

Selective biotransformations in continuous flow reactors

*Cs. Csajági¹, G. Szatzker², M. Szigeti², A. Tomin², E. R. Tőke²,
S. Pilbák², L. Ürge¹, F. Darvas¹ and L. Poppe^{*2}*

*¹ ThalesNano Inc., Budapest, Hungary; ² Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, Hungary;
E-mail: poppe@mail.bme.hu*



Synthetic biocatalysis

Lipases as biocatalysts for synthetic biotransformations

- Among the available **biocatalysts**, several characteristics make **hydrolases** useful for **synthetic biotransformations**.^[1]
- **Hydrolases** often exhibit **broad substrate specificity** and accept as substrates various synthetic intermediates. At the same time, they often show **high stereoselectivity**, even toward un-natural substrates.
- Besides hydrolysis, **hydrolases** can also catalyze several **related reactions** such as condensations (**reversal of hydrolysis**) and alcoholysis (a **cleavage using an alcohol in place of water**).
- **Lipases** proved to be **highly versatile biocatalysts** in stereoselective biotransformations such as kinetic resolutions,^[2] deracemisations and dynamic kinetic resolutions.^[3]

[1] U. T. Bornschauer, R. J. Kazlauskas, *Hydrolases in Organic Synthesis: Regio- and Stereoselective Biotransformations*, Wiley-VCH, Weinheim-New York, 2006.

[2] A. Ghanem, H. Y. Aboul-Enein, *Chirality*, 2005, 17, 1-15.

[3] N. J. Turner, *Curr. Opin. Chem. Biol.*, 2004, 8, 114-119.

Synthetic biocatalysis

Mode of process in synthetic biotransformations

- Despite the strong trend for automation, the major part of high-throughput chemistry is still carried out in batches, whereas flow-through processes are rather restricted to production processes. This is not well understandable because the main advantages of the flow-through approach are facile automation, reproducibility, safety, and process reliability are not exploited.
- Numerous lipase-catalyzed reactions in **continuous packed-bed reactor systems** were studied in **connection with food and related industries**. In this respect, mostly biotransformations of triglycerides and related compounds were performed in continuous-flow reactors.
- The vast majority of the **enzymatic enantioselective processes** were performed in **batch mode**.^[1,4] Surprisingly, there are **only a few examples for hydrolase-catalyzed enantioselective processes carried out in various continuous-flow systems**. Most of these studies aimed at the biocatalytic syntheses of chiral pharmaceutical intermediates and were performed at relatively large scale using immobilized lipase in packed-bed reactor.^[5]

[1] U. T. Bornschauer, R. J. Kazlauskas, *Hydrolases in Organic Synthesis: Regio- and Stereoselective Biotransformations*, Wiley-VCH, Weinheim-New York, 2006.

[4] a) L. Poppe, L. Novák, *Selective Biocatalysis: A Synthetic Approach*, VCH, Weinheim-New York, 1992;
b) K. Faber, *Biotransformations in Organic Chemistry*, 4th ed., Springer, Berlin, 2004.

[5] R. N. Patel, A. Banerjee, L. J. Szarka, *J. Am. Oil Chem. Soc.*, 1996, 73, 1363-1375.

Synthetic biocatalysis

The Evonik (Degussa) case:

Process design & Pilot production

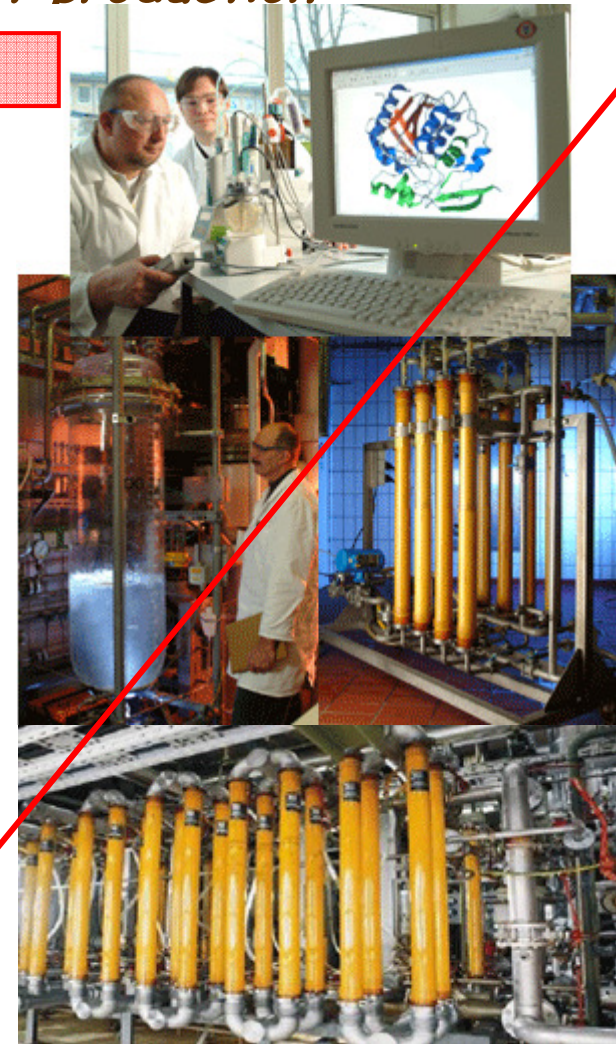
Batch mode

Industry don't waste resources on lab curiosities. The job of the process design team is to **rapidly provide economic solutions that work at scale.**

DoE-strategies are used to set optimal process parameters which are tested at meaningful scale using flexible and scalable unit operations.

Economies are analyzed based on pilot production runs which include required downstream processing steps to match the specificity of your molecule. Only this will provide you with a meaningful answer to the economy of your process!

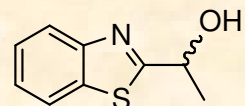
Evonik - Degussa



Continuous flow mode

Biocatalysis in continuous systems

Enantiomer separation by continuous enzyme reactor



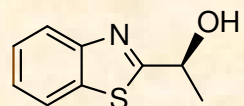
50 % conv.

~ 90 % separation in one extraction step

bioreactor

hexane
vinyl acetate

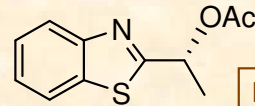
>99 %ee



40 % MeOH
-H₂O

mixing cell

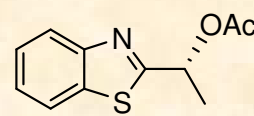
>99 %ee



separator

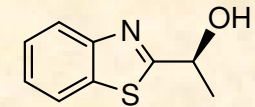
hexane

>99 %ee



40 % MeOH
- H₂O

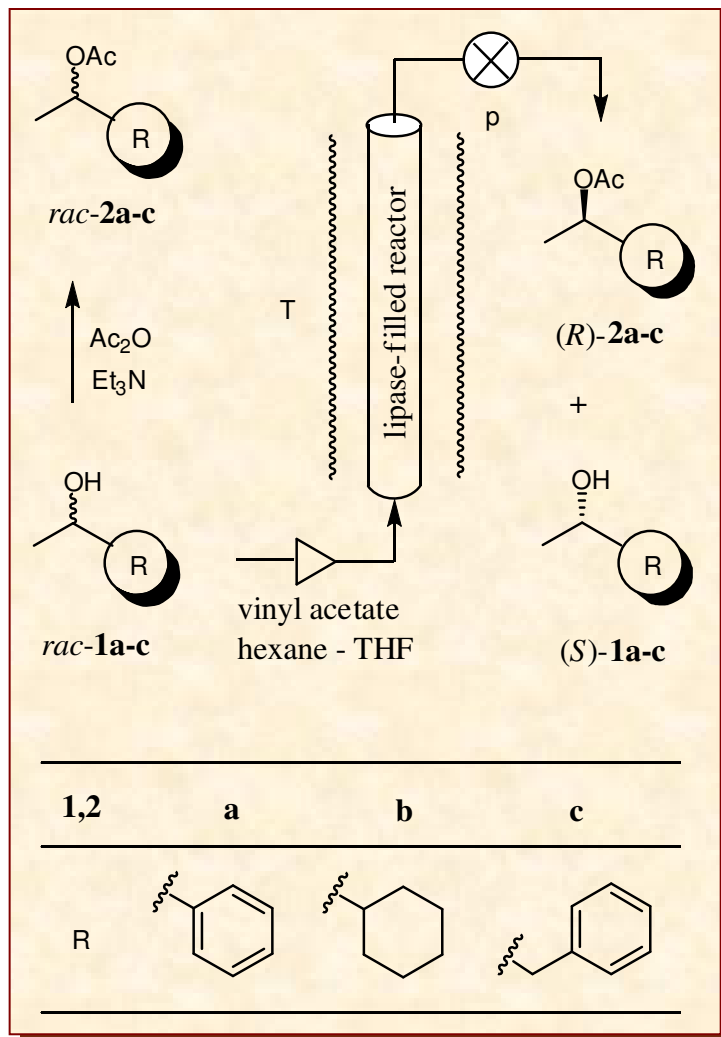
>99 %ee



Pilbák, S.; Poppe, L.: *Unpublished results.*

Biocatalysis in X-Cube

Enantiomer separation by lipases in the X-Cube



Lipase-catalyzed kinetic resolutions of secondary alcohols *rac-1a-c* in X-Cube

Csajági, Cs.; Szatzker, G.; Tőke, E. R.; Üрге, L.; Darvas, F.; Poppe, L, *Tetrahedron:Asymmetry*, 2008, 19, 237-246 .

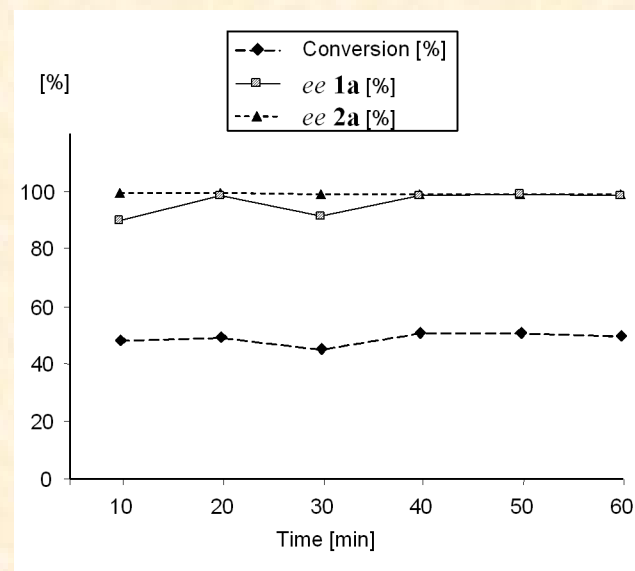
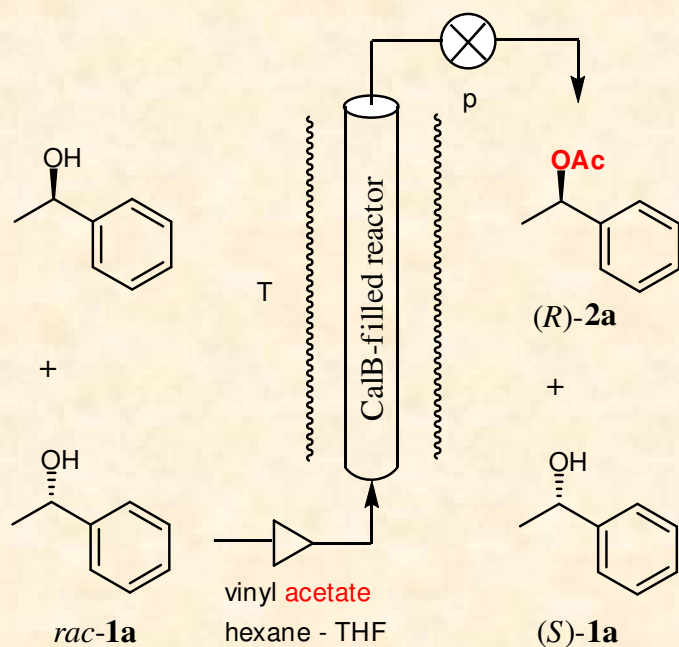
18 October 2008

ThalesNano UGM 2008, Budapest

6

Kinetic resolution in X-Cube

Enantiomer separation by CalB in the X-Cube



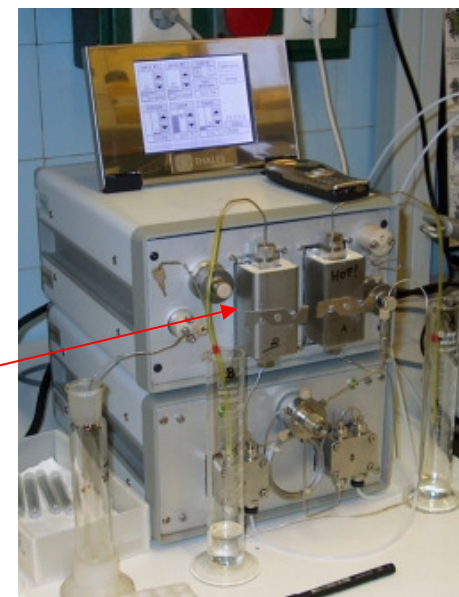
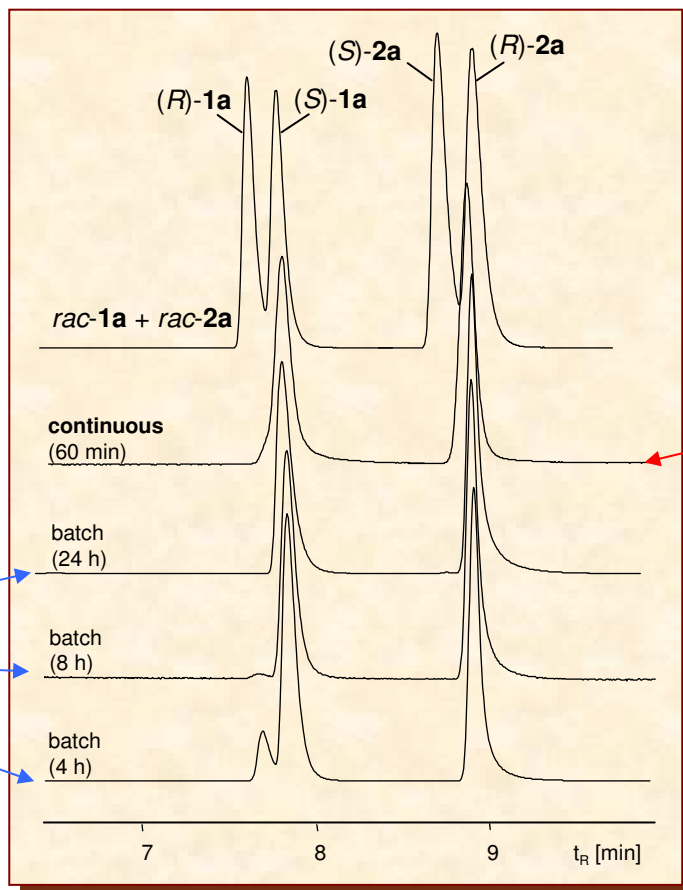
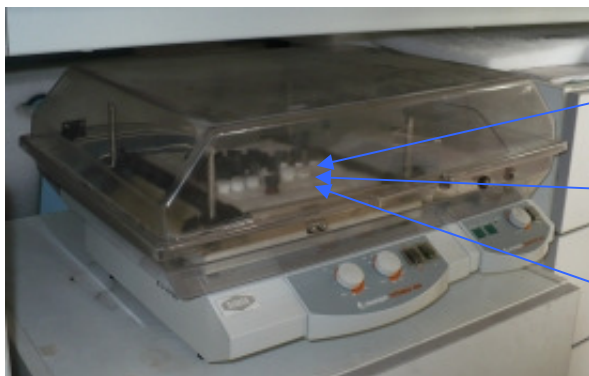
The **stationary state** of kinetic resolution in CalB-filled CatCart™ can be reached within 60 min

Kinetic resolution in X-Cube

Batch mode vs. continuous mode reactions: selectivity

CalB-catalyzed reactions of *rac*-1a analyzed by GC on chiral phase

Batch mode reaction
in shake flasks (vials)



Continuous reaction
in X-Cube

In the batch reactions, the high degree of enantiomeric purity of the unreacted enantiomer (*S*)-1a can be reached only after 24 h.

Biocatalysis in X-Cube

Batch mode vs. continuous mode reactions: productivity

$$r_{\text{flow}} = \frac{[P] \times f}{m_e} \left[\frac{\mu\text{mol}}{\text{min} \times \text{g}} \right]$$

Equation 1.

The **specific reaction rate** for a **continuous-flow system** (r_{flow}) can be calculated^[6] from the concentration of the product ($[P]$ [$\mu\text{mol mL}^{-1}$]), the flow rate (f [mL min^{-1}]) and the mass of the applied enzyme (m_e [g]) according to Equation 1.

$$r_{\text{batch}} = \frac{n_p}{t \times m_e} \left[\frac{\mu\text{mol}}{\text{min} \times \text{g}} \right]$$

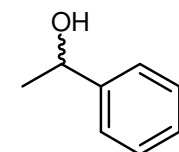
Equation 2.

A **stirred (or shake flask) batch reaction** can also be characterized by the **specific reaction rate** (r_{batch}) which can be calculated from the amount of the product (n_p [μmol]), the reaction time (t [min]) and the mass of the applied enzyme (m_e [g]) according to Equation 2.

[6] E. J. Tomotani, M. Vitolo, *J. Food Engin.*, 2007, 80, 662-667.

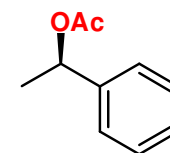
No	Enzyme (in packed bed column)	<i>c</i> [%]	<i>ee</i> _{(S)-1a} [%]	<i>ee</i> _{(R)-2a} [%]	<i>E</i>	<i>r</i> [μmol min ⁻¹ g ⁻¹]
1	CaLB	50	98.8	99.2	»200	10.2
2	Lipase, <i>Pseudomonas cepacia</i> , IM	50	96.8	97.4	>200	10.2
3	Lipozyme™ <i>Mucor miehei</i> , IM	19	31.1	99.1	>200	4.0
4	Lipozyme TL IM	18	28.9	98.9	>100	3.7
5	Amano Lipase AK	52	99.8	94.6	>100	10.6
6	Amano Lipase PS	24	39.6	99.6	»200	5.0
7	Lipase, <i>Candida rugosa</i>	15	21.7	53.2	4.0	3.1
8	Lipase, porcine pancreas	6	13.6	97.2	81	1.2
9	Lipase B, <i>C. antarctica</i> , on CS ^[d]	10	33.5	93.4	41	2.0
10	Lipase, <i>T. lanuginosus</i> , on CS ^[d]	20	50.0	97.6	>100	4.1
11	Lipase, porcine pancreas, on CS ^[d]	1	0.9	54.1	3.4	0.2

No	Enzyme (in shake flask)	<i>c</i> [%]	<i>ee</i> _{(S)-1a} [%]	<i>ee</i> _{(R)-2a} [%]	<i>E</i>	<i>r</i> [μmol min ⁻¹ g ⁻¹]
12	CaLB	51	99.6	98.3	»200	5.7
13	Lipase, <i>Pseudomonas cepacia</i> , IM	57	99.5	80.6	53	7.5
14	Lipozyme™ <i>Mucor miehei</i> , IM	42	78.3	98.7	>200	4.1
15	Lipozyme TL IM	31	55.4	97.7	>100	2.5
16	Amano Lipase AK	75	99.0	47.0	13	17.3
17	Amano Lipase PS	44	84.4	99.4	»200	4.4
18	Lipase, <i>Candida rugosa</i>	16	22.4	53.7	4.1	1.1
19	Lipase, porcine pancreas	16	32.1	98.5	>100	1.0
20	Lipase, <i>T. lanuginosus</i> , on CS ^[d]	34	58.6	95.9	87	2.9
21	Lipase, <i>T. lanuginosus</i> , precip. ^[d]	51	71.5	69.6	12	5.2



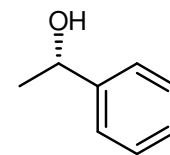
rac-1a

↓ vinyl acetate
lipase



(R)-2a

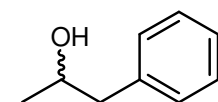
+



(S)-1a

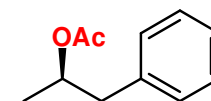
Similar enantiomer selectivities (*E*) with *rac* 1-phenylethanol (*rac*-1a) in continuous-flow or batch reactions

No	Enzyme (in packed bed column)	<i>c</i> [%]	<i>ee</i> _{(S)-1c} [%]	<i>ee</i> _{(R)-2c} [%]	<i>E</i>	<i>r</i> [μmol min ⁻¹ g ⁻¹]
1	CaLB	42	57.2	84.9	23	7.7
2	Lipase, <i>Pseudomonas cepacia</i>, IM	50	92.4	94.3	>100	9.2
3	Lipozyme™ <i>Mucor miehei</i> , IM	24	30.8	98.9	>100	4.4
4	Lipozyme TL IM	16	17.4	98.4	>100	2.9
5	Amano Lipase AK	38	61.0	98.7	>200	7.0
6	Amano Lipase PS	6	5.7	98.6	>100	1.1
7	Lipase, <i>Candida rugosa</i>	8	2.6	0.3	1.0	1.4
8	Lipase, porcine pancreas	3	2.1	89.4	18	0.5
9	Lipase B, <i>C. antarctica</i> , on CS ^[d]	19	21.2	93.1	34	3.5
10	Lipase, <i>T. lanuginosus</i> , on CS ^[d]	30	41.7	97.4	>100	5.6
No	Enzyme (in shake flask)	<i>c</i> [%]	<i>ee</i> _{(S)-1c} [%]	<i>ee</i> _{(R)-2c} [%]	<i>E</i>	<i>r</i> [μmol min ⁻¹ g ⁻¹]
11	CaLB	71	99.2	39.4	10.7	12.5
12	Lipase <i>Pseudomonas cepacia</i>	52	99.4	89.7	>100	5.6
13	Lipozyme™ <i>Mucor miehei</i>	49	93.4	97.9	>200	4.8
14	Lipozyme TL IM	49	97.1	95.7	>100	4.9
15	Amano Lipase AK	52	99.5	89.7	>100	5.5
16	Amano Lipase PS	44	80.1	97.6	>100	4.0
17	Lipase <i>Candida rugosa</i>	16	2.4	12.4	1.3	1.0
18	Lipase, porcine pancreas	14	7.6	87.5	20	0.9



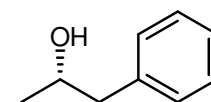
rac-1c

↓ vinyl acetate
lipase



(R)-2c

+

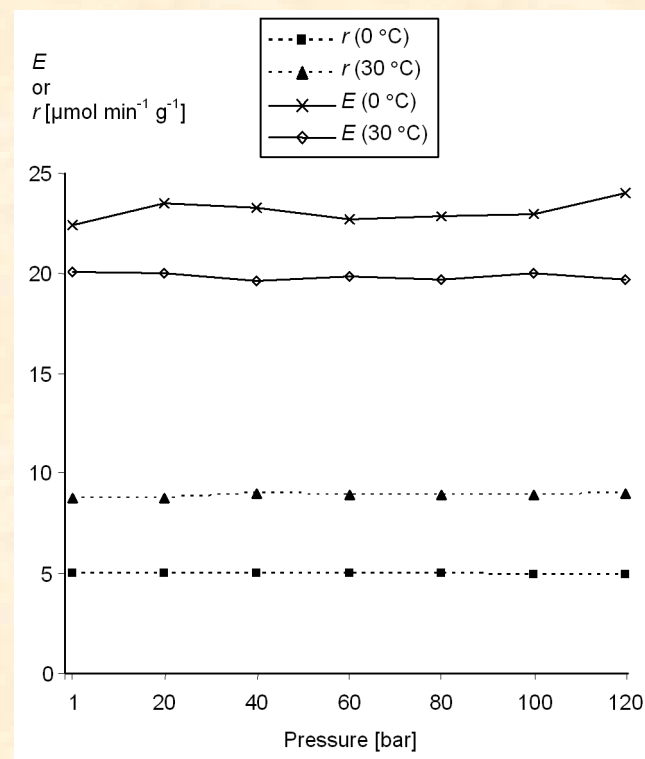
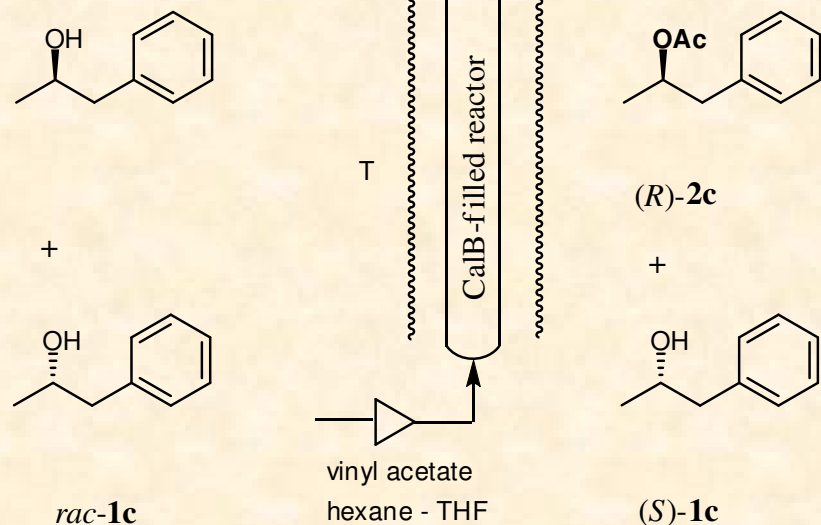


(S)-1c

With 1-phenylpropan-2-ol (1c) most lipases retained their high enantiomer selectivities except CaLB

Kinetic resolution in X-Cube

Effect of *pressure* on continuous mode lipase-reaction

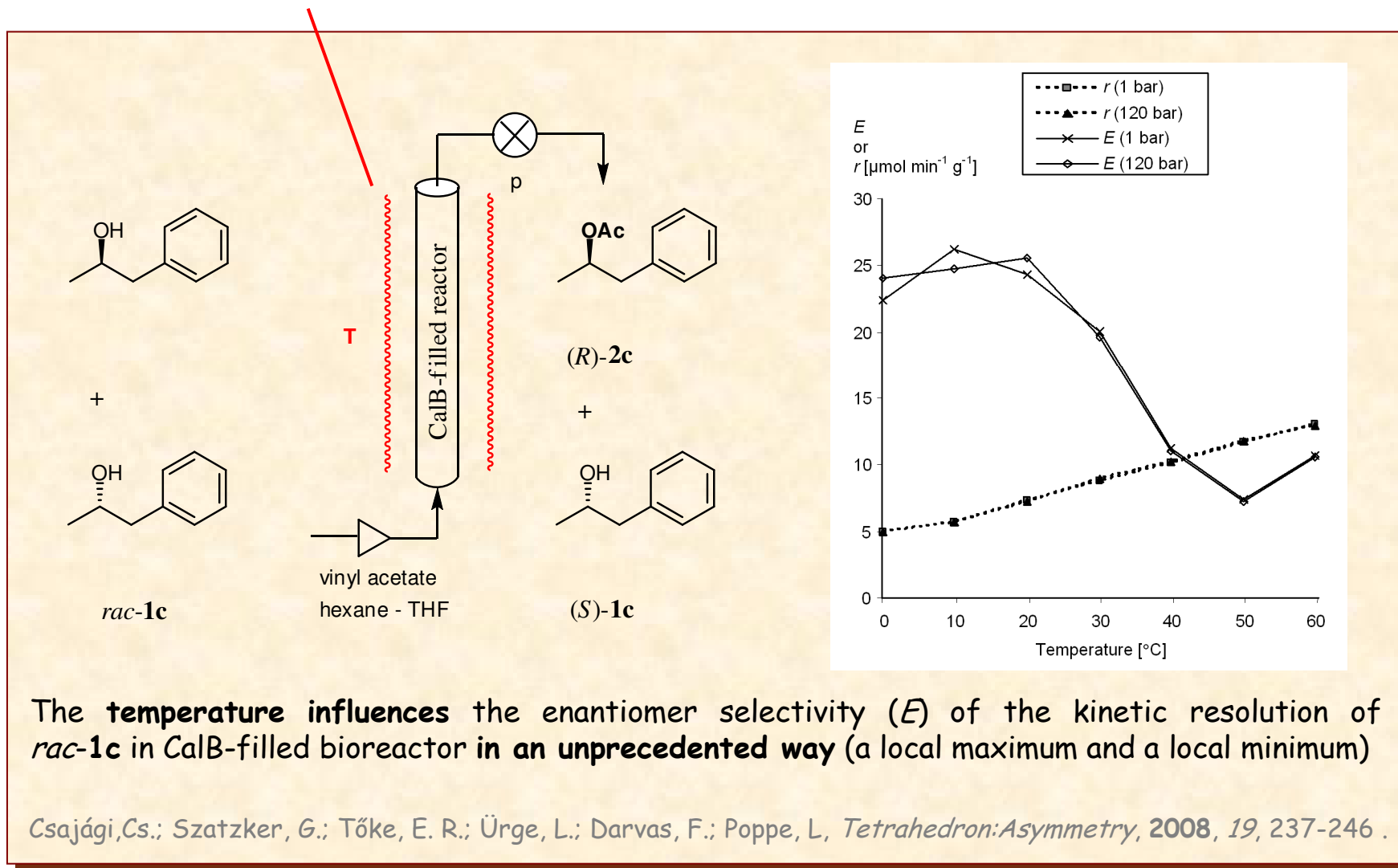


The pressure has negligible effect on the kinetic resolution of *rac-1c* in CalB-filled bioreactor

Csajági, Cs.; Sztzker, G.; Tőke, E. R.; Üрге, L.; Darvas, F.; Poppe, L, *Tetrahedron:Asymmetry*, 2008, 19, 237-246 .

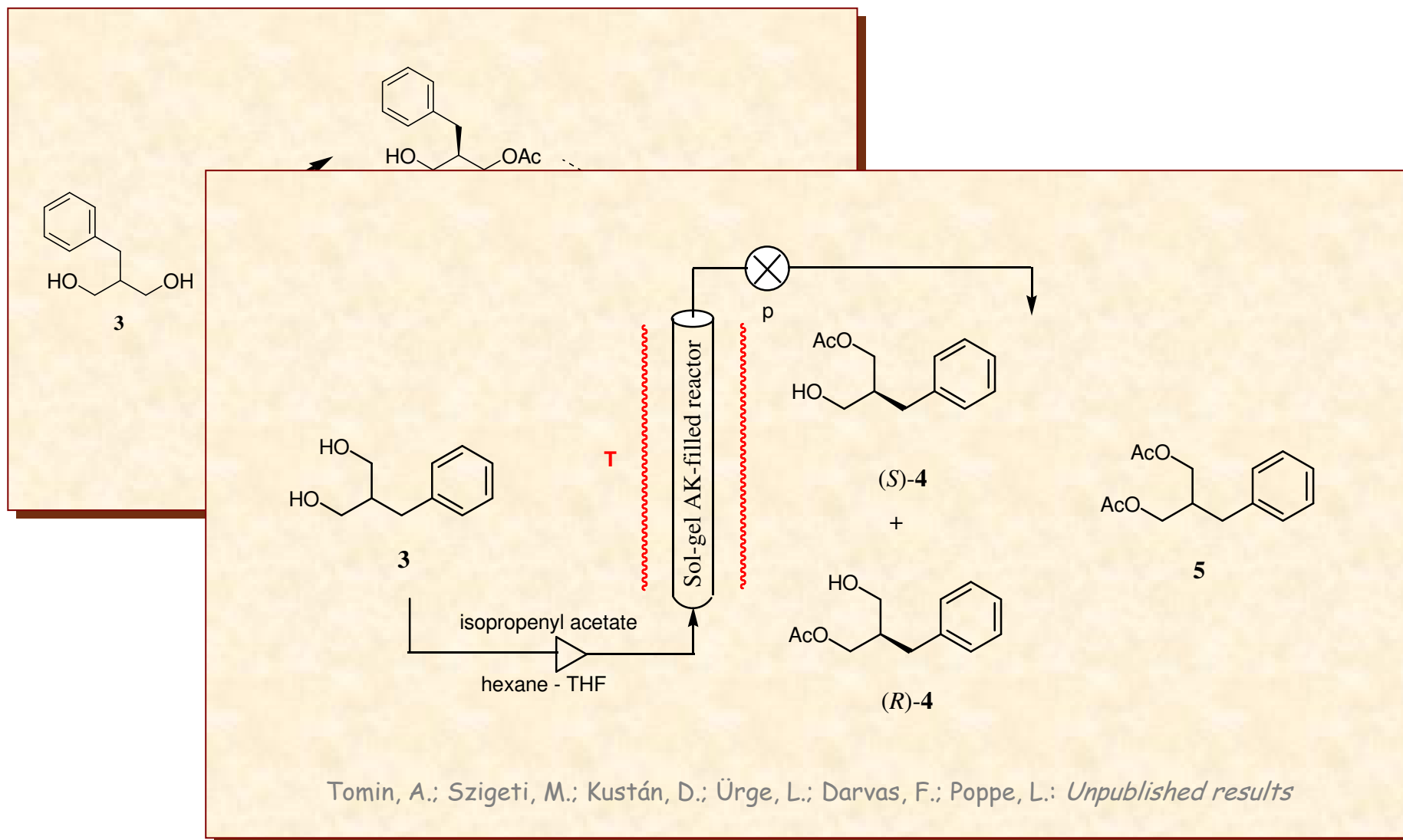
Kinetic resolution in X-Cube

Effect of *temperature* on continuous mode lipase-reaction



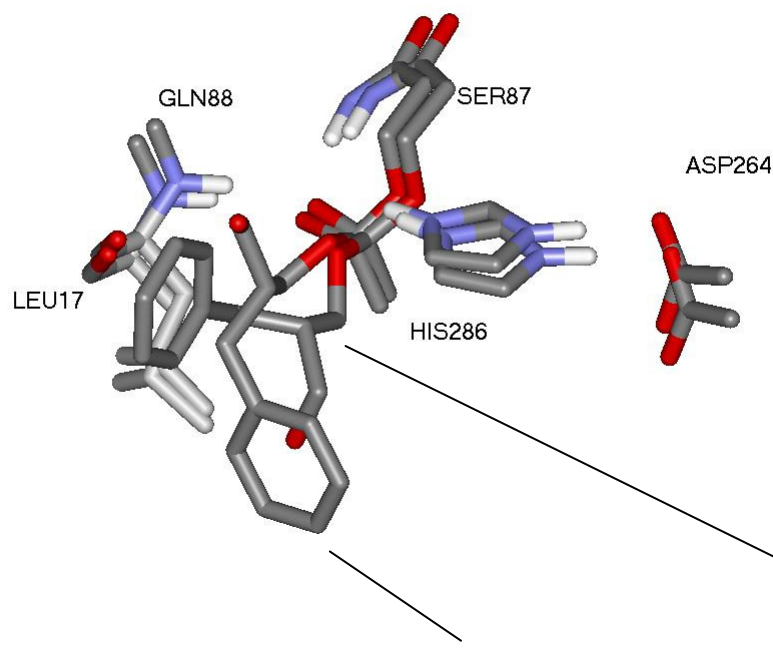
Asymmetric acylation in X-Cube

Asymmetric acylation of prochiral diol by lipase-reaction



Absolute configuration prediction

*THI states for acylation of diol 3 within *P. cepacia**



The **QM/MM** calculations for THI states of the acylation of the prochiral diol **3** within the active site of lipase from *Burkholderia cepacia* (*Pseudomonas cepacia*, PDB: 1YS1) predicted that the **(*R*)-4 monoacetate** is the favored acylation product.

The calculations are in full agreement with the absolute configuration assignment based on optical rotation [$+$ \rightarrow (*R*)].

Relative energies for THI states (kcal/mol) determined by QM/MM calculations

QM part	<i>R</i> -alc- <i>R</i> -THI	<i>R</i> -alc- <i>S</i> -THI	<i>S</i> -alc- <i>R</i> -THI	<i>S</i> -alc- <i>S</i> -THI
pm3	0.0	7.8	5.3	22.2
hf	0.0	30.6	19.1	27.0

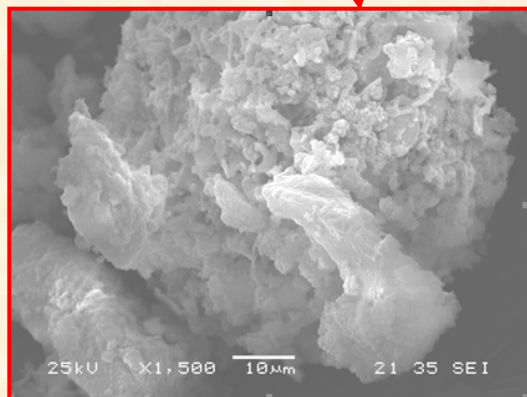
Biocatalysis in X-Cube

Novel combined sol-gel immobilized lipases (Lipase AK)

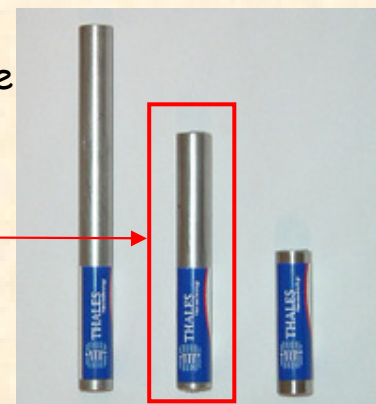
SilaneI/SilaneII (1:1)	Time (hours)	Method ^a	Preparate amount (mg)	Enzyme amount ^b (mg)	Specific activity ^c (μmol/h·mg enzyme)	Relative activity ^d
OcTMOS/TEOS	48	A	388.0	10.05	0.27	5.08
PhTMOS/TEOS	48	A	536.7	7.26	0.18	3.37
OcTMOS/TMOS	48	B	530.0	7.36	0.42	7.80
OcTMOS/TMOS	48	C	372.5	10.47	0.15	2.71
OcTMOS/TMOS	48	D	476.5	8.18	0.43	7.97
Free Lipase AK	48	-	-	50.00	0.05	1

^a Method B: Lipase AK containing sol-gel material composed of OcTMOs/TMOS, polymerized onto Celite

^b Enzyme amount in activity test; ^{c,d} Enzyme activity is related to kinetic resolution of 1-phenylethanol *rac*-1a



The OcTMOS/TMOS/Celite Lipase AK biocatalysts **retained most of its activity after stirring in refluxing hexane for 1h**

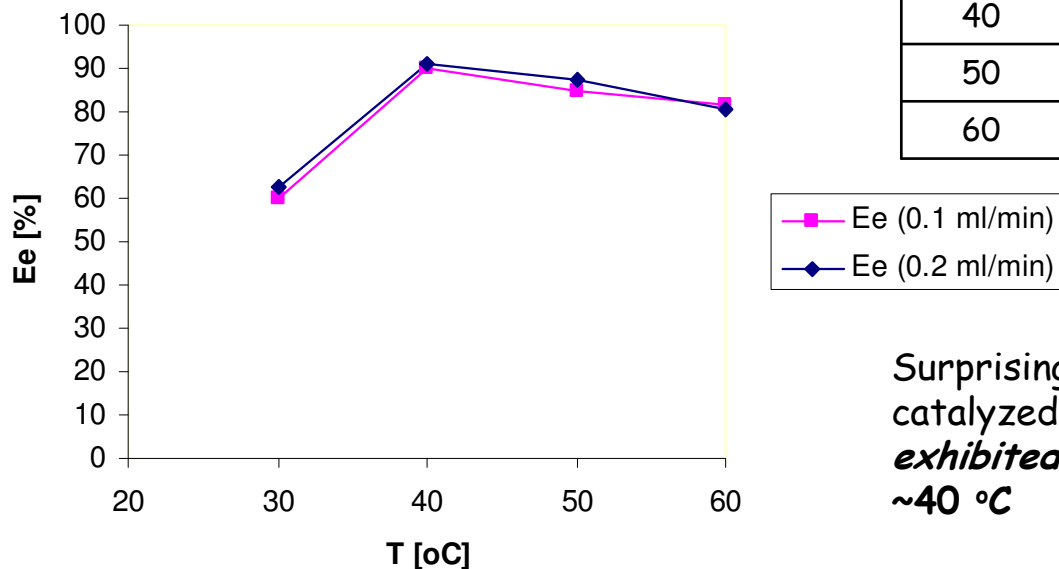


Tomin, A.; Szigeti, M.; Bata Zs.; Corici, L.; Kustán, D.; Üрге, L.; Darvas, F.; Poppe, L.: *Unpublished results*

Asymmetric acylation in X-Cube

Asymmetric acylation of prochiral diol by lipase-reaction

The OcTMOS/TMOS/Celite Lipase AK biocatalysts exhibited good but not extremely high enantioselectivity -> dependence of the selectivity on temperature can be studied



T [°C]	f [ml/min]	c [%]	[a] _b	ee [%]
30	0.1	70	9.2	60
40	0.1	73	13.8	90
50	0.1	74	13	85
60	0.1	85	12.5	82
30	0.2	33	9.6	63
40	0.2	35	13.9	91
50	0.2	51	13.4	88
60	0.2	53	12.3	80

Surprisingly, the acylation of prochiral diol **3** catalyzed by OcTMOS/TMOS/Celite Lipase AK exhibited enantioselectivity maximum at **~40 °C**

Tomin, A.; Szigeti, M.; Kustán, D.; Üрге, L.; Darvas, F.; Poppe, L.: *Unpublished results*

Take home message

- Continuous mode biotransformations catalyzed by lipases **do not alter the selectivity** of the biotransformations compared to the batch mode reactions
- The **productivity of the biocatalysts is superior in continuous mode** biotransformations compared to the batch mode reactions
- Pressure has negligible effect on the lipase catalyzed acylations
- The **dependence of the stereoselectivity on temperature is significant and often unpredictable**



Thank you for your attention

BME:

Gábor Szatzker, Mariann Szigeti, Anna Tomin,
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Csaba Csajági, László Ürge, Ferenc Darvas

Politehnica University, Timisoara:

Livia Corici, Francisc Peter



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