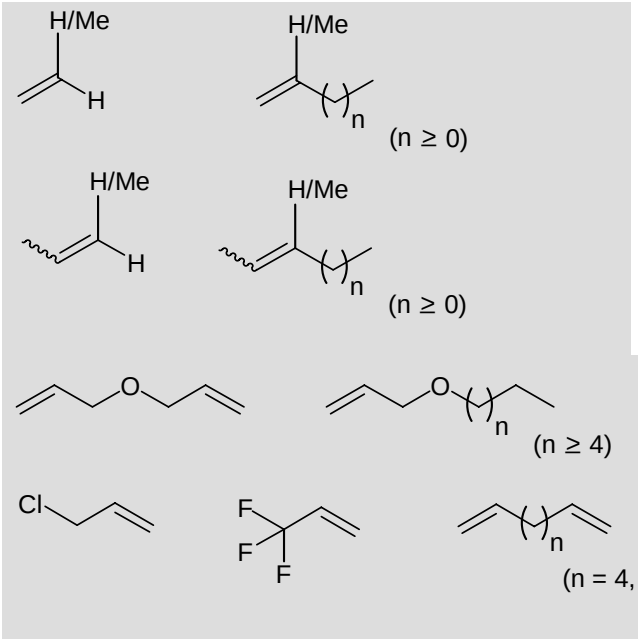
1 **2**

Nippon Mining

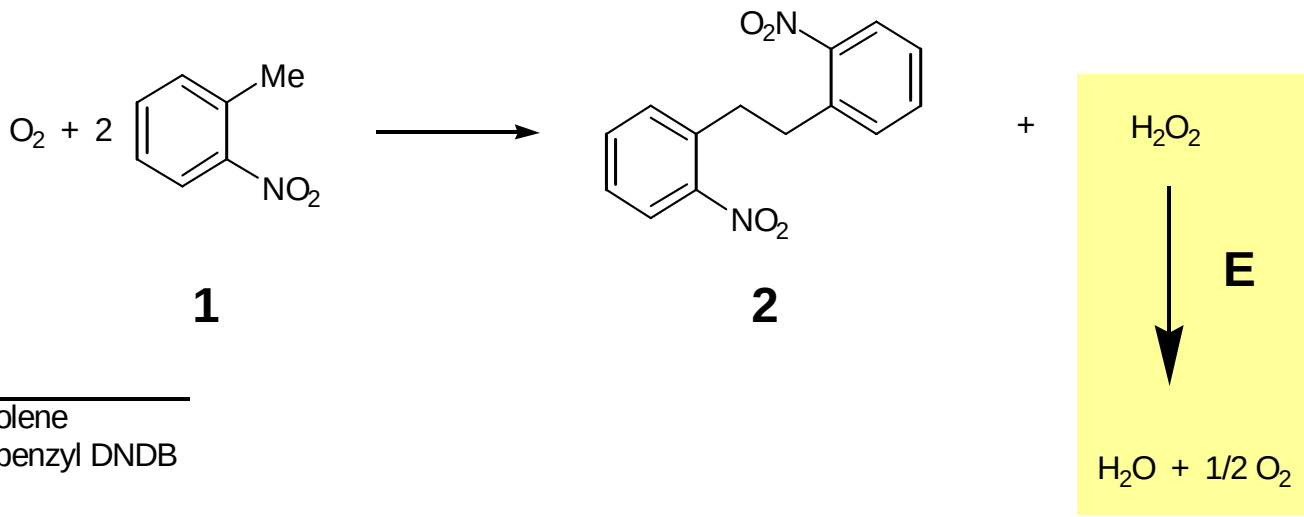
reaction conditions:		***process parameters:***	
[1]:		**yield:**	92 %
two phase system		**selectivity:**	94 %
pH:		**reactor type:**	batch
T:	30 °C	**rate of aeration is raised to strip the short chain toxic epoxide**	
catalyst:	suspended whole cells		

Substrate range





Microbial source

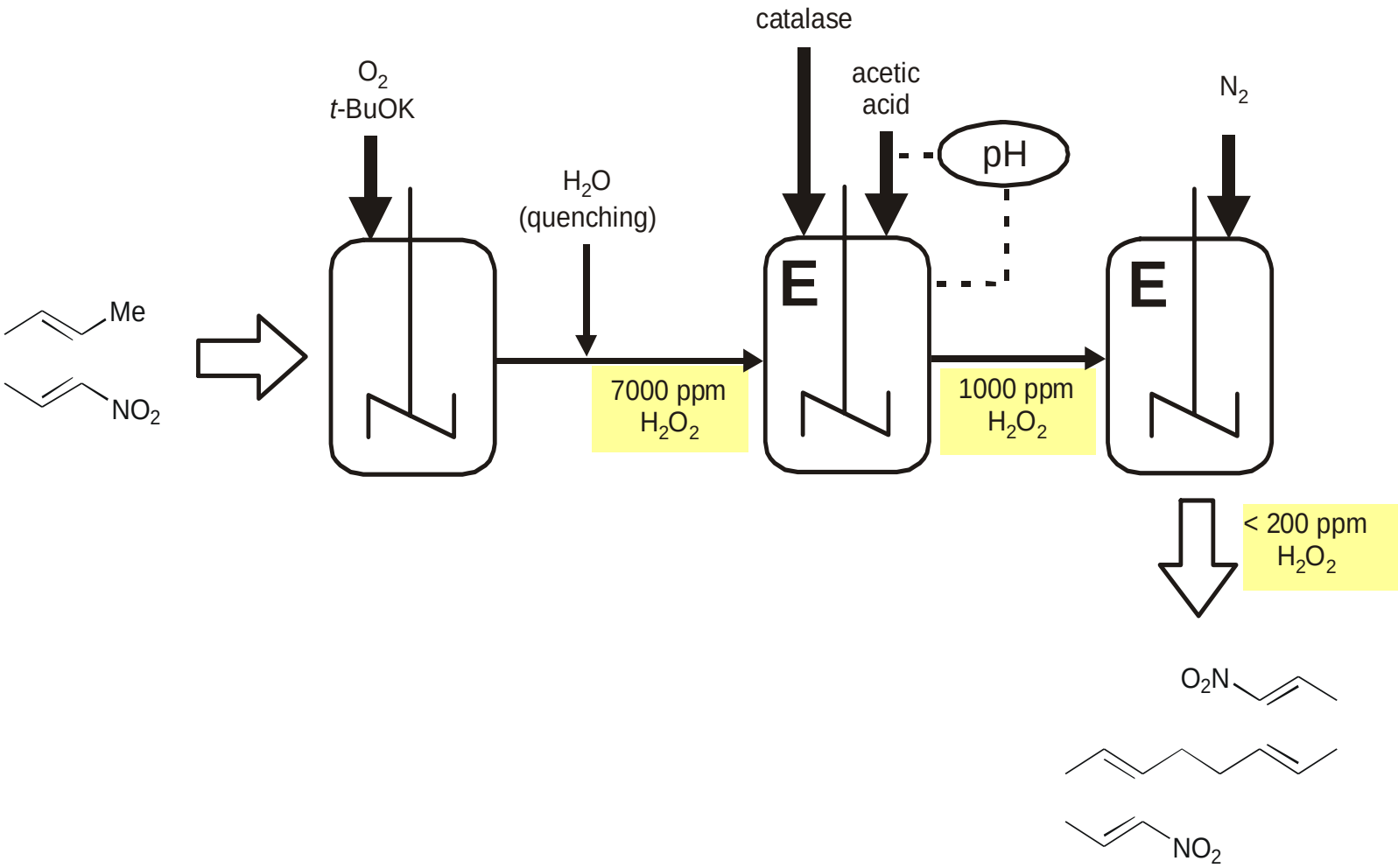


1 = nitrotoluolene
2 = dinitrodibenzyl DNDB

Novartis

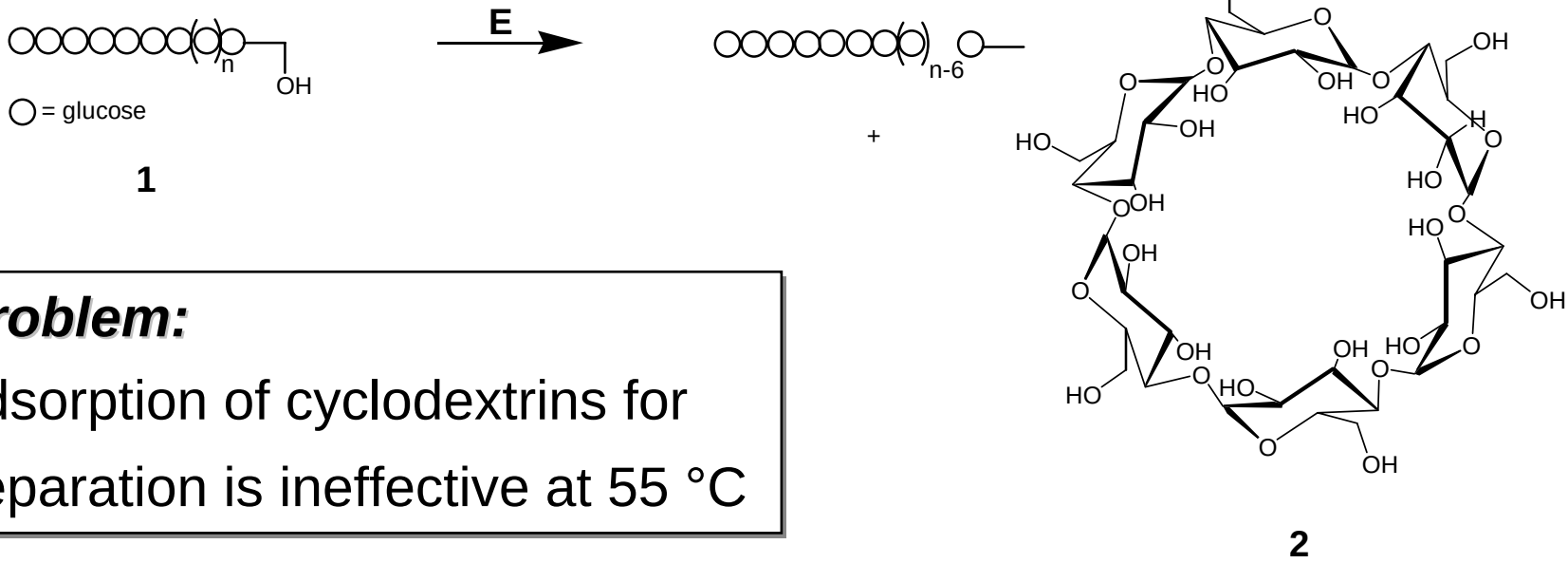
- **removal of side-product**
- only incomplete conversion with heavy-metal catalysts
- following process steps with DNDB are problematic by contamination with heavy-metal catalyst.

Flow Scheme


catalase
O_2
t-BuOK
acetic
acid
N_2
pH
H_2O
(quenching)
Me
NO_2
E
E
7000 ppm
H_2O_2
1000 ppm
H_2O_2
< 200 ppm
H_2O_2
O_2N
NO_2

Cyclodextrin Glycosyltransferase EC 2.4.1.19

Bacillus circulans



1 **2**

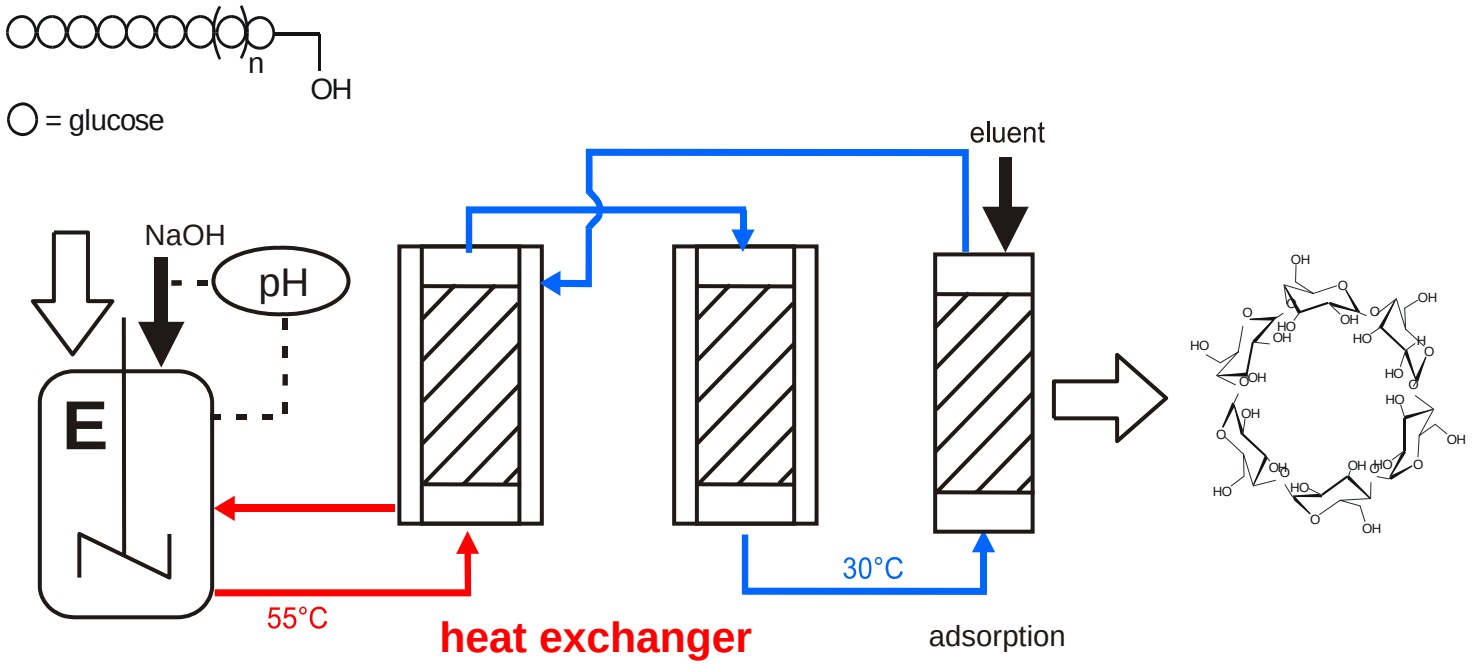
Problem:

adsorption of cyclodextrins for separation is ineffective at 55 °C

reaction conditions:		***process parameters:***	
[1]:	8.3 w/v % liquified starch	**yield:**	22.3 % (α-cyclodextrin)
pH:	5.8 – 6.0		10.8 % (β-cyclodextrin)
T:	**55 °C**		5.1 % (γ-cyclodextrin)
catalyst:	soluble enzyme	**chemical purity:**	94.9 %
		reactor type:	batch

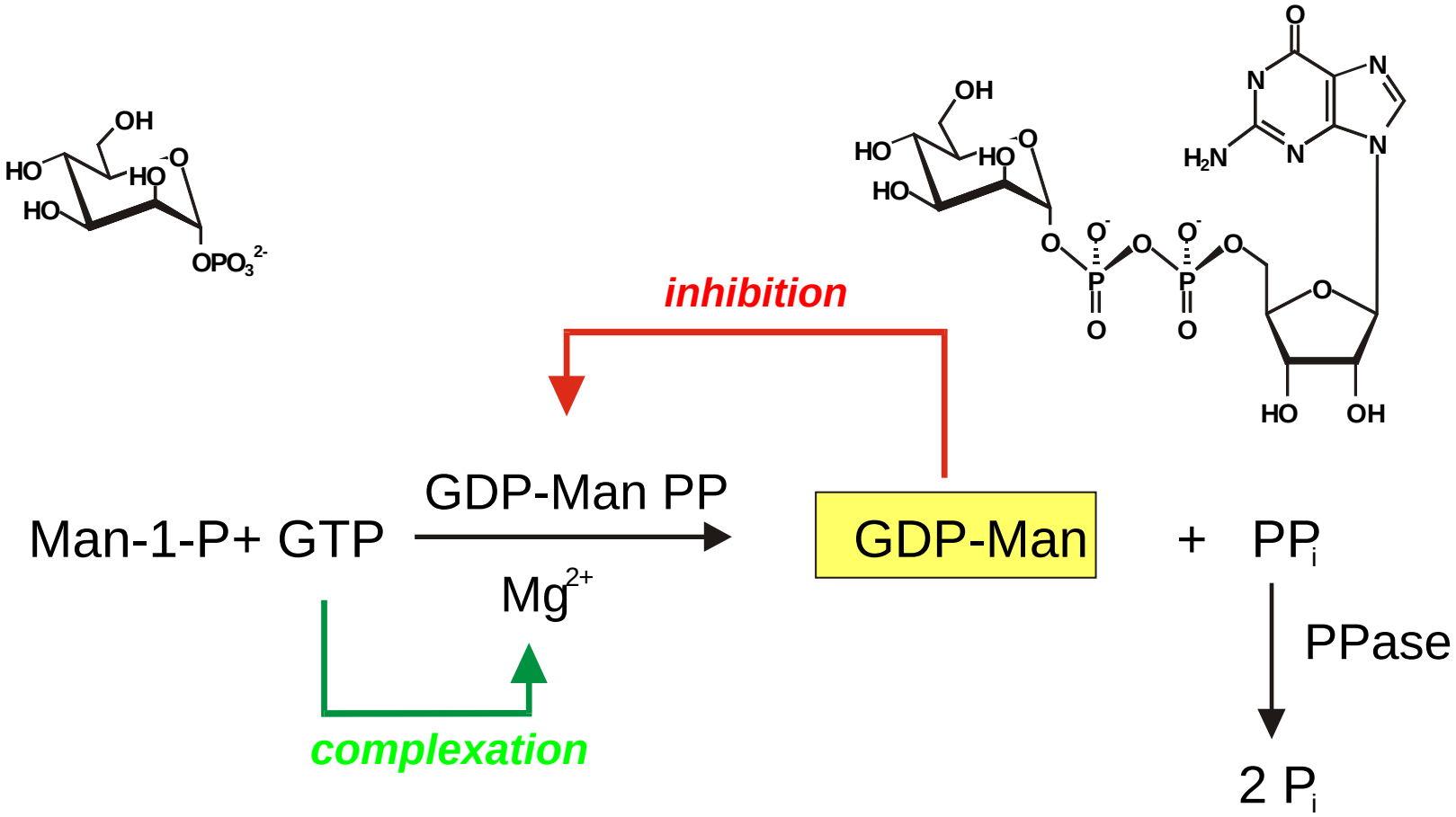
Chin, P.J., Chein, S. (1989), J. Chem. Technol. Biotechnol. **46**, 283-294

Flow Scheme - Integrated Process



- **Temperature is lowered to 30 °C for effective adsorption**. (At this temperature almost no cyclodextrins are formed during circulation.)
- Before reentering the main reactor the temperature of the solution is again adjusted to 55 °C.
- To **prevent adsorption of the cyclodextrin glycosyltransferase** on the columns 3 w/v % NaCl are added.

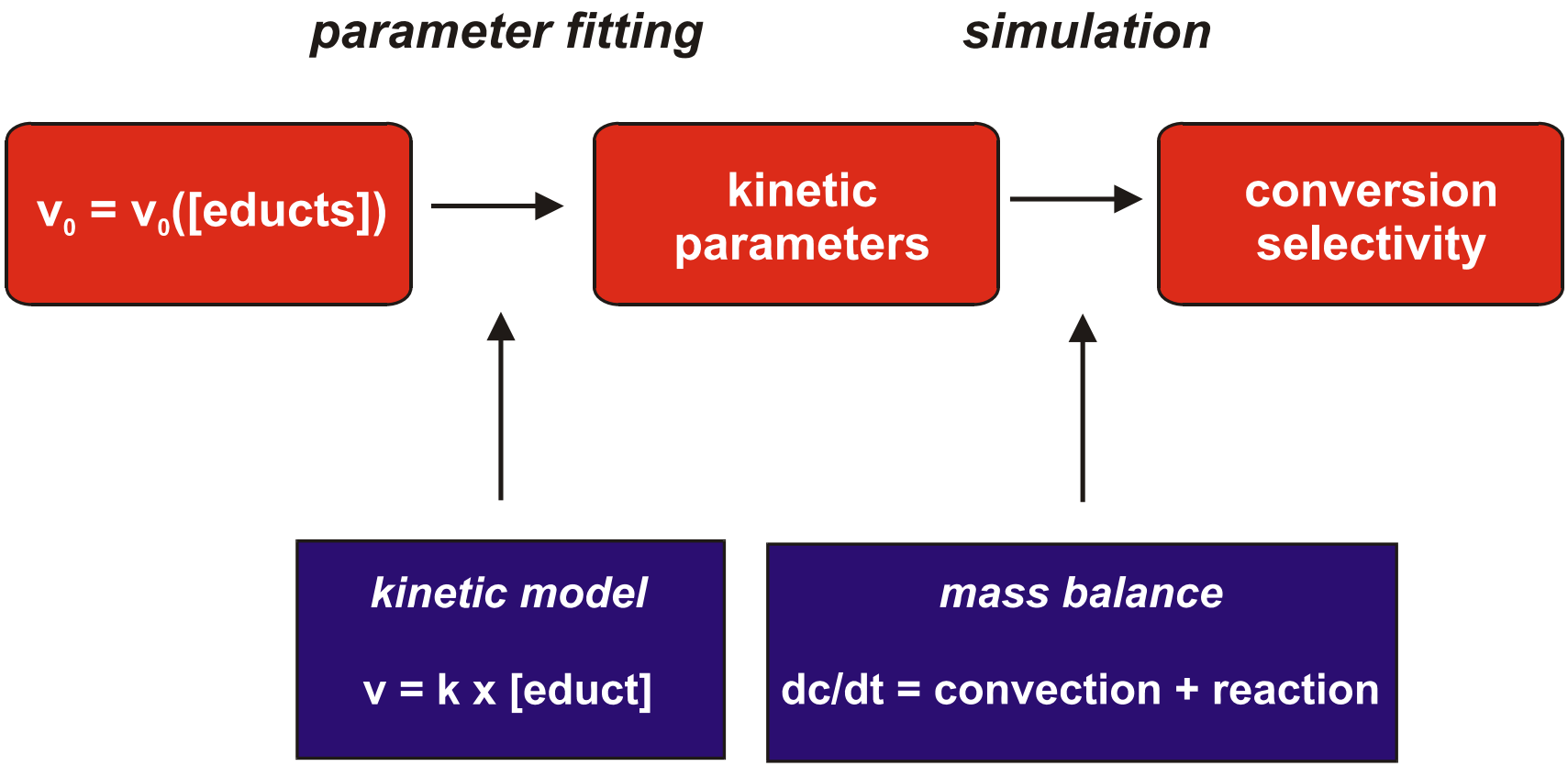
Enzymatic Synthesis of GDP-Mannose



- GDP-Man-pyrophosphorylase
- inorganic pyrophosphatase

Elling et al., Glycobiology **6**, 591 (1996)

Modelling of Processes

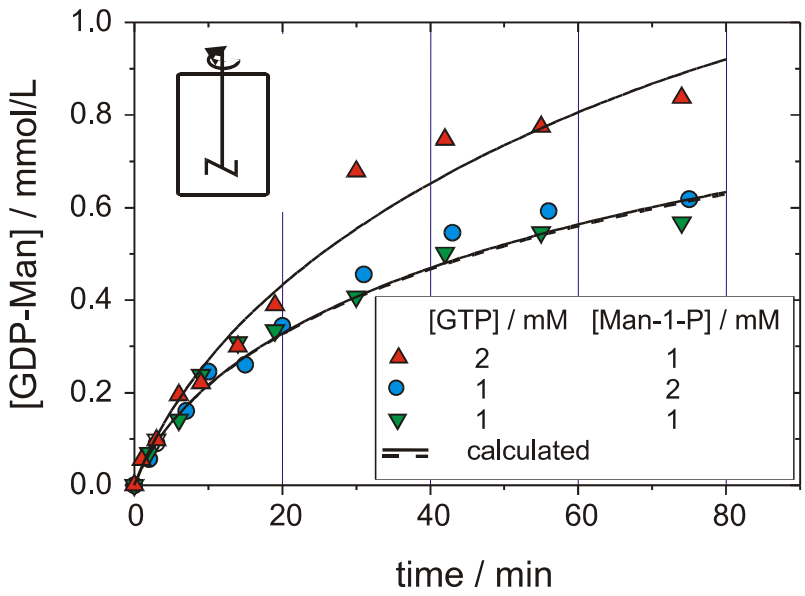


Kinetics of GDP-Man Pyrophosphorylase

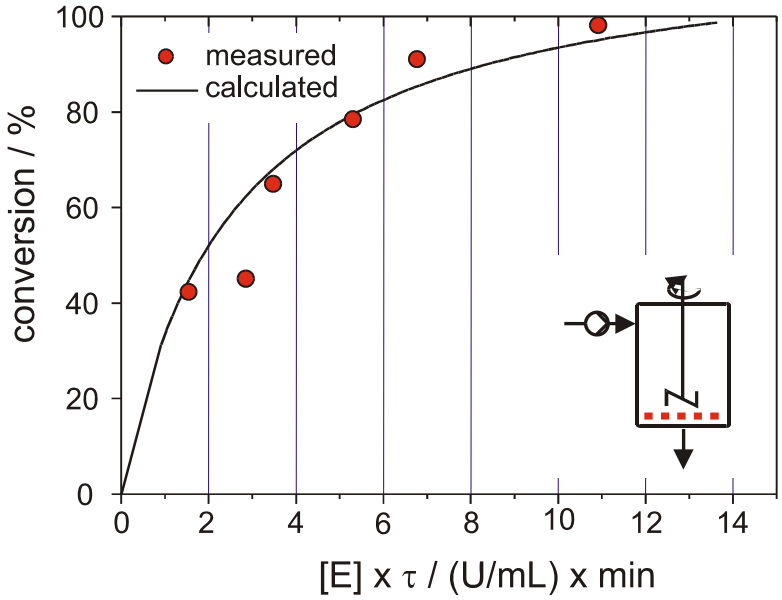
$$v_1 = [E] \times A_{max}^{GMPP} \times \frac{1}{1 + \frac{K_M^{Mg}}{\left(\frac{[Mg]}{[GTP]}\right)^2} + \frac{[Mg]}{K_i^{Mg} \times [GTP]}} \times \frac{[GTP]}{K_M^{GTP} \times \left(1 + \frac{[GDP-Man]}{K_i^{GDP-Man}} + \frac{[PP_i]}{K_i^{PP_i}}\right) + [GTP]} \times \frac{[Man-1-P]}{K_M^{Man-1-P} + [Man-1-P]}$$

A_{max}^{GMPP}	15.0	U/mg	±	12.2	U/mg
K_M^{GTP}	40	µmol/L	±	9	µmol/L
$K_i^{GDP-Man}$	9	µmol/L	±	2	µmol/L
K_i^{PPi}	16	µmol/L	±	4	µmol/L
$K_M^{Man-1-P}$	15	µmol/L	±	3	µmol/L
K_M^{Mg}	2.9	-	±	2.6	-
K_i^{Mg}	0.1	-	±	0.09	-

Verification of Kinetic Model

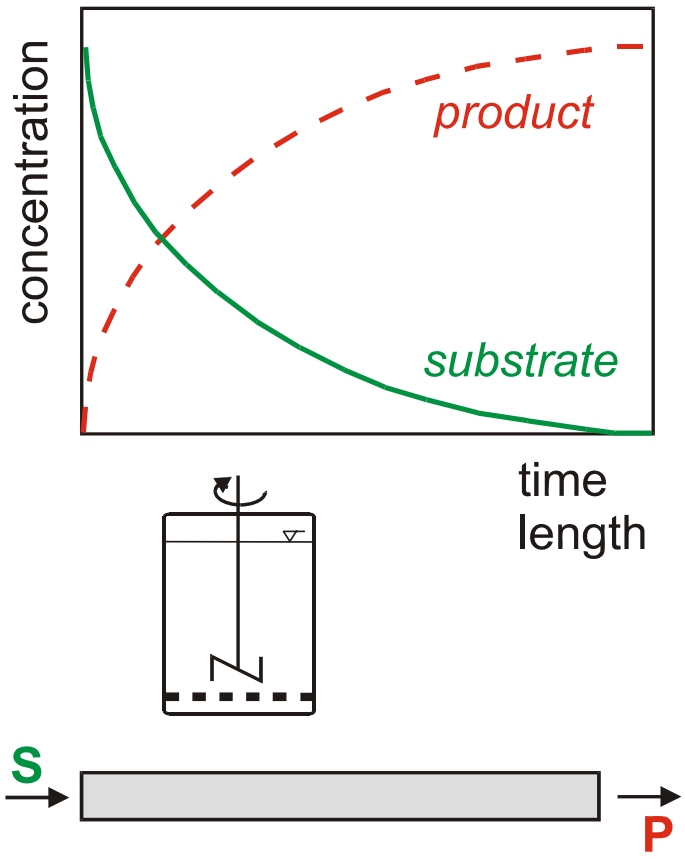


GDP-Man PP	0.044	U/mL
Pyrophosphatase	1.0	U/mL
$MgCl_2$	5.0	mM

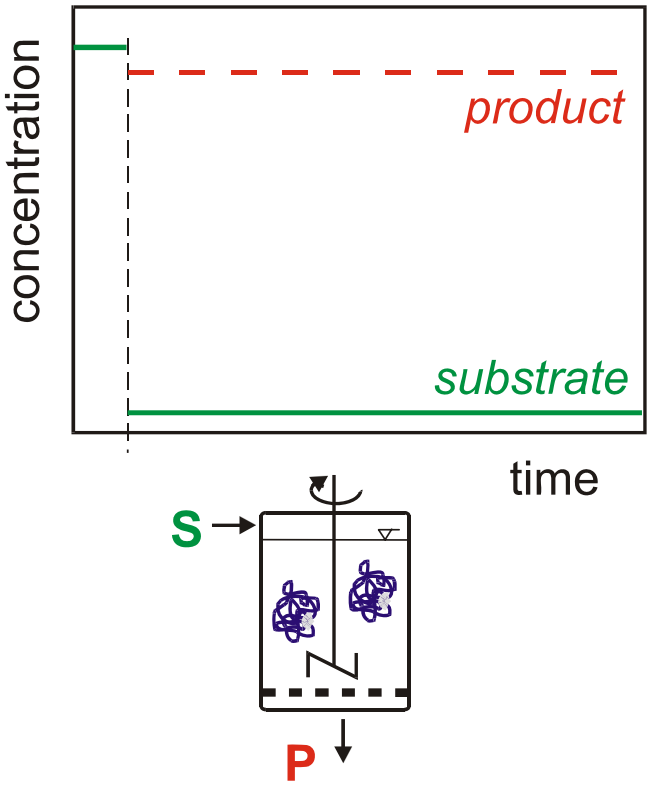


Man-1-P	2	mM
GTP	1	mM
25 °C, pH 8.0		

Concentration Profiles of Different Reactors

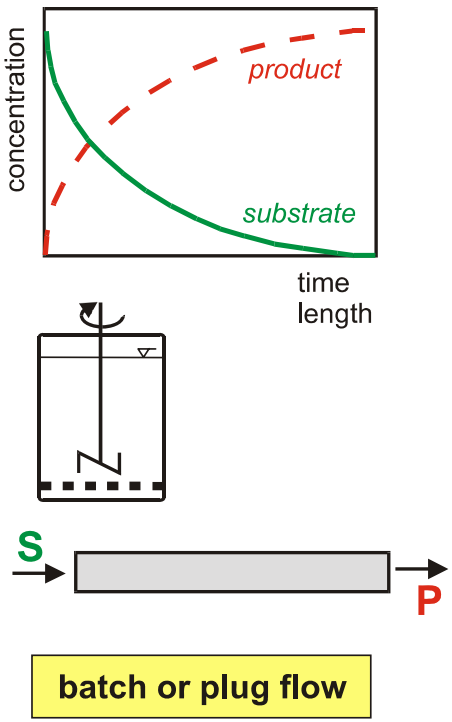


batch or plug flow

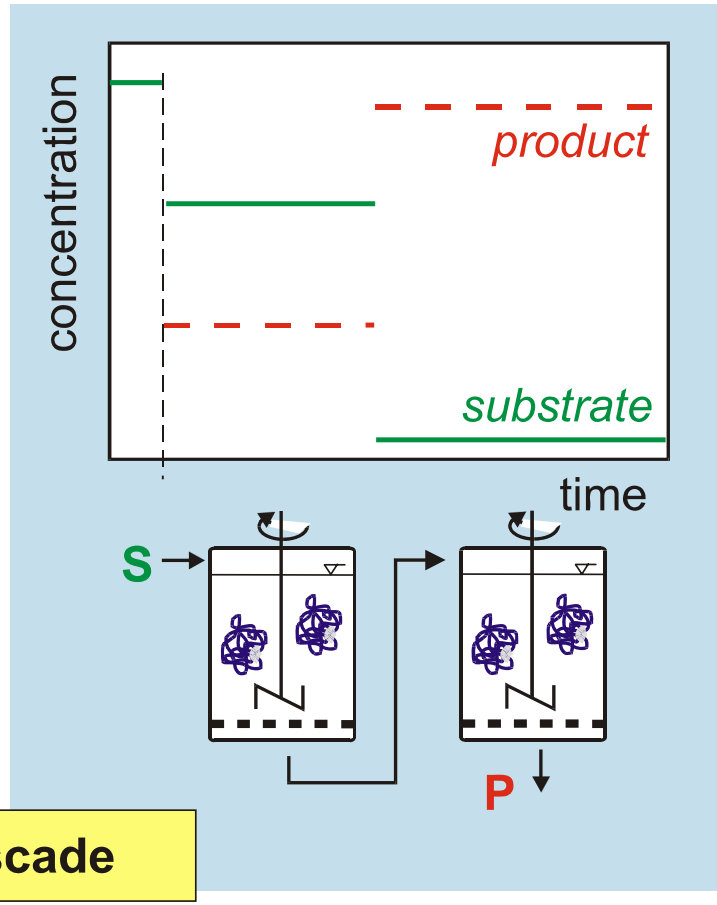


CSTR

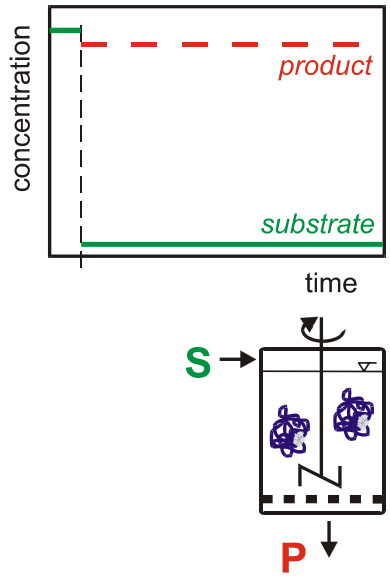
Concentration Profiles of Different Reactors



batch or plug flow

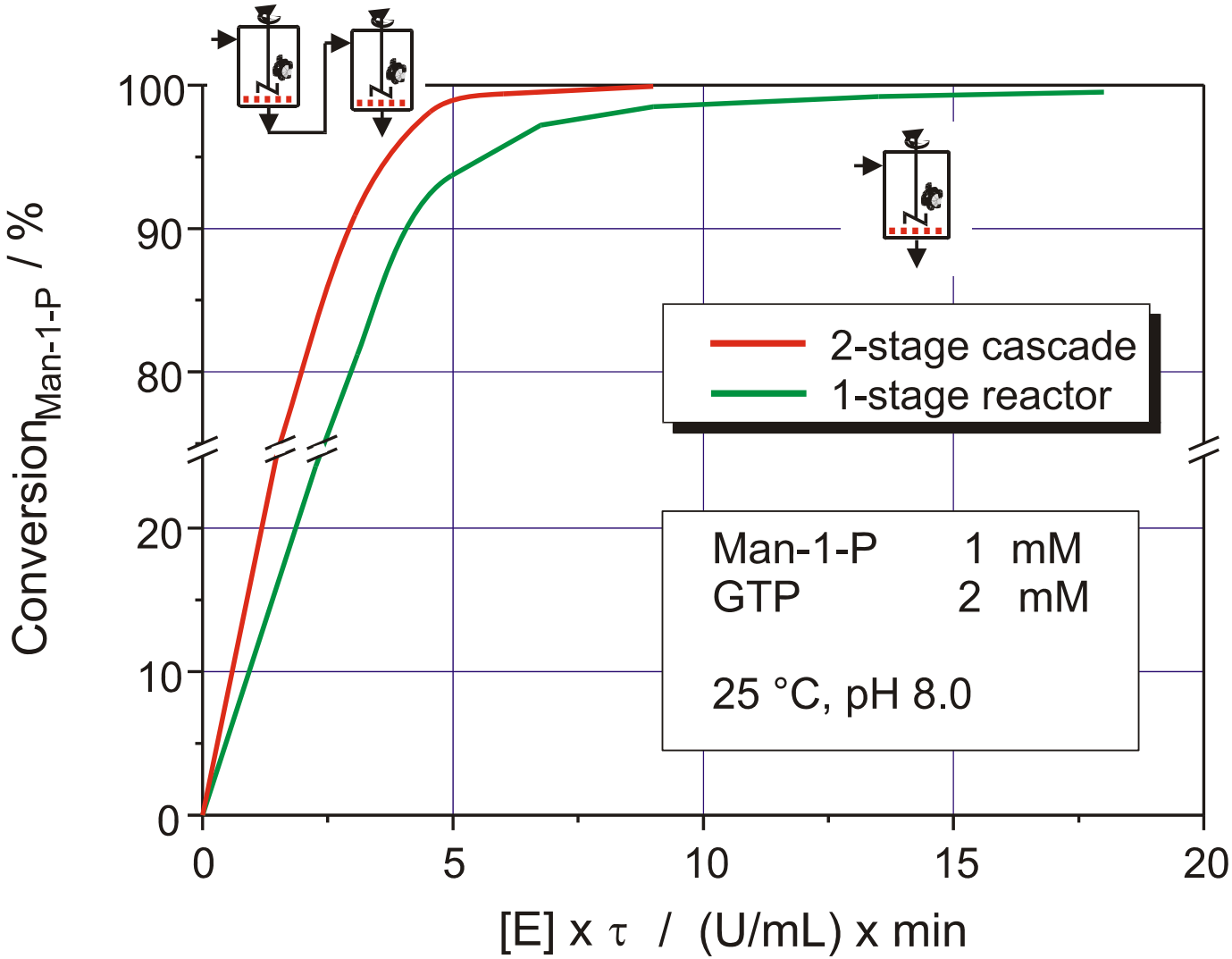


CSTR-cascade



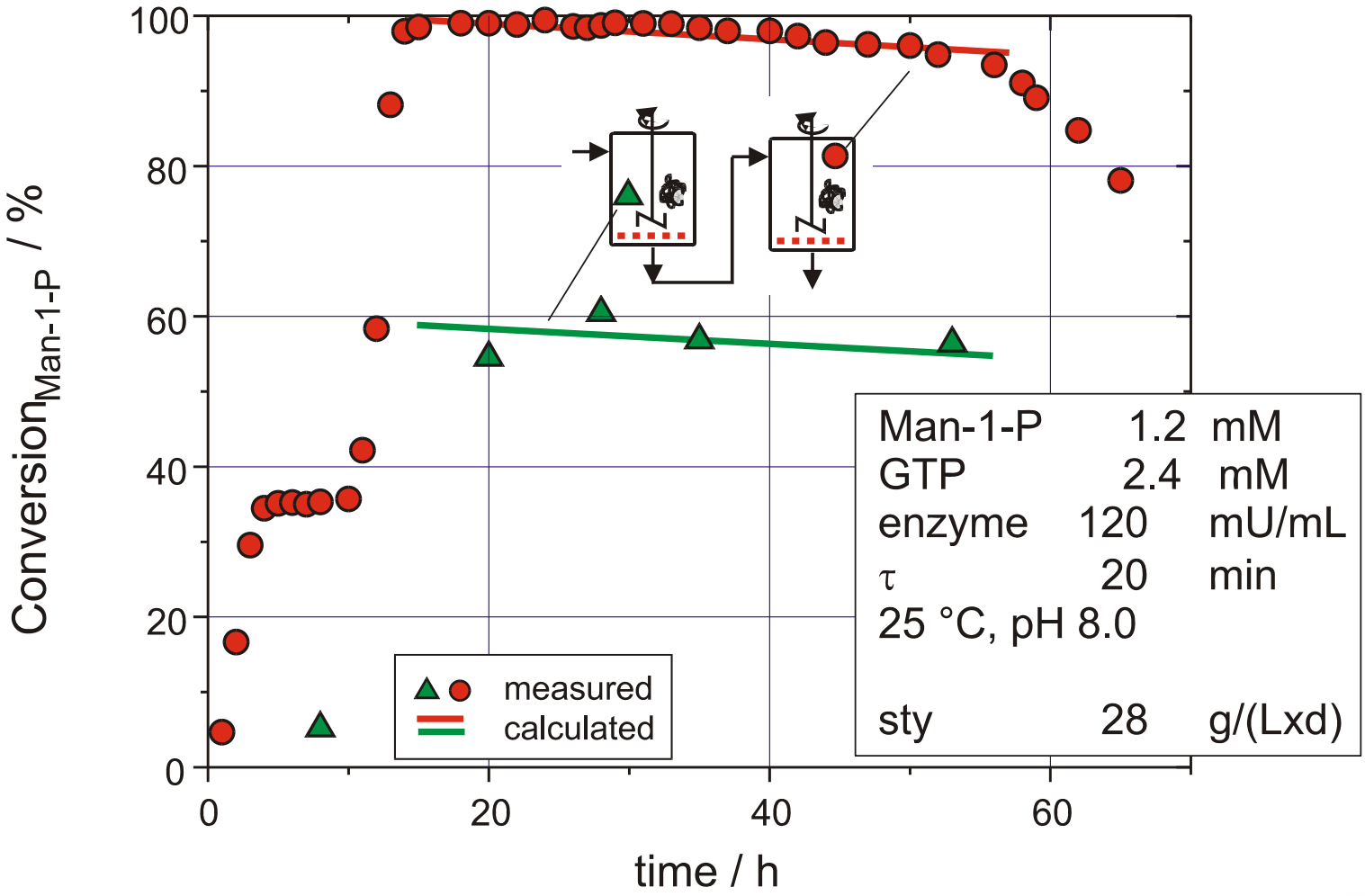
CSTR

Two-Stage CSTR-Cascade to Reduce Product Inhibition

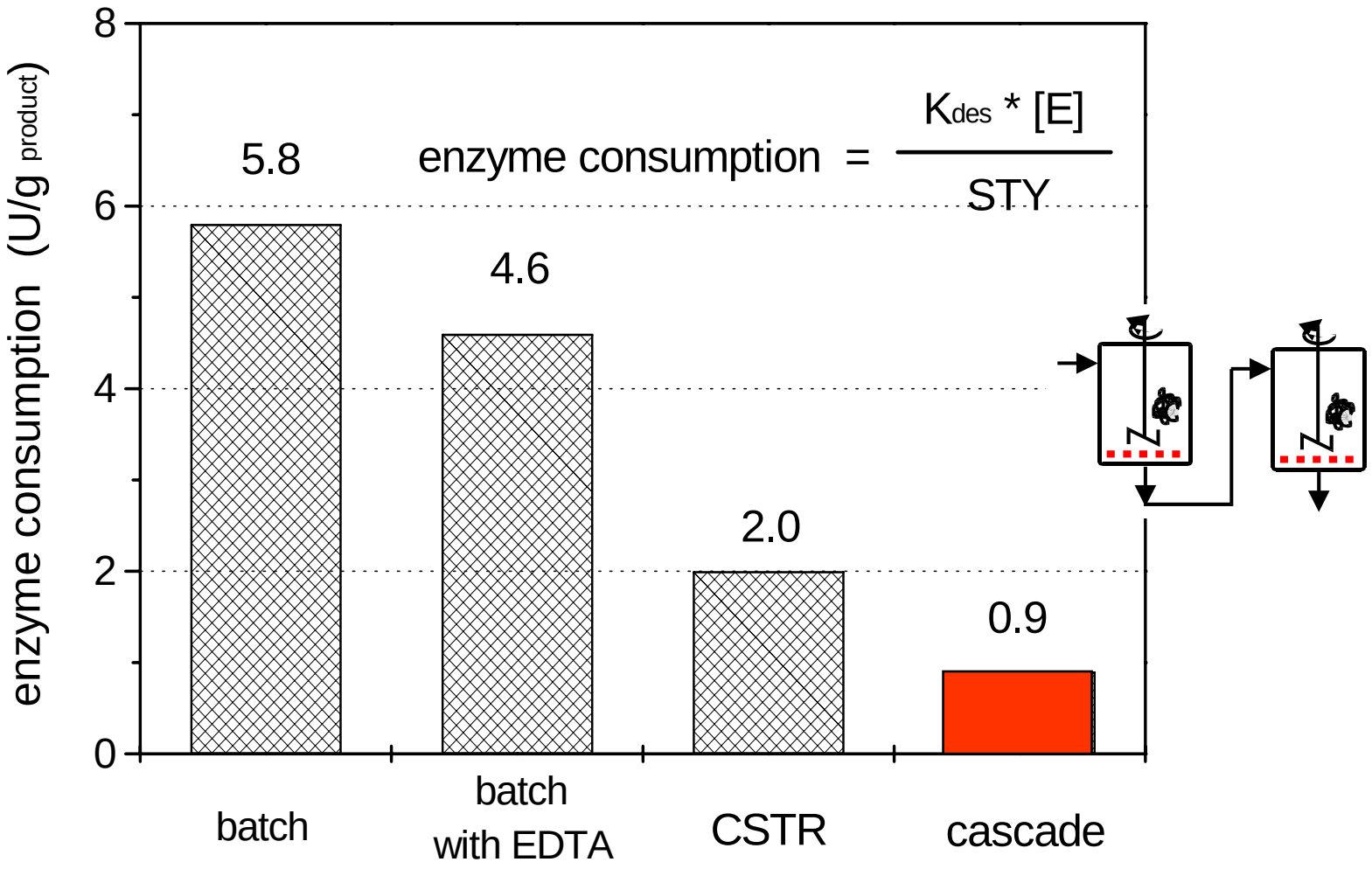


Fey et al., Carbohydr. Res. **305**, 475 (1997))

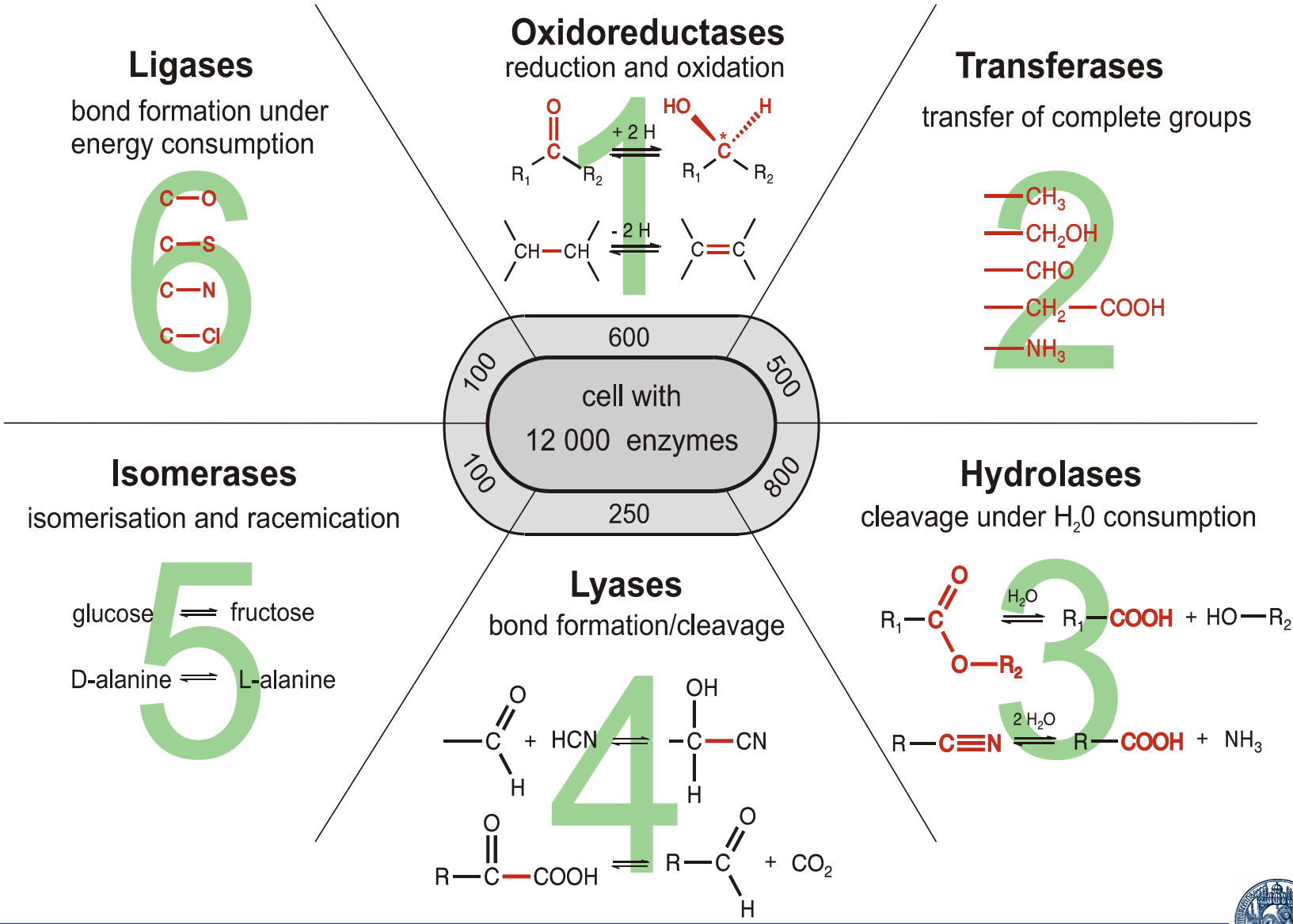
GDP-Man Production in a 2-Stage CSTR-Cascade



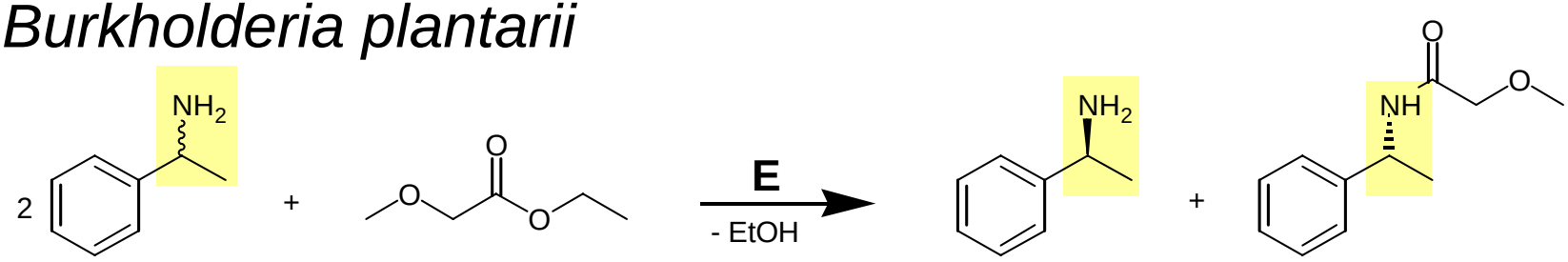
GDP-Man Production - Enzyme Consumption



Classification of Enzymes



Burkholderia plantarii



(*R,S*)-**1** **2** (*S*)-**1** *(R)*-**3**

freeze drying of the enzyme solution together with fatty acids to increase enzyme activity

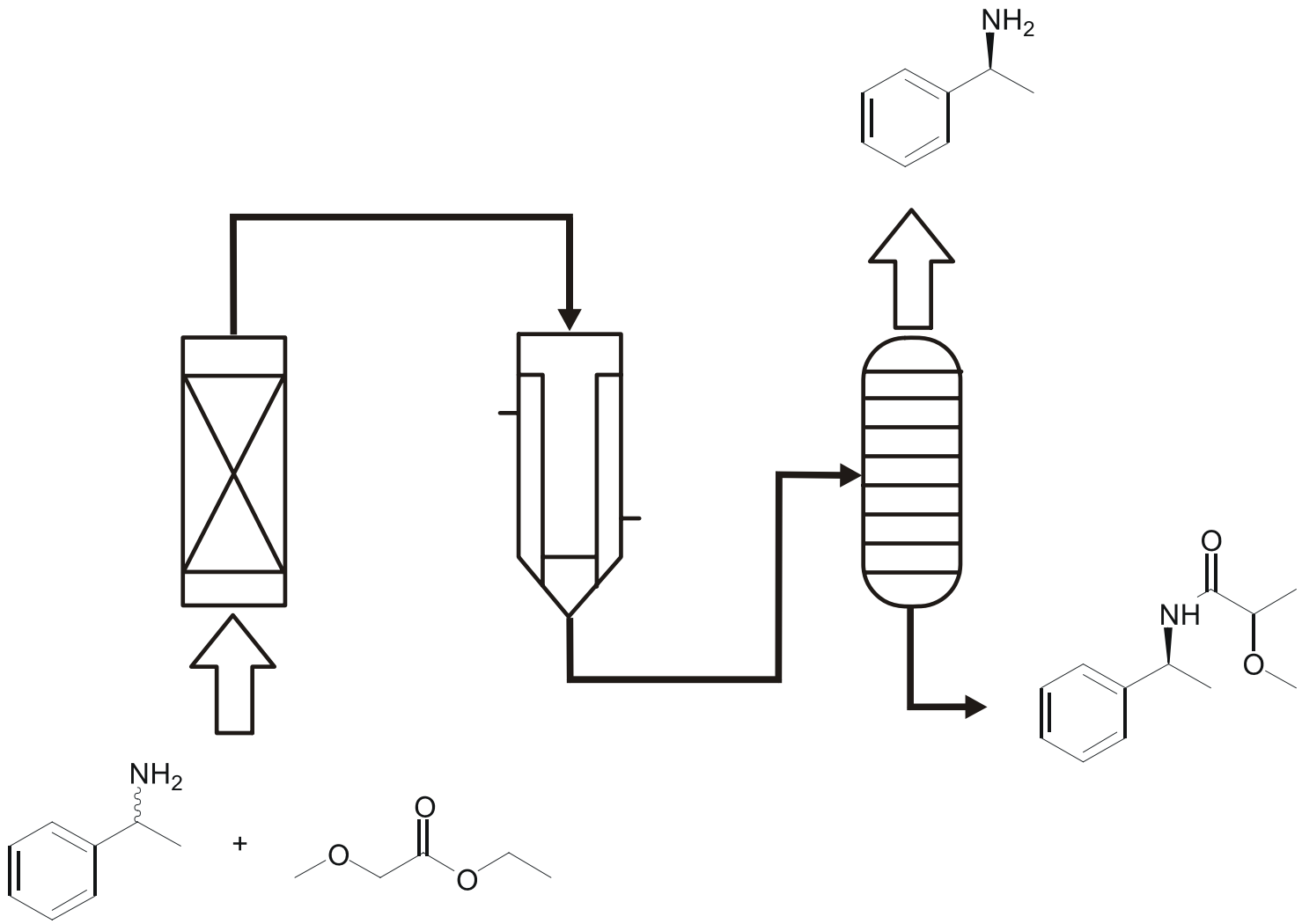
1 = 1-phenylethylamine
2 = ethylmethoxyacetate
3 = phenylethylmethoxyamide

BASF

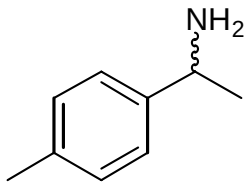
reaction conditions:		***process parameters:***	
[(R/S)-1]:	1.65 M, 200 g·L^{-1}	**conversion:**	50 %
		yield:	> 90 %
pH:	8.0-9.0	**selectivity:**	> 500 (E-Value)
medium:	MTBE-ethylmethoxyacetate	**ee:**	> 99% (S); 93 % (R)
		capacity:	> 100 t·a^{-1}
catalyst:	**immobilized enzyme**	**residence time:**	5-7 h

Balkenhohl, F. et al. (1995), BASF AG, DE 4329293

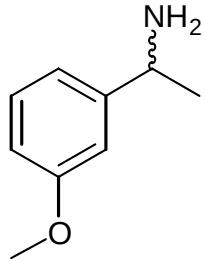
Flow Scheme



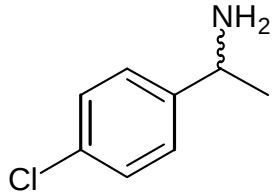
Other Starting Materials



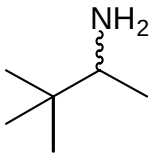
1-*p*-tolyl-ethylamine



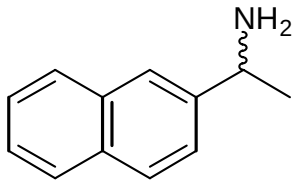
1-(3-methoxy-phenyl)-ethylamine



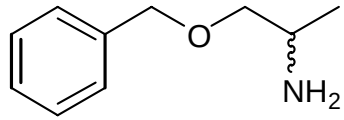
1-(4-chloro-phenyl)-ethylamine



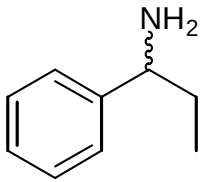
3,3-dimethyl-butyl-2-amine



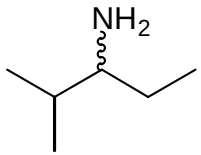
1-naphthyl-ethylamine



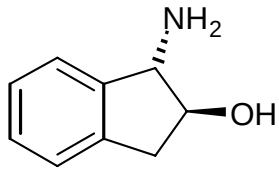
2-benzyloxy-1-methyl-ethylamine



1-phenyl-propylamine



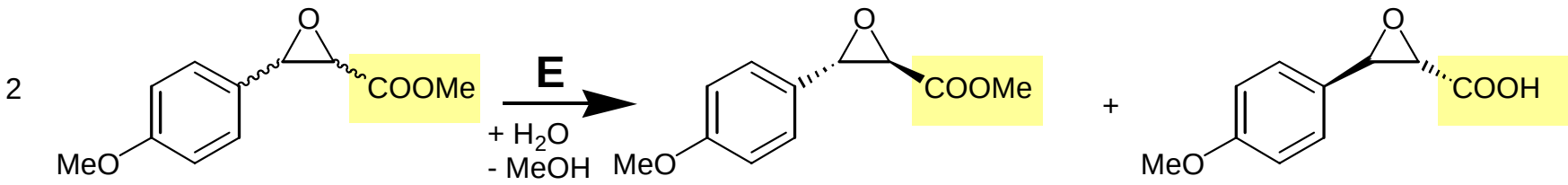
1-ethyl-2-methyl-propylamine



1-amino-indan-2-ol

Balkenhohl, F., Ditrich, K., Hauer, B., Lander, W. (1997), J. prakt. Chem. **339**, 381-384

Serratia marescens



1 (*2R,3S*)-**1** (*2S,3*R)-**2**

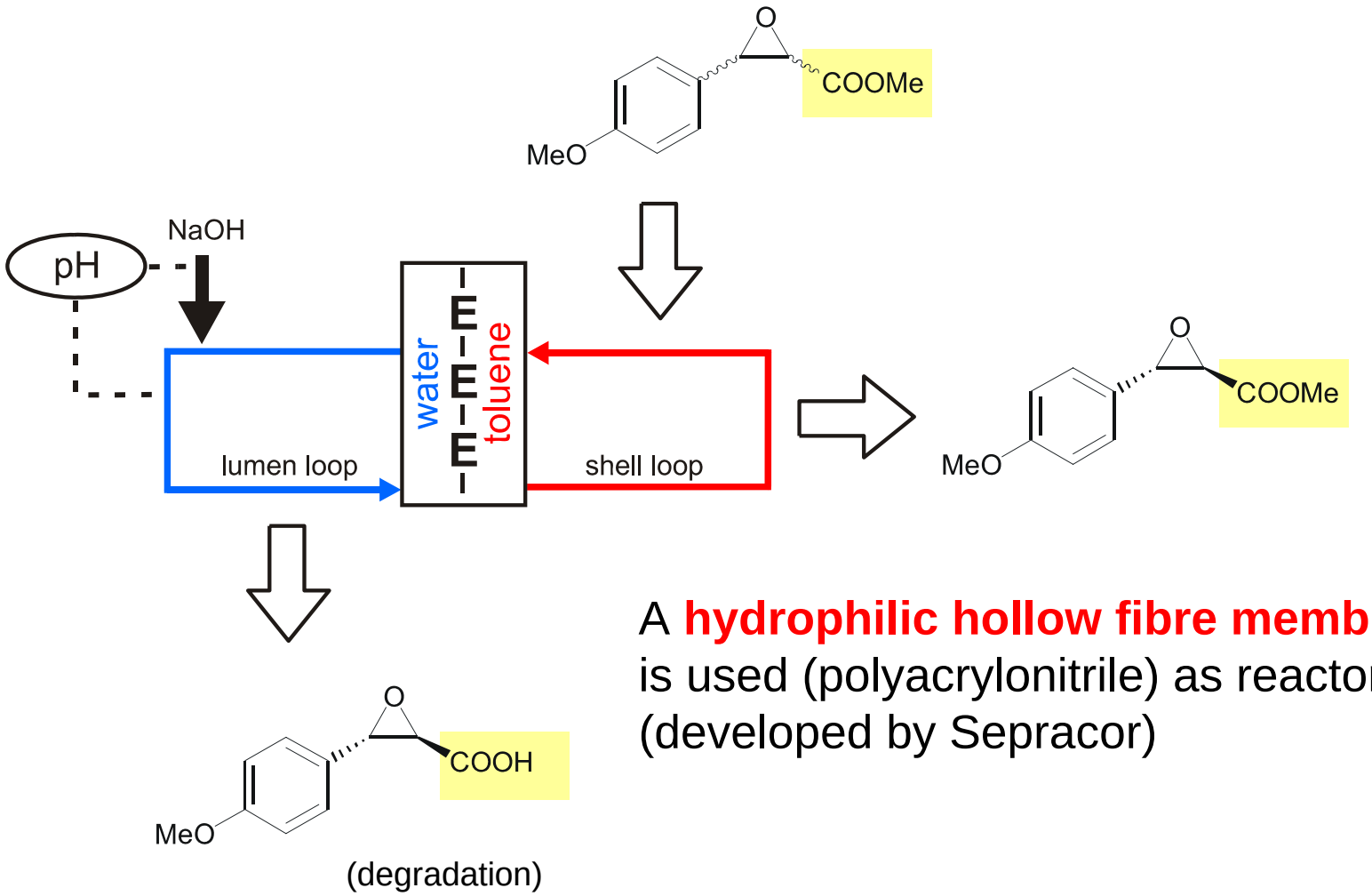
1 = 3-(4-methoxyphenyl) glycidic acid methyl ester = MPGM
2 = 3-(4-methoxyphenyl) glycidic acid

Tanabe Seiyaku Co., Ltd.

reaction conditions:		***process parameters:***	
[1]:	< 0.6 M, < 125 g·L^{-1}	**yield:**	40-45 %
pH:	8.5	**ee:**	100 %
T:	22 °C	**reactor type:**	batch
medium:	aqueous/toluene	**capacity:**	40 kg (-)-MPGM /(m^2·a)
catalyst:	immobilized enzyme		50 t a^{-1}
		start-up date:	1993

Matsumae, H., Furui, M., Shibatani, T., Tosa, T. (1994), J. Ferment. Bioeng. **78**, 59-63
Matsumae, H., Shibatani, T. (1994), J. Ferment. Bioeng. **77**, 152-158

Flow Scheme

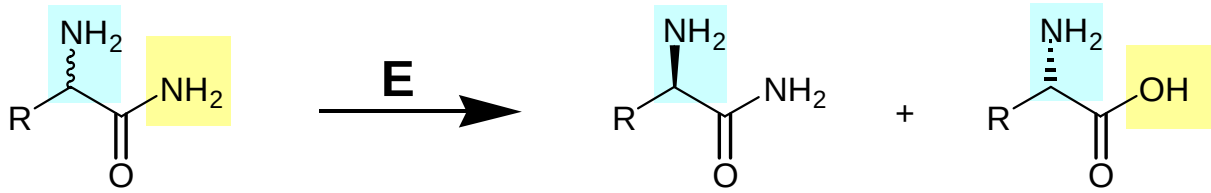


A **hydrophilic hollow fibre membrane** is used (polyacrylonitrile) as reactor unit (developed by Sepracor)

Matson, S. L. (1987), PCT WO 87/02381, PCT US 86/02089

Aminopeptidase EC 3.4.11.1

Pseudomonas putida



D,L-**1** D-**1** L-**2**

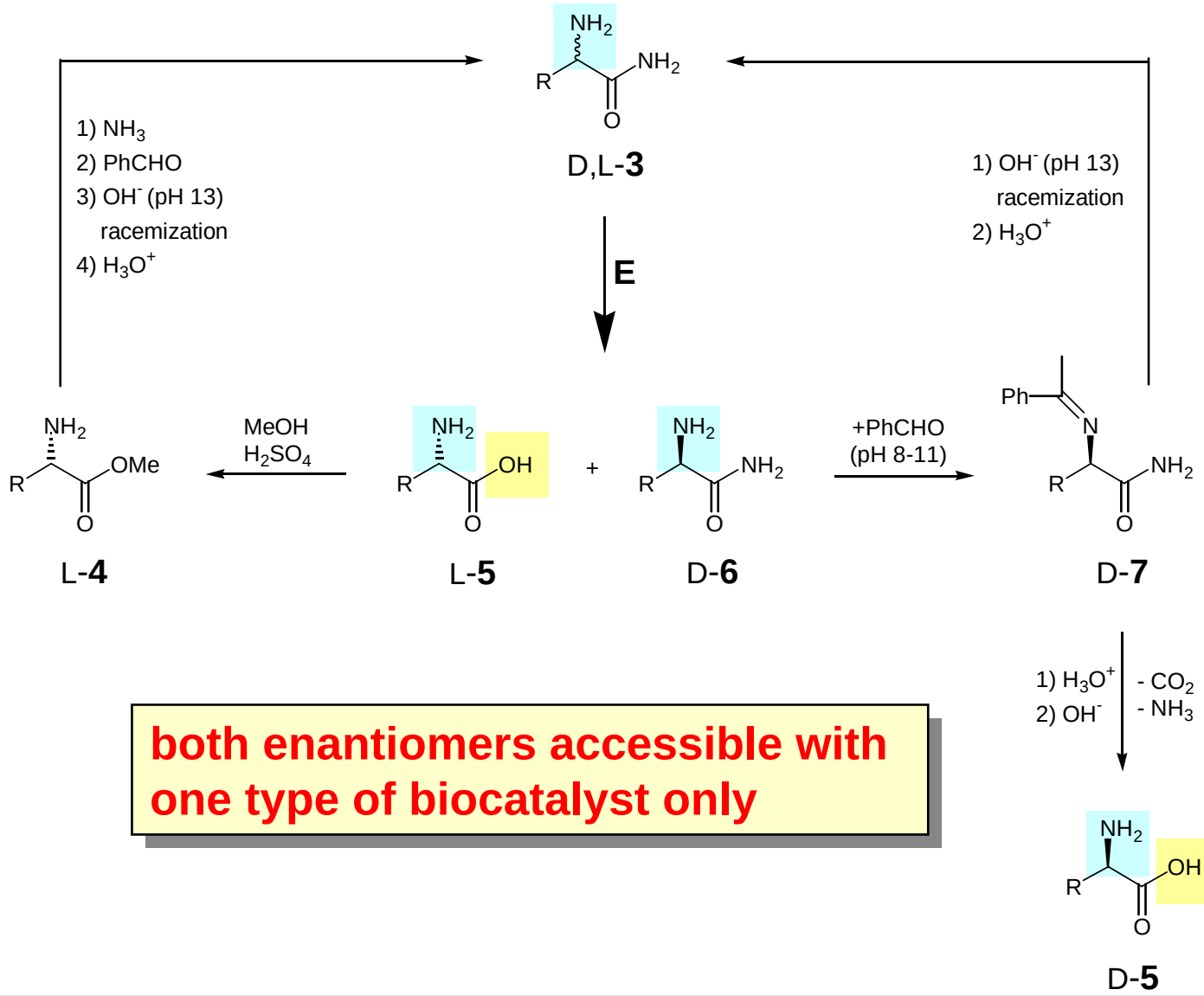
1 = α-amino acid amide
2 = α-amino acid

DSM

reaction conditions:		***process parameters:***	
[1]:	up to 20 g·L^{-1}	**ee:**	> 99 %
pH:	8.0 – 10.0	**reactor type:**	batch
T:		**capacity:**	ton scale
medium:	aqueous	**enzyme activity:**	338,000 U·g$_{protein}^{-1}$
catalyst:	suspended whole cells	**enzyme supplier:**	Novo Nordisk, Denmark
		start-up date:	1988

Schoemaker, H.E. et al. (1992), Pure & Appl. Chem. **64**, 1171-1175

“Dynamic Resolution” (not really)

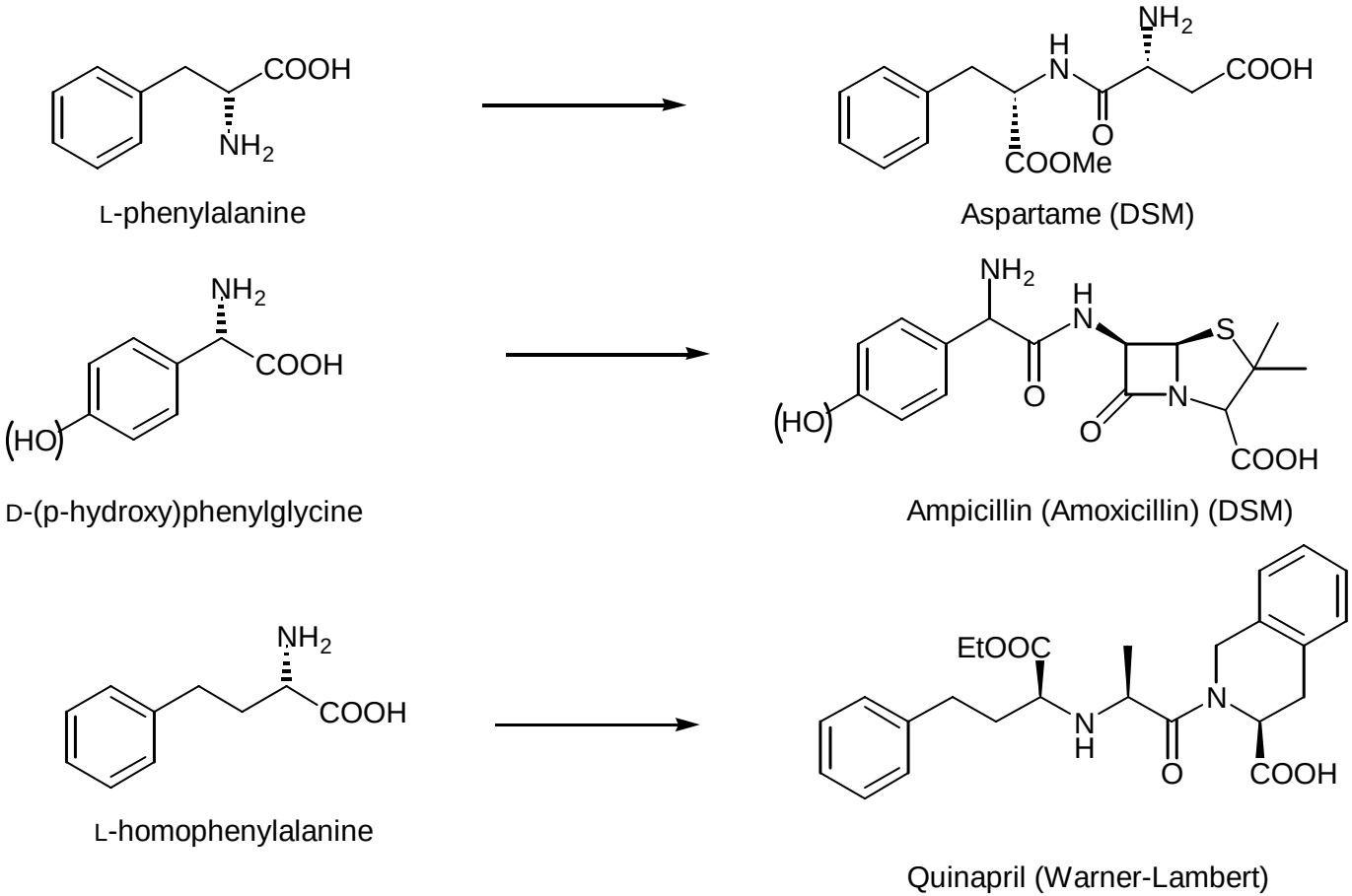


formation of Schiff base for easy separation (precipitation)

both enantiomers accessible with one type of biocatalyst only

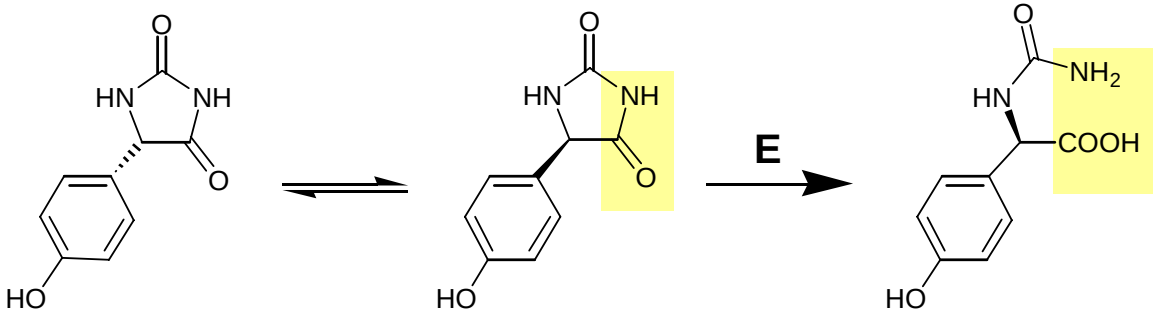
Product Application

DSM uses the amino acids produced by this biotransformation in different product syntheses



D-Hydantoinase EC 3.5.2.2

Aspergillus oryzae



L-**1** D-**1** D-**2**

key raw material for semisynthetic penicillins and derivatives

- **unreacted L-hydantoins racemize easily under these conditions**.
- L-Specific hydantoinases are also known.

real dynamic resolution

1 = 5-(p-hydroxybenzyl)-hydantoin
2 = D-*N*-carbamoyl amino acid

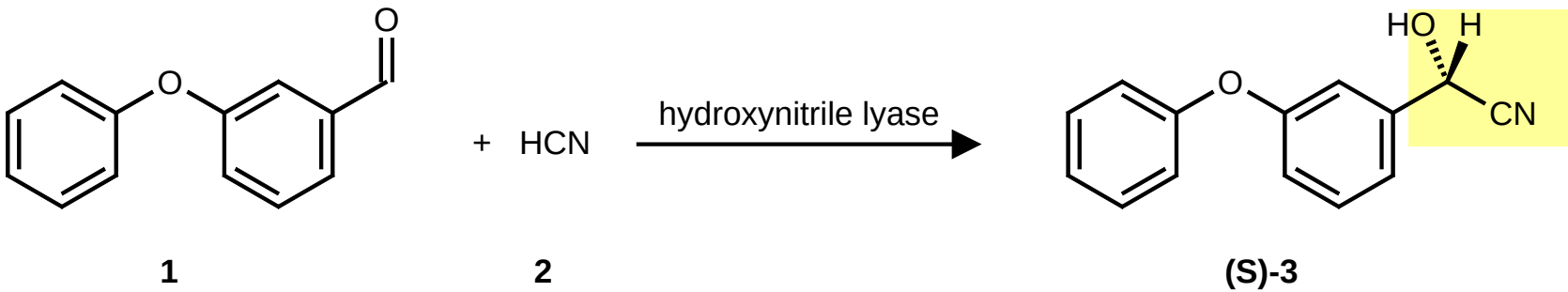
Kanegafuchi

reaction conditions:		***process parameters:***	
pH:	8.0	**conversion:**	100%
catalyst:	immobilized whole cells	**capacity:**	200 $t \cdot a^{-1}$
		enzyme activity:	17.14 $U \cdot g^{-1}$
		start-up date:	1983

Ikemi, M. (1994), Bioprocess Technology **19**, pp. 797-813

Hydroxynitrile Lyase (S-Oxynitrilase) EC 4.1.1.11

Havea brasiliensis



1 **2** **(S)-3**

DSM Linz

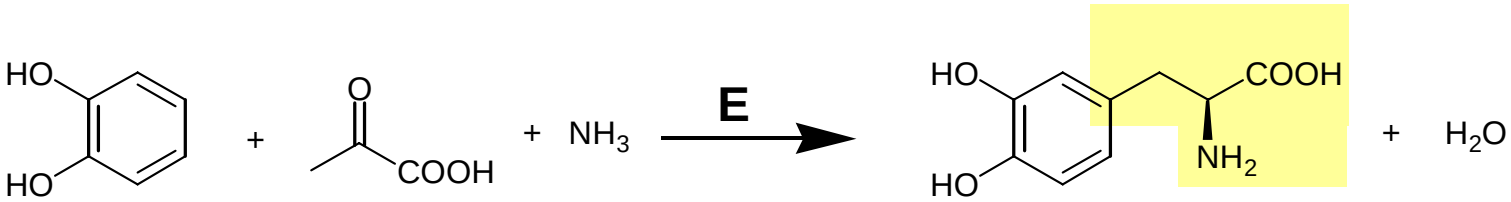
(R-Oxynitrilase: Rosenthaler 1908)

reaction conditions:		***process parameters:***	
[1]:	30% w/w	**reactor type:**	batch
[2]:	1.3 equ.	**ee:**	**>97%**
catalyst:	soluble enzyme (rec.)		
	two phase system		

P. Pöchlauer, Chem. today **16**, 15, 1998

Tyrosine Phenol Lyase EC 4.1.99.2

Erwinia herbicola



1 **2** L-**3**

L-DOPA

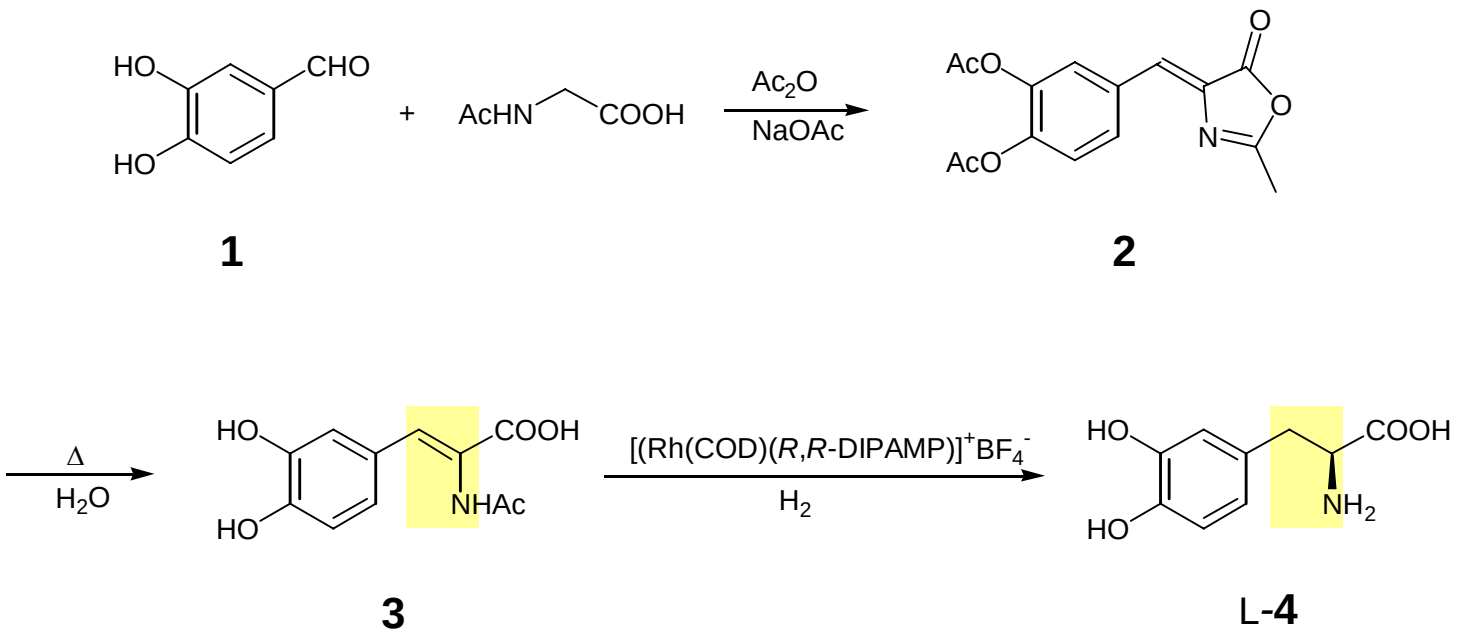
1 = catechol
2 = pyruvic acid
3 = dopa

Ajinomoto Co., Ltd.

reaction conditions:		***process parameters:***	
[3]:	0.558 M, 110 g·L^{-1}	**reactor type:**	fed batch
catalyst:	suspended whole cells	**reactor volume:**	60 000 L
		capacity:	250 t·a^{-1}

Tsuchida, T. et al. (1993) Ajinimoto Co., Ltd., JP 5123177A
Yamada, H. (1998), pp. 13-17, Studies in Organic Chemistry **53**, Elsevier, Amsterdam

Competing with Monsanto's Chemical Process



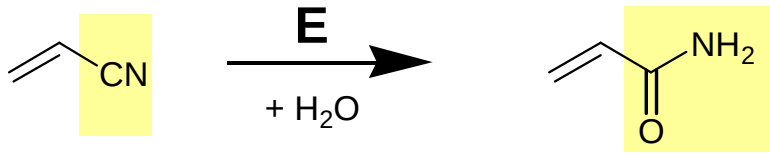
for the treatment of Parkinson

1 = vanillin
2 = hydantoin
3 = Z-enamide
3 = dopa

Ager, D.J. (1999) Handbook of Chiral Chemicals, Marcel Dekker, New York

Nitrile Hydratase EC 4.2.1.84

Rhodococcus rhodochrous



1 **2**

1 = acrylonitrile
2 = acrylamide

Nitto Chemical Industry

reaction conditions:		***process parameters:***	
[1]:	0.11 M, 6 g·L^{-1} (fed batch)	**conversion:**	> 99.99 %
[2]:	**5.6 M, 400 g·L^{-1}**	yield:	> 99.99 %
pH:	7.0	**selectivity:**	> 99.99 %
T:	5 °C	**reactor type:**	fed batch
catalyst:	immobilized whole cells	**capacity:**	> 30,000 t·a^{-1}
		residence time:	5 h
		space-time-yield:	1.920 g·L^{-1}·d^{-1}
		start-up date:	1991

Yamada, H., Kobayashi, M (1996), Biosci. Biotech. Biochem. **60** (9), 1391-1400
Yamada, H., Tani, Y. (1987), Nitto Chemical Industry Co., Ltd., US 4637982

Chemical Synthesis was Substituted

- The chemical synthesis uses **copper salt** as catalyst for the hydration of acrylonitrile and has several disadvantages:

  1) The rate of acrylamide formation is lower than the **acrylic acid formation**,

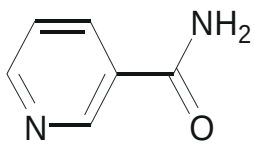
  2) The double bond of educt and products causes **by-product formations** such as ethylene, cyanohydrin and nitrylotrispropionamide and

  3) at the double bonds occur polymerization.

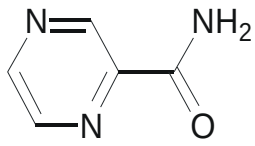
- The biotransformation has the advantages that recovering of unreacted nitrile is not necessary since the **conversion is 100 %** and the **no copper catalyst removal** is needed.

Shimizu, H., Ogawa, J., Kataoka, M., Kobayashi, M. (1997), in: New Enzymes for Organic Synthesis, Adv. Biochem. Eng. Biotechnol. **58** (Ghose, T. K., Fiechter, A., Blakebrough, N. eds.), pp. 56-59

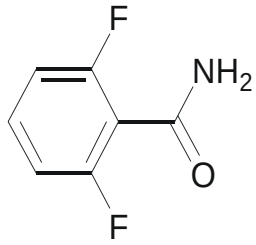
Product Spectrum



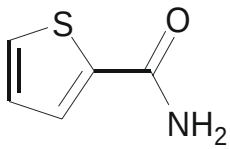
1,465 g L^{-1}



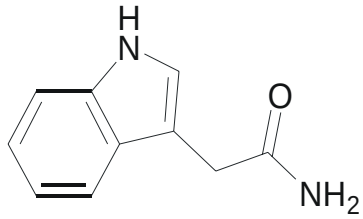
985 g L^{-1}



306 g L^{-1}



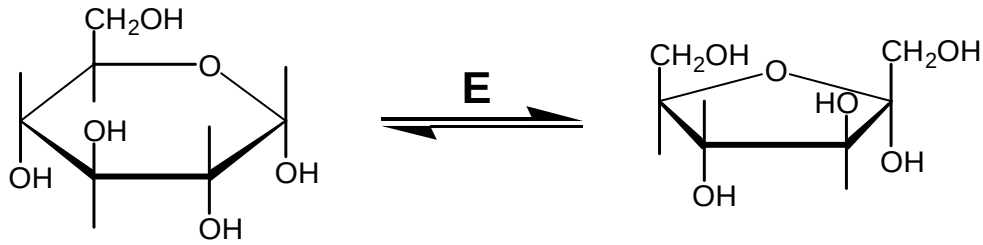
210 g L^{-1}



1,045 g L^{-1}

Xylose Isomerase EC 5.3.1.5

Bacillus coagulans …



1 **2**

1 = glucose
2 = fructose

Novo-Nordisk
Gist-brocardes
Miles Kali-Chemie
Finnsugar
Nagase

reaction conditions:		***process parameters:***	
[1]:	> 95 % dry matter	**reactor type:**	continuous, fixed bed
pH:	7.5 - 8.0	**capacity:**	**> $7 \cdot 10^6$ t·a^{-1}**
T:	50 – 60 °C	**residence time:**	0.17 – 0.33 h
reaction type:	isomerization	**start-up date:**	1967 by Clinton Corn Processing Co. (USA)
catalyst:	immobilized whole cell or isolated enzyme		

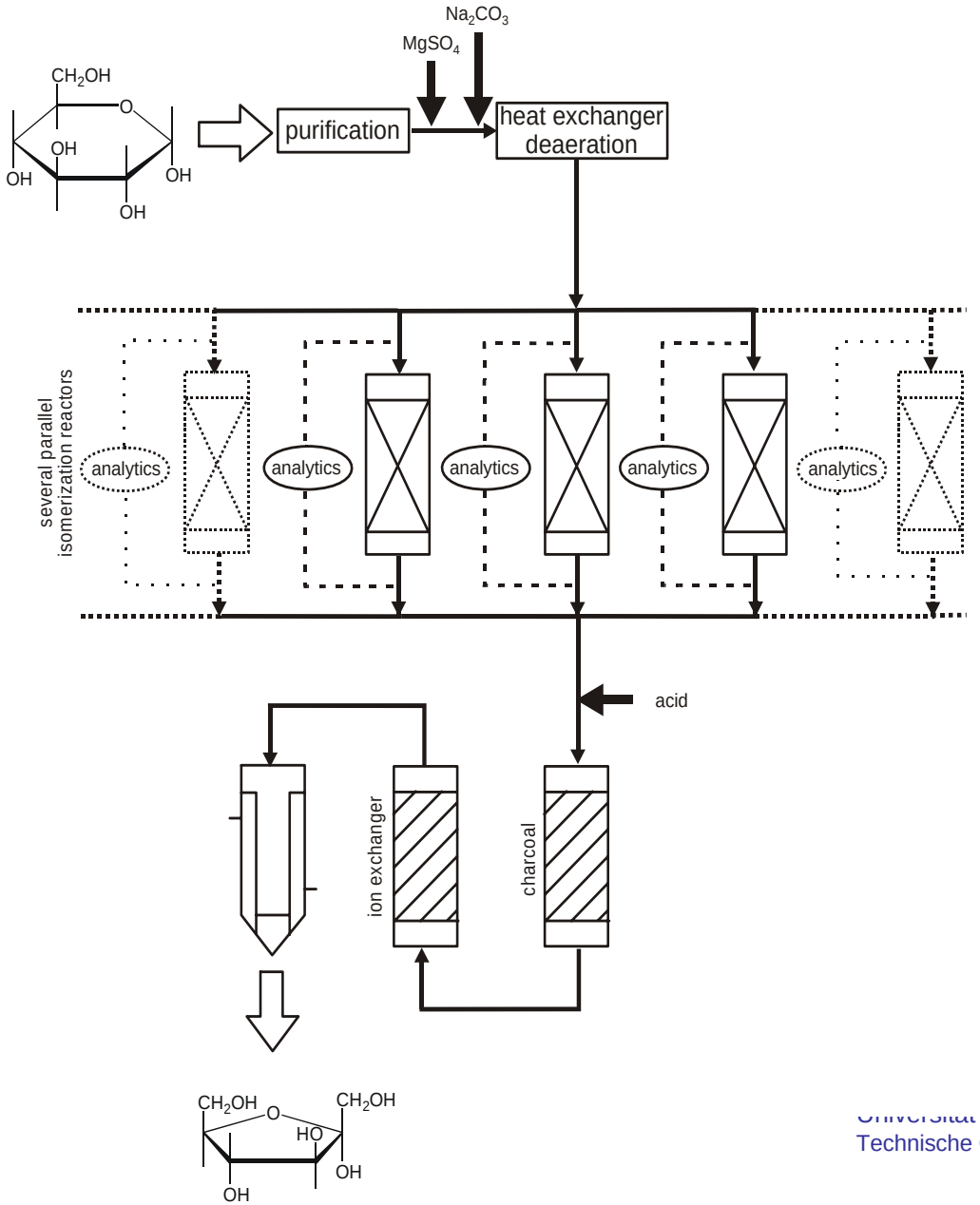
Blanchard, P. H., Geiger, E. O. (1984) **11**, Sugar Technol. Rev. 1-9

Remarks

- Since these isomerases belong to the group of metallo enzymes, Co^{2+} and Mg^{2+} is required.
- The **reaction enthalpy is slightly endothermic and reversible**. The equilibrium conversion is about 50 % at 55 °C.
- **Several reactors are operated in parallel** or in series, containing **enzymes of different ages**. The feed to a single reactor is controlled by the conversion of this reactor.
- Plants proceeding more than 1000 t of HFCS (based on dry matter) per day typically use at least 20 individual reactors.
- The product HFCS contains 42 % fructose (53 % glucose) or 55 % fructose (41 % glucose)(dry matter).

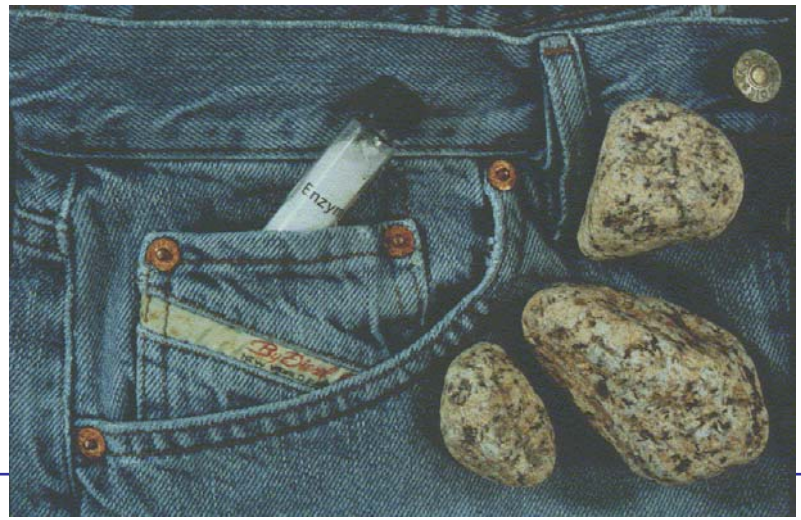
Straatsma, J., Vellenga, K., Witt, H. G. J. de, Joosten, G. E. (1983) Ind. Eng. Chem. Process Des. Dev. **22**, 356-361

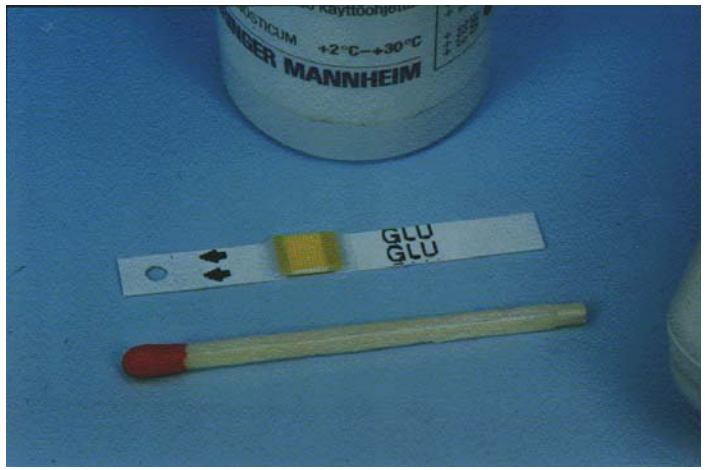
Flow Scheme


Na_2CO_3
$MgSO_4$
CH_2OH
O
OH
OH
OH
OH
purification
heat exchanger
deaeration
several parallel
isomerization reactors
analytics
analytics
analytics
analytics
analytics
acid
ion exchanger
charcoal
CH_2OH
O
CH_2OH
HO
OH
OH

Other Applications of Enzymes

purpose	**type**
washing powder	proteases, lipases etc.
food technology	pectinases, amylases
textile industry	leather, „biobleaching“
biosensors	glucose oxidase

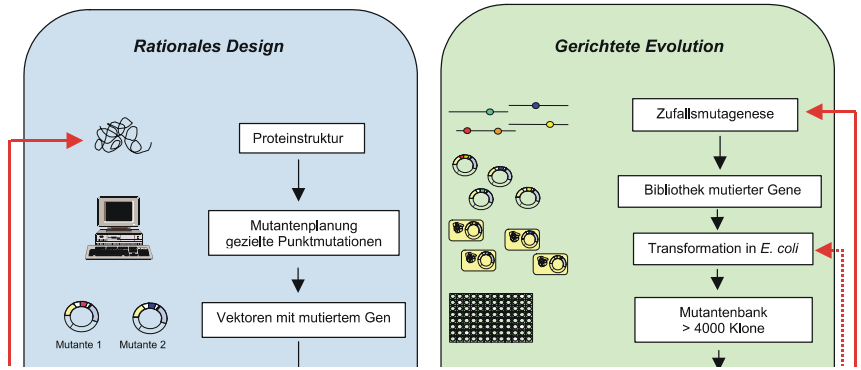




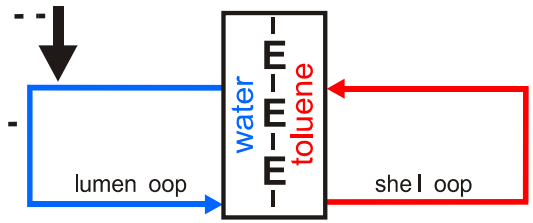
Process Devopment & Improvement

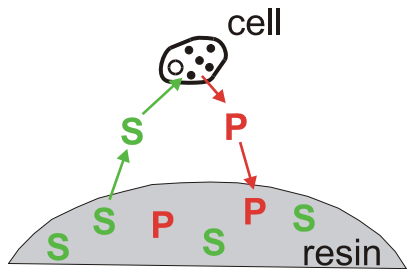
- **modification of biocatalyst by genetic engineering**
  - rational protein design
  - directed evolution

Review: M. Pohl, U. Bornscheuer, Curr. Opin. Chem. Biol. **5**, 37 (2001)



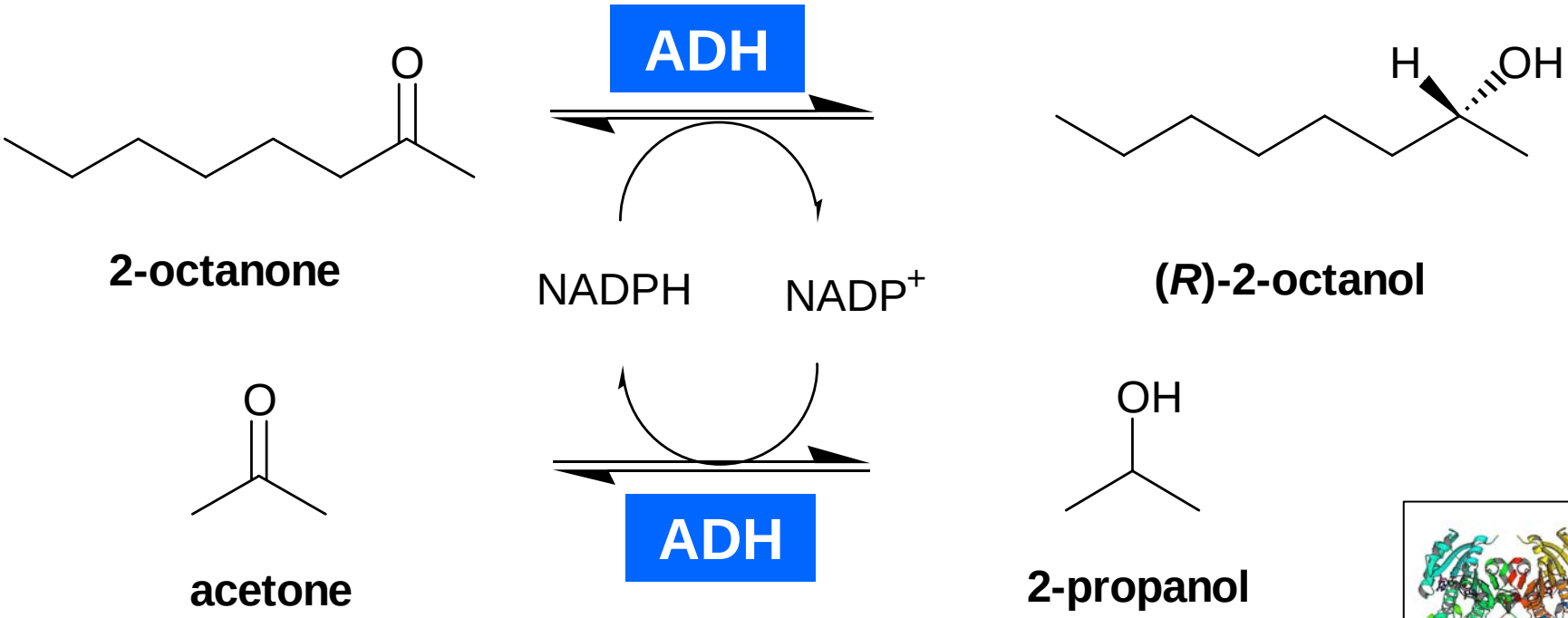
- **biochemical engineering**
  - reactor design (to overcome product inhibition)
  - outline of process





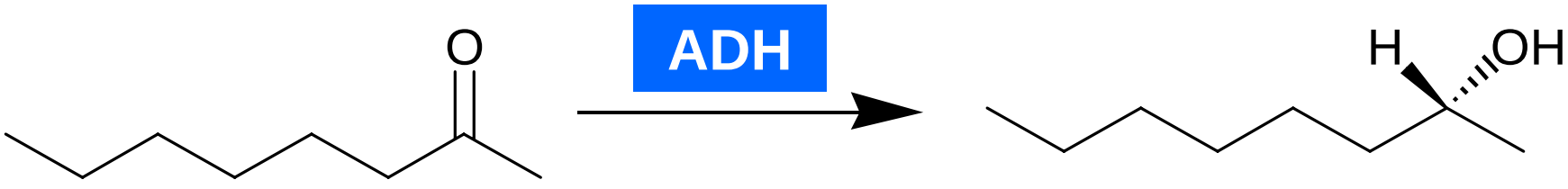
novel approaches such as Ionic Liquids

Alcohol Dehydrogenase for Production of Chiral Alcohols



- rec ADH from: *Lactobacillus brevis* → **(*R*)**-alcohol
  Thermoanaerobium spec. → **(*S*)**-alcohol
- used for production of chiral alcohols on 10 - 100 kg scale
  eg (R)-ethyl 3-hydroxybutyrate or (R)-2-octanol

Solubility of 2-Octanone / 2-Octanol



water-solubility:

1.1 g/L

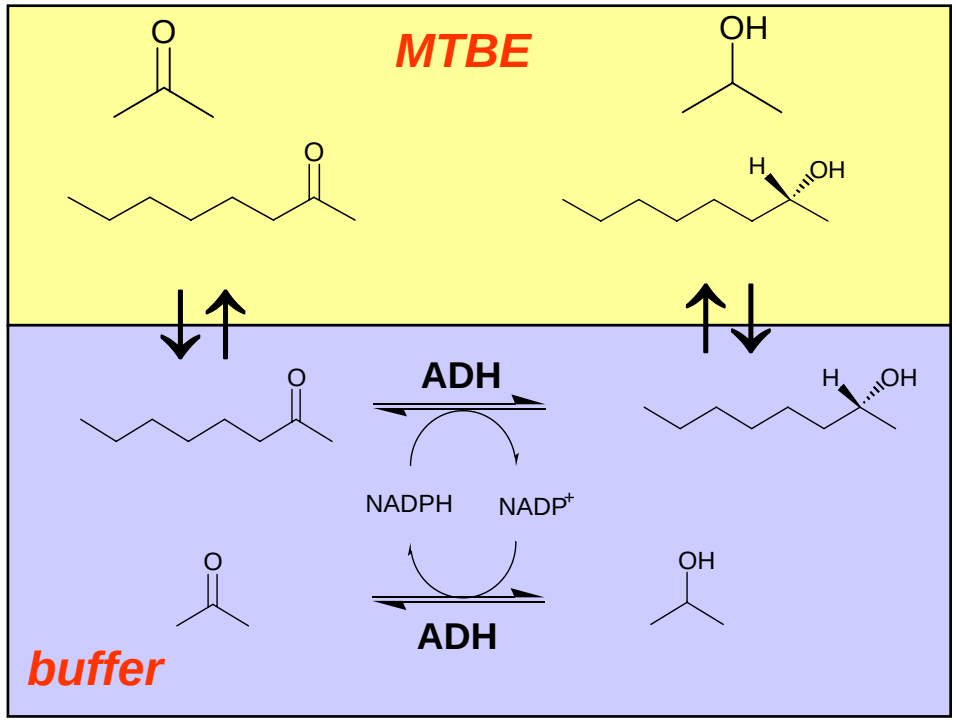
addition of water-soluble organic solvent
→ **one phase system**

pure organic solvent (mainly lipases)

water + organic solvent
→ **two phase system**

MTBE for Two-Phase Enzymatic Reduction

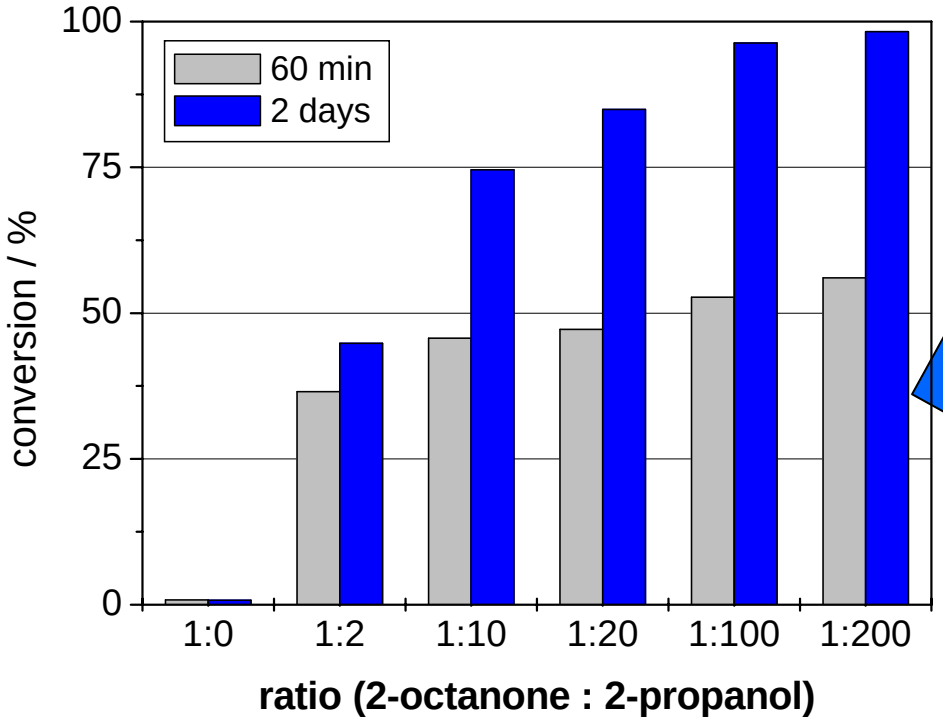
Partition coefficients MTBE / H_2O	
2-propanol	1.0
acetone	1.1
2-octanone	> 100
2-octanol	> 100



- thermodynamic equilibrium
- distribution between phases
- excess of 2-propanol is necessary

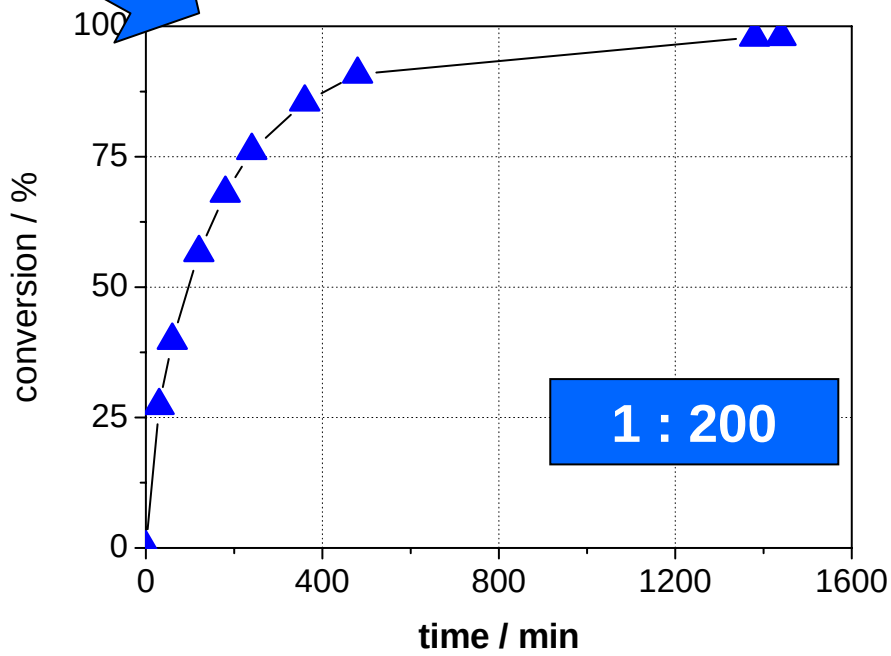
High Excess of Regeneration Substrate Necessary

two phase



MTBE / buffer
10 mM 2-octanone
2 M 2-propanol
30 °C
2.5 ml each phase

ADH



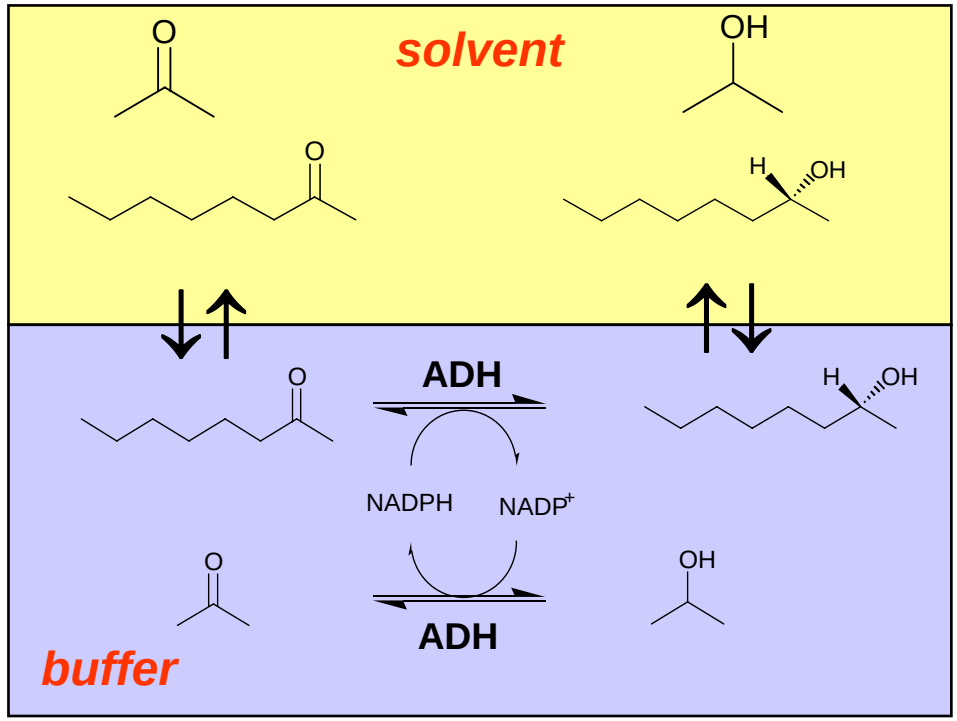
max. conversion = 98 % in 24 h

Favorable Partition Coefficient when Using an IL

Partition coefficients solvent / H_2O

	MTBE	IL
2-propanol	1.0	0.4
acetone	1.1	2.0

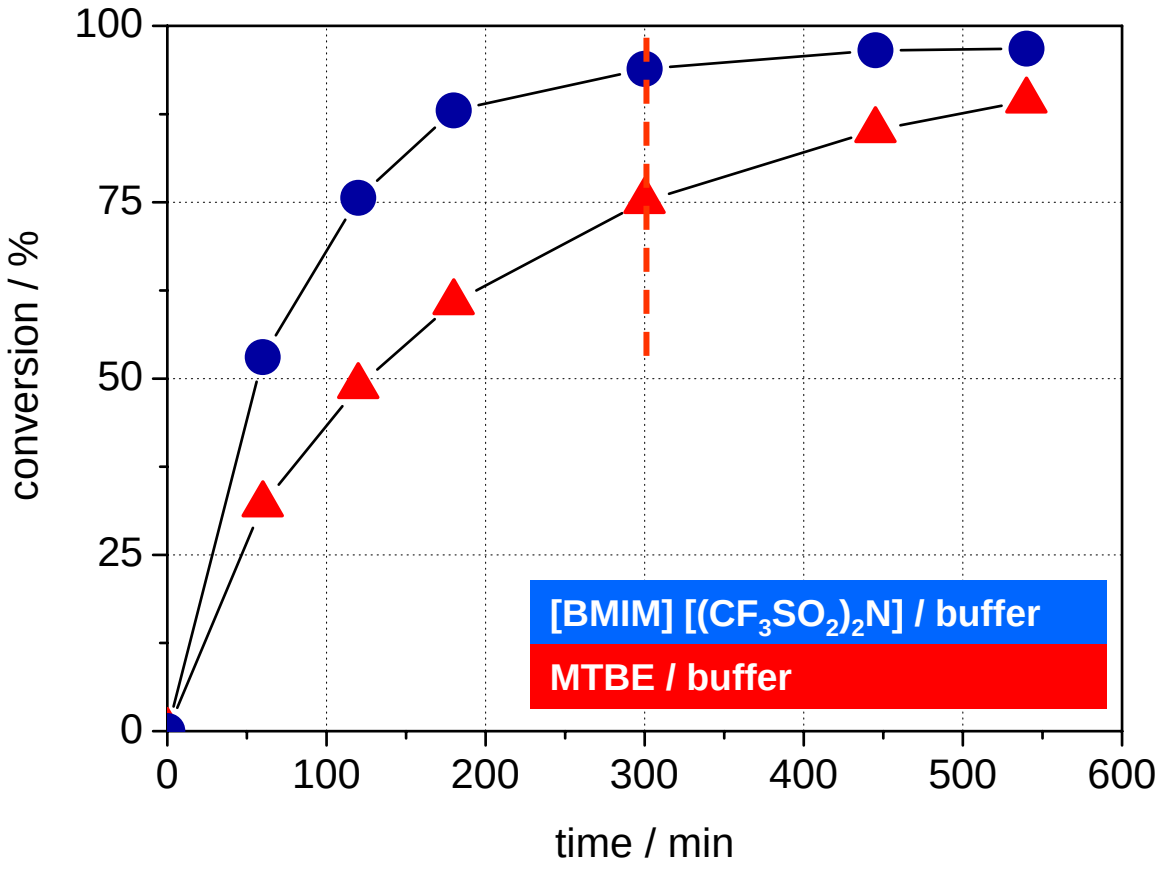
[BMIM] [$(CF_3SO_2)_2N$]
[BMIM] [BTA]



- **regeneration substrate stays in water phase**
- **regeneration product removed from water phase**
  → reduction of product inhibition

Eckstein et al.; Chem. Comm. 1084 (2004)

Increased Productivity of Octanone Reduction

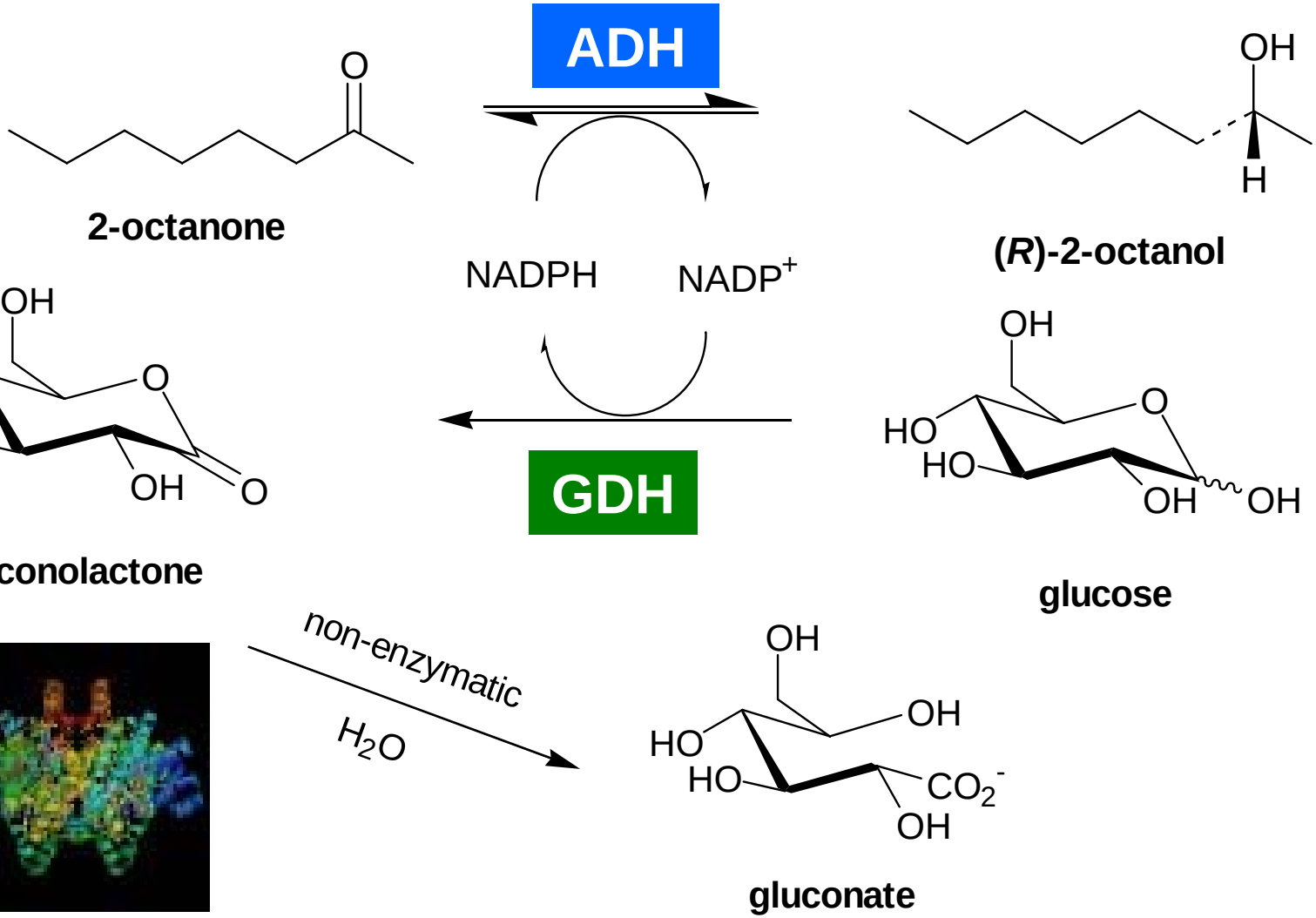


- higher reaction velocity due to reduced product inhibition
- in both cases max. conversion 95-98 %

Eckstein et al.; Chem. Comm. 1084 (2004)

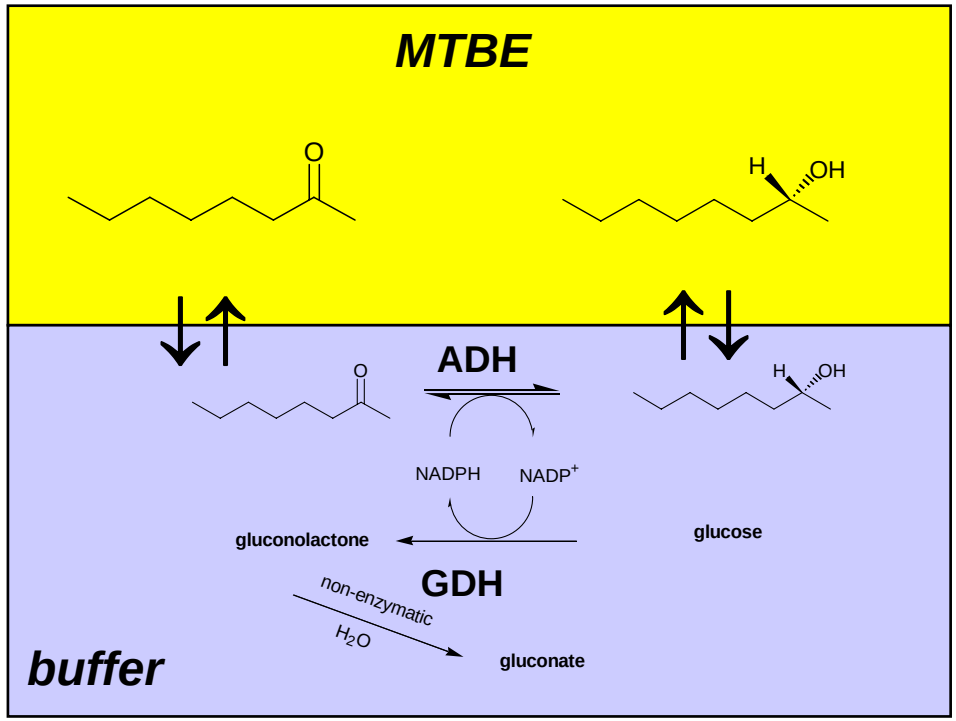
Second enzyme for cofactor regeneration

two phase



Enzyme-coupled cofactor regeneration

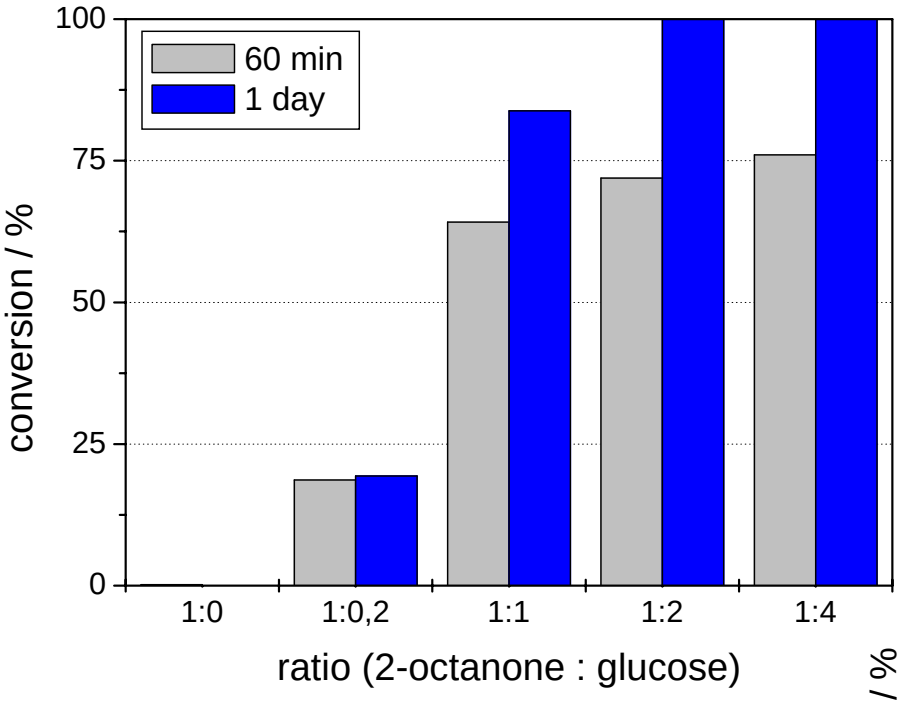
Partition coefficients MTBE / H_2O	
glucose	**-**
gluconate	**-**
2-octanone	> 100
2-octanol	> 100



- irreversible cofactor regeneration
- glucose / gluconate stay in aq. phase

Reduced ecxess of regeneration substrate

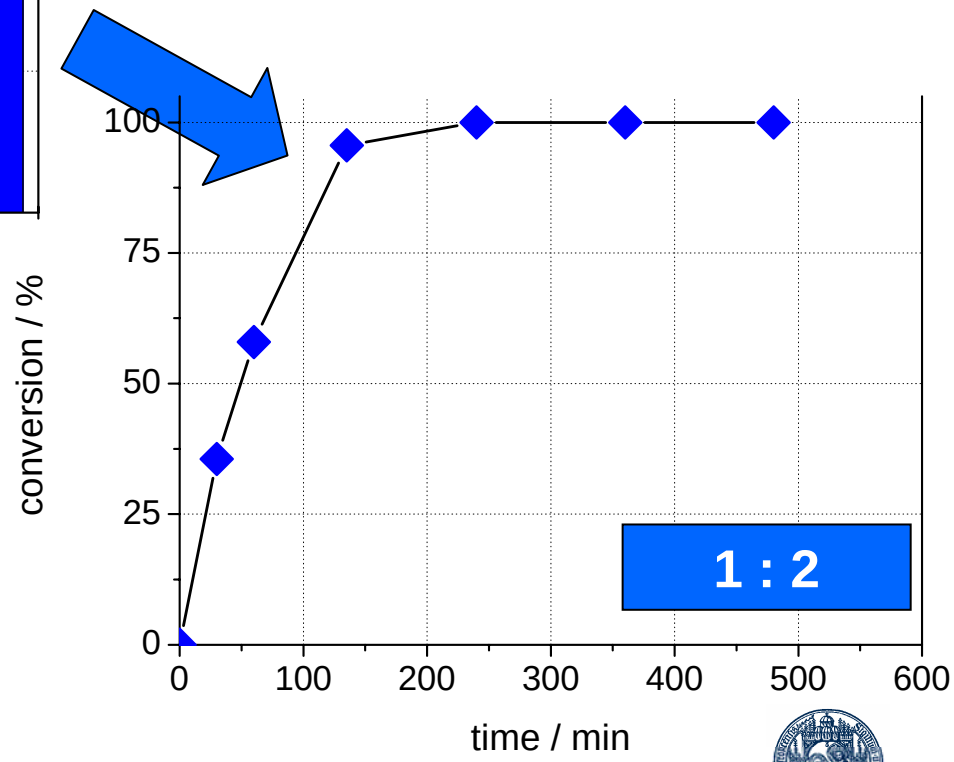
two phase



MTBE / buffer
10 mM **2-octanone**
20 mM glucose
30 °C
2.5 ml each phase

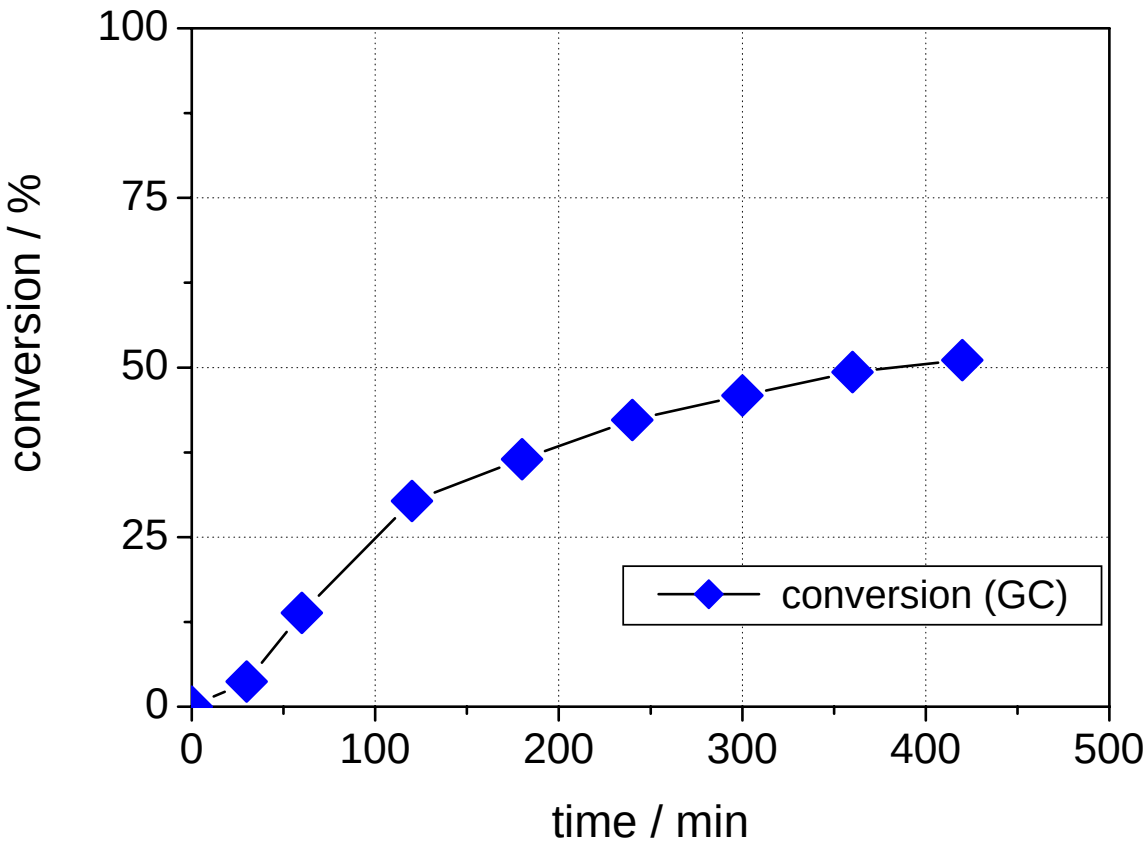
ADH
GDH

max. conversion >99 % in 6 h



Increasing the substrate concentration

two phase

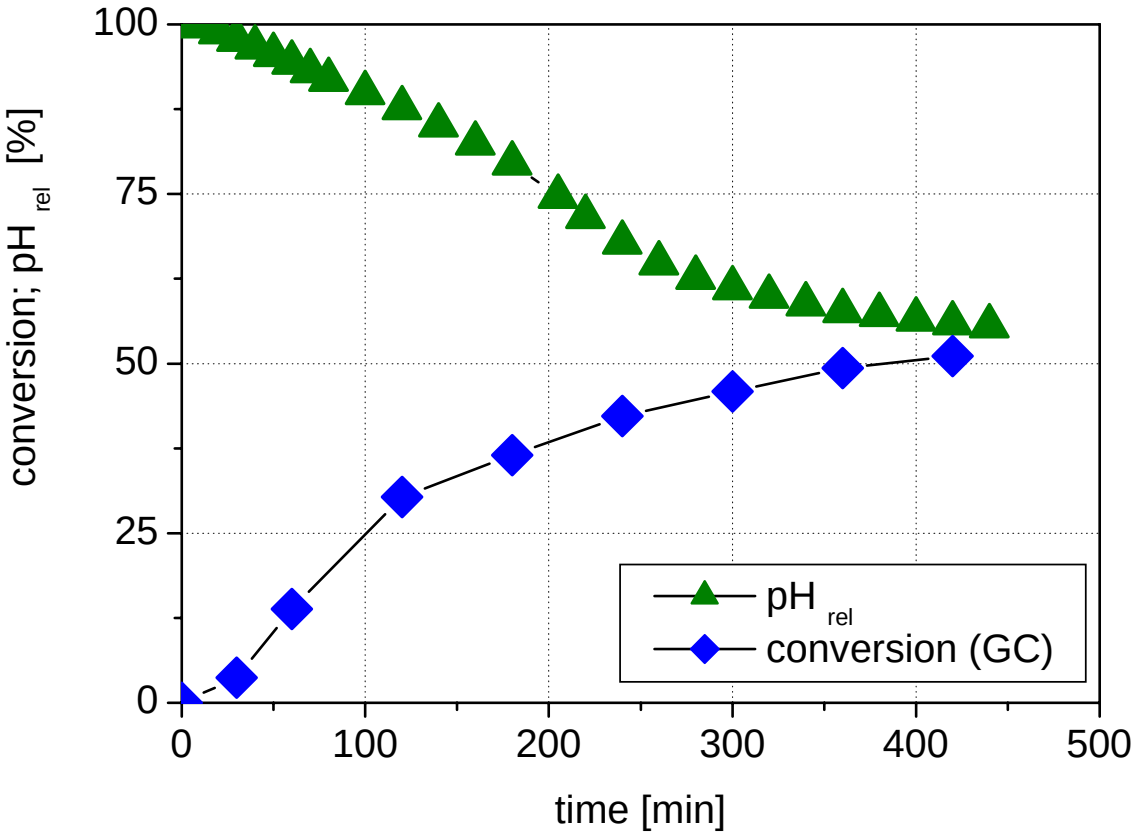


MTBE / buffer
100 mM 2-octanone
200 mM glucose
30 °C
2.5 ml each phase

ADH
GDH

Increasing the substrate concentration

two phase



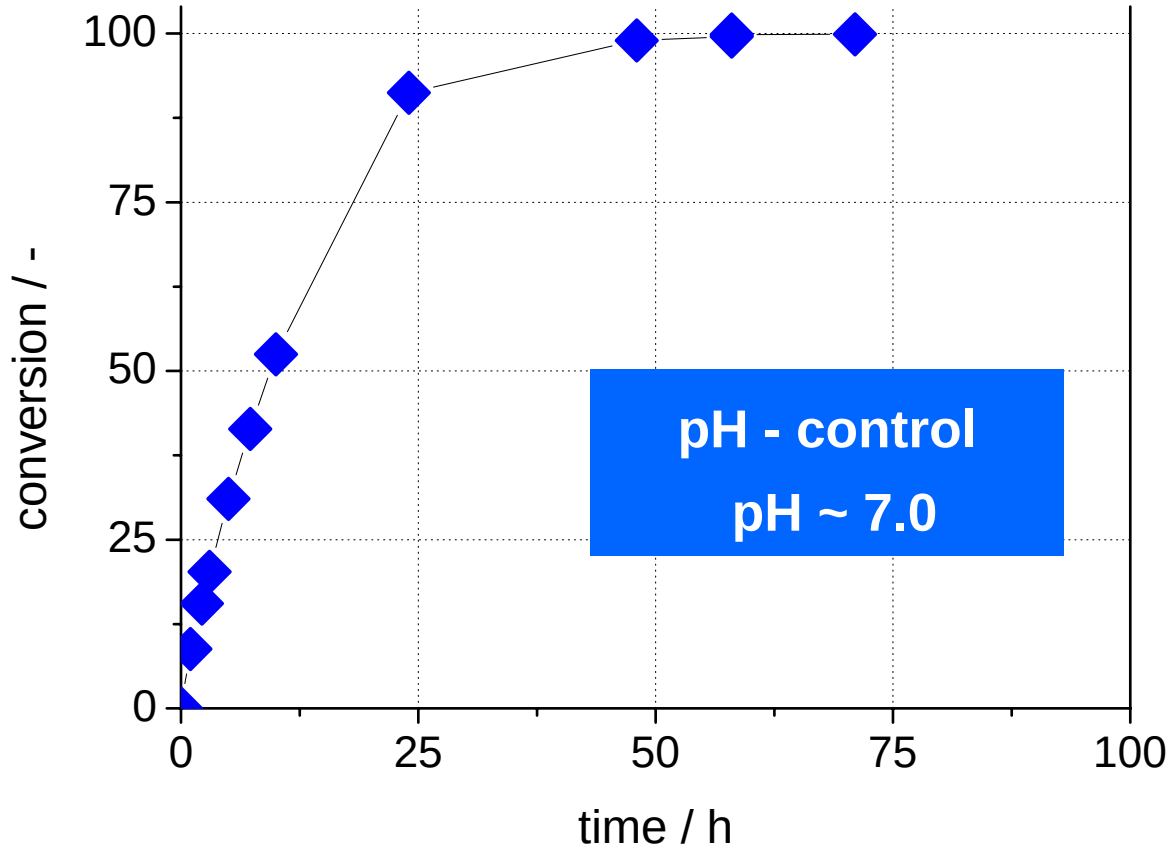
MTBE / buffer
100 mM 2-octanone
200 mM glucose
30 °C
2.5 ml each phase

ADH
GDH

end of reaction
pH ~ 3.8

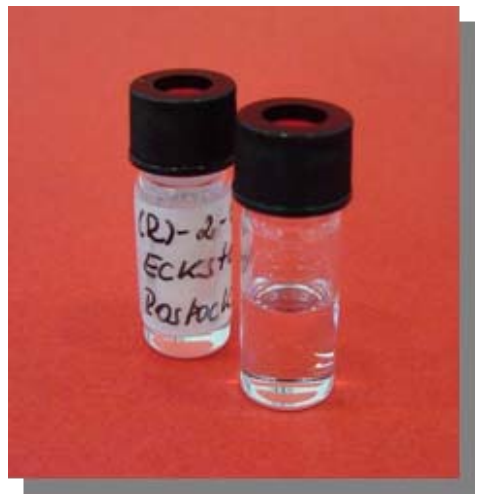
Scale up

two phase



MTBE / buffer
400 mM 2-octanone
500 mM glucose
30 °C
100 ml each phase

ADH
GDH



max. conversion = 99.9 %
~ 4.5 ml product
isolated yield after distillation ~72 %
space time yield 50 g/(L×d)

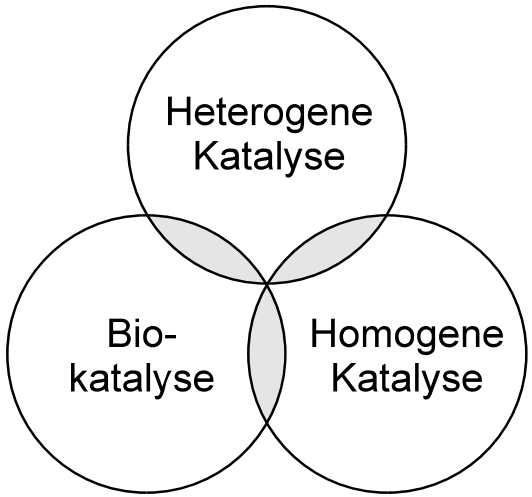
Take Home Message

- Biotransformations are gaining importance due to their better **selectivity** and **„cleaner“** reaction conditions.
  They replace and complement chemical processes.
- Measures have to taken to meet **special properties** and **requirements** of biocatalysts (product inhibition, toxicity).
- More than **100 industrial biotransformations** known
  - most of them use **hydrolases**.
  - **oxidoreductases** often as whole cells (cofactor!).
- Biotransformations are possible in **organic solvents & IL´s**.
- **Protein metabolic engineering** for improved
  - availability.
  - selectivity and specificty

`Bacteria are capable of bringing about chemical reactions of amazing variety and sublety in an extremely short time... Many bacteria are of very great importance to industry where they perform tasks which would take much time and trouble by ordinary chemical methods.´

<Sir Cyril Hinshelwood, 1956>

(The New Scientist, 1st issue 22 Nov. 1956)



UNIVERSITÄT ROSTOCK

Mathematisch-Naturwissenschaftliche Fakultät
FB Chemie – Abt. für Analytische, Technische und Umweltchemie
Technische Chemie - **Prof. Dr. Udo Kragl**

U. Kragl, Universität Rostock, D-18051 Rostock

Albert-Einstein-Str. 3A
D-18059 Rostock
Tel. 0381-498-6450
Fax. 0381-498-6452
Email udo.kragl@uni-rostock.de

Biocatalysis - References

10.01.2008

Bailey, J. E.; Ollis, D. F.; 1986; Biochemical Engineering Fundamentals, McGraw-Hill, New York.

Bisswanger, H. 1994; Theorie und Methoden der Enzymkinetik. Verlag Chemie, Weinheim. 2. Aufl.

Bornscheuer, U.; Kazlauskas, R. J.; 1999; Hydrolases in Organic Synthesis, Wiley-VCH.

Chmiel, H. (Ed.); 1991; Bioprozeßtechnik 1 & 2, Gustav Fischer Verlag, Stuttgart und neuere Auflage.

Collins, A. N.; Sheldrake, G. N.; Crosby, J. (Eds.); 1992; Chirality in Industry; John Wiley & Sons, Chichester.

Cornils, B.; Herrmann, W. A.; Schlögl, R.; Wong, C.-H., Catalysis from A to Z, Wiley-VCH, Weinheim, 2000 und neuere Ausgaben.

Cornish-Bowden, A. 1995; Fundamentals of enzyme kinetics. 2nd ed. Portland Press.

Drauz, K.; Waldmann, H. (Eds.); 2002; Handbook of Enzyme Catalysis in Organic Synthesis; VCH, Weinheim.

Faber, K.; 2000; Biotransformations in organic chemistry, 4th ed. Springer Verlag, Berlin.

Fluka, Spezialbroschüre Enzymes; Fluka, Buchs, CH.

Godfrey, T.; West, S. (Eds.); 1996, Industrial Enzymology - Application of Enzymes in Industry; 2nd Edition, Macmillan, London.

Holland, H. L.; 1992; Organic synthesis with oxidative enzymes; VCH Weinheim.

Karlson, P.; 1984; Kurzes Lehrbuch der Biochemie; Georg Thieme Verlag, Stuttgart.

Liese, A.; Seelbach, K.; Wandrey, C., Industrial Biotransformations, VCH-Wiley, Weinheim, 2000, 2004.

Patel, R. N., Stereoselective Biocatalysis, Marcel Dekker, New York, 2000.

Roberts, S. M.; Casy, G.; Nielsen, M.-B.; Phythian, S.; Todd, C., Wiggins, K.; 1999; Biocatalysts for fine chemicals synthesis, John Wiley & Sons; previously published as Preparative biotransformations: Whole cells and isolated enzymes in organic chemistry. Wiley, Chichester, 1997.

Schmid, R. D. 2001, Taschenatlas der Bio- und Gentechnologie, Wiley-VCH. (Engl. Version 2003)

Schomburg, D.; 1993; Enzyme Handbook. Springer, Berlin; www.brenda.uni-koeln.de

Segel, I. H.; 1975; Enzyme kinetics. John Wiley & Sons, New York.

Sheldon, R. A.; 1993; Chirotechnology: industrial synthesis of optically active compounds; Marcel Dekker, New York.

A. J. J. Straathof, P. Adlercreutz (Eds.), 2000: Applied Biocatalysis, 2nd. Edition, Harwood Academic Publishers, Amsterdam, ISBN 90-5823-023-6

Tanaka, A.; Tosa, T.; Kobayashi, T. (Eds.); 1993; Industrial application of immobilized biocatalysts; Marcel Dekker, New York.

Wong, C.-H.; Whitesides, G. M. 1994; Enzymes in synthetic organic chemistry. Pergamon Press, Oxford.

Selected Chapters/Keywords in (print, online)

Roempp´s Chemie Lexikon
Ullmann´s Encyclopedia of Industrial Chemistry
Winnacker-Küchler Winnacker-Küchler: Chemische Technik 5. Aufl. pp 1377-1416 (2005)
Handbok of Heterogenous Catalysis, 2. Aufl. (2008).