

## Excellence in Biocatalysis

Full service from screening to large-scale production

- 40 years of experience in biotechnology
- 20 different enzymes applied in commercial scale
- Commercial production of various amino acids and other products using fermentation based processes
- Evonik produces commercial products based on fermentative and biocatalytic technologies globally on five sites

**Many of these enzymes are also applied as whole cell catalysts**

### Screening and Optimization

- Development, screening and optimization of new enzymes
- Based on bacteria, algae, fungi/yeast

### Applied technologies

- Directed evolution
- Rational design
- OMICs
- HTS analysis

The optimization process always takes the requirements of a later production into account and yields a stable, scalable process.

### Large scale production

- Various Scalable expression systems
- In-house fermenters from 2 l to 50 m<sup>3</sup>
- Batch-, EMR- and fixed bed conversions
- Ton scale production using several enzyme classes and reactor concepts have been implemented at Evonik

### Process development beyond enzyme supply

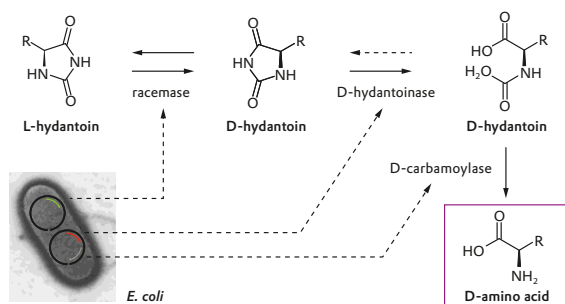
Examples for large scale enzymatic processes:

- L- and D-amino acids by hydantoinases
- R- and S-alcohols by alcohol dehydrogenases
- D- and L-serine by aldolases
- L-tert-leucine by amino acid dehydrogenase

### Cascade reactions established for several products

Up to four reaction steps in one reactor using one multi-enzyme whole-cell biocatalyst

#### Example 1: Heterologous Enzyme Cascades / Hydantoinase Platform Technology



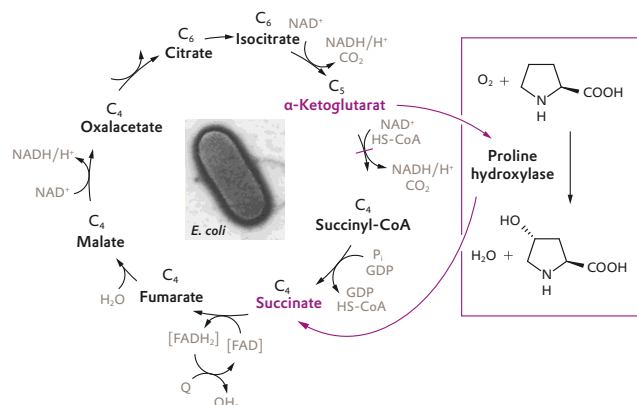
Combination of three enzymatic steps:

- Hydantoin cleavage by hydantoinase
- Hydantoin racemization by racemase
- Intermediate hydrolysis by carbamoylase

"One-pot" dynamic kinetic resolution reaching virtually 100 % conversion

Library of > 40 enzymes ready to use for the synthesis of a broad range of aromatic and aliphatic D- and L-amino acids. Application of whole cell catalysts simplifies enzyme production and application. Technology proved efficiency at 50 m<sup>3</sup>-/10- tons-scale

## Example 2: Combination of Biocatalysis and Co-substrate supply from primary metabolism



The *E. coli* strain produces the heterologous enzyme proline hydroxylase

The Co-substrate  $\alpha$ -ketoglutarate is recycled via *E. coli*'s Citric acid cycle

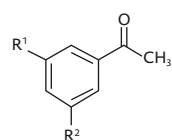
Knock-out mutations provide for a increased  $\alpha$ -ketoglutarate pool and the elimination of proline degradation

## Platform technologies

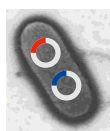
Evonik's enzymatic tool box comprises approx. 20 enzymes on a commercial scale:

- Acylases
- Aldolases
- Amidases
- Amino acid oxidases
- Carbamoylases
- Hydantoinases and -racemases
- Monooxygenases
- Hydroxynitril lyases
- Lipase, esterases
- Nitrilases
- Proteases
- Threonin Hydratases
- Transaminases
- Dehydrogenases: Alcohol, amino acid, formate, glucose

## Example 3: Process development using "off the shelf" whole cell catalysts



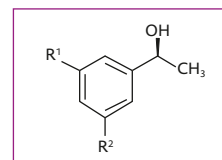
Substrate concentration:  
200g/L



Whole cell-catalyst (40 g/L)

containing:  
• ADH  
• GDH  
• NADH

Glucose (1.05eq.)  
NAD<sup>+</sup> (1.0g/L)



>98 % conv.  
80% Yield  
>94,4 % ee

0	3	6	9	12	15	weeks
Customer inquiry	Evaluation of biocatalysts	Synthesis of a 50g-sample	Process optimization			Production at 1m <sup>3</sup> scale

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