Novel Biocatalyst Development and Their Application Methodology

Roland Wohlgemuth
Overview

Introduction
Epoxidehydrolases
Alcoholdehydrogenases/Ketoreductases
Transketolases
Kinases
Dehydratases
Outlook
Manufacturing Performance, Selectivity, Sustainability by Molecular Economy

Revitalizing Old Routes

Finding New Routes
Epoxidehydrolases
Selective Biocatalytic Epoxide Hydrolysis

Epoxide Hydrolase Catalyzed Resolutions of (+)- and (−)-cis/trans-Limonene Oxides

Solvent-free preparative resolution of (+)- and (-)-limonene oxide mixtures catalyzed by LEHs after process optimization\(^a\)

<table>
<thead>
<tr>
<th>Process parameter</th>
<th>(+)-Limonene oxide</th>
<th>(-)-Limonene oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>E: Re-LEH</td>
<td>F: Tomsk-LEH</td>
<td>G: CH55-LEH</td>
</tr>
<tr>
<td>H: Re-LEH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>substrate volume [mL]</td>
<td>6.55</td>
<td>1.64</td>
</tr>
<tr>
<td>substrate total amount [g]</td>
<td>6.09</td>
<td>1.52</td>
</tr>
<tr>
<td>substrate loading [mol L(^-1)]</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>enzyme [mg mL(^-1)]</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>reaction temperature [°C]</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>reaction time [h]</td>
<td>4.5</td>
<td>24</td>
</tr>
<tr>
<td>epoxide yield [%](^b)</td>
<td>44 (2)</td>
<td>33 (1)</td>
</tr>
<tr>
<td>diol yield [%](^c)</td>
<td>40 (3)</td>
<td>59 (3)</td>
</tr>
<tr>
<td>STY(^{d,e}) [mmol L(^-1) h(^-1)]</td>
<td>167.3</td>
<td>6.6</td>
</tr>
<tr>
<td>specific productivity [µmol mg(^-1) h(^-1)](^e)</td>
<td>837</td>
<td>9.4</td>
</tr>
<tr>
<td>ECN(^e,f) [mg mmol(^-1)]</td>
<td>0.26</td>
<td>4.4</td>
</tr>
</tbody>
</table>

\(^a\) Reactions (20 mL) performed in KP\(_i\) buffer (pH 8.0). \(^b\) Recovery yield estimated based on the unreacted epoxide isomer indicated in brackets. \(^c\) Recovery yield estimated based on the formed diol indicated in brackets. \(^d\) STY: space-time yield. \(^e\) Calculated based on epoxide recovery. \(^f\) ECN: enzyme consumption number.
Alcoholdehydrogenases/Ketoreductases
Selective Biocatalytic Reductions

Retrosynthetic Analysis in the Biodomain for D- and L-Lactaldehyde
Enantiomerically Pure and Stable Lactaldehydes

Enantiomer Analysis of D- and L-Lactaldehyde

Transketolases
Transketolases for Biocatalytic C2-Chain Elongation

Modular Microfluidic Reactor and Inline Filtration System

Output = Pure Product

B.O’Sullivan et al., Journal of Molecular Catalysis B: Enzymatic 77 (2012) 1–8
Synthesis of L-Erythrulose in Microfluidic Reactor using Transketolase (TK)

- Full conversion achieved in the microfluidic reactor.
- Conversion rate in microfluidic reactor is slightly faster than in the microwell batch reactor.
- Further work will investigate non-specific adsorption of Transketolase (TK) onto the microchannel surface.
- The mass balance of product formation and substrate disappearance corresponds to initial substrate concentration.

50 mM substrates
0.65 mg/ml E. coli transketolase (TK) wild type (WT)
2.4 mM thiamine pyrophosphate (TPP)
9.8 mM MgCl₂

Room temperature, pH 7

Error bars represent standard deviation from three experiments.
Synthesis of PKD in Microfluidic Reactor using Transketolase

- Model reaction system with a more hydrophobic substrate yielding (3S)-1,3-dihydroxypentan-2-one (PKD).
- 50 mM substrates
- 0.56 mg/mL E. coli transketolase mutant D469T
- 2.4 mM thiamine pyrophosphate (TPP)
- 9.8 mM MgCl₂

40°C, pH 7

- Demonstrate microfluidic enzymatic reactor accepts substrates of different hydrophobic properties and underpins applicability of the reactor for organic synthesis
- High conversion to product measured.
- Rate of conversion to PKD in the microfluidic reactor slightly faster than in the batch reactor.

B.O’Sullivan et al., Journal of Molecular Catalysis B: Enzymatic 77 (2012) 1–8
Multi-Input Reactor (MIR) Setup

200 mM HPA

- HPA / TK / co-factors fed through primary, comingled with first GA input
- Residence time chosen according to model estimation of complete conversion
- Position of further inputs selected to best match those predictions of depletion in model

Kinases
Biocatalytic Asymmetric Phosphorylation

Time course of 5-Phosphomevalonate concentration [MVAP] in the enzymatic phosphorylation reaction by $^{31}$P-NMR

- [MVAP] from R-mevalonolactone
- [MVAP] from racemic R,S-mevalonolactone
- [MVAP] from S-mevalonolactone

R. Matsumi et al., RSC Advances, 4(25), 12989-12994 (2014)

Mevalonate kinase from Thermococcus kodakaraensis TK1474, overexpressed in E.coli
Reducing the Number of Reaction Steps in Synthesis

R. Matsumi et al., RSC Advances, 4(25), 12989-12994 (2014)
Biocatalytic Asymmetric Synthesis of D-GAP and L-GAP and Enantiomer Analysis

D-GAP

D-Glyceraldehyde-3-phosphate (D-GAP)

L-GAP

D.Gauss, B.Schoenenberger, R.Wohlgemuth, Carbohydrate Res. 389, 18-24 (2014);
D.Gauss et al. (2015) in press
Expanding Phosphorylated Metabolites

B. Schönenberger et al.

N. Hardt et al.
Dehydratases
One-Step Enzymatic Synthesis of 2-Keto-3-deoxy-gluconate (KDG)

Navigation Tools for Biocatalysts
Enzyme Explorer

Find enzymes / proteins, substrates, activators, and inhibitors. Resources for metabolic pathways, kinases, proteolytic enzymes and inhibitors, carbohydrate analysis as well as new cell signaling, analytical and diagnostic enzymes and detection reagents.

https://www.sigmaaldrich.com/life-science/metabolomics/enzyme-explorer.html

Metabolic Pathways

http://www.sigmaaldrich.com/life.science/metabolomics.html
Micro- and Macroscale Analytical Modes

Micro- and Macroscale Manufacturing Modes

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EU FP7-Project Biointense