Development of an in situ-product crystallization (ISPC) concept to shift the reaction equilibria of selected transaminase-catalyzed reactions

Philipp Süß
Company Info

Areas of Activity

Development of bio-based products and processes
Production of enzymes, tailor made chemicals and APIs
Customer specific solutions in enzyme technology

Location

Biotechnikum Greifswald, Germany

Management

Dr. Ulf Menyes (CEO)
Mr. Steffen Weber (CFO)

Ownership structure

Shareholders and strategic investors
**Introduction**

**Goals**

- overcome unfavorable reaction equilibria
- overcome inhibitions, side reactions, etc.
- improve downstream processing

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12.02.2020  4th DECHEMA-PRAXISforum “Enzymes for Industrial Applications”  3
Biocatalysis & Crystallization

insufficient equilibrium

substrate(s) → product(s)

product salt = equilibrium displacement + simplified downstream processing
Biocatalysis & Crystallization

\[ R^1 \text{amide acceptor} \xrightarrow{\text{TA, PLP}} R^1 \text{product amine} \]

- Unfavorable reaction equilibrium
  - Non-stoichiometric use of donor amine
  - Or (bio)synthetic solutions = low atom efficiency

- Product isolation
  - E.g. Extraction or distillation

- TA - Transaminase-catalyzed reaction

- Simplified TA reaction*

- In situ product removal (ISPR)

* Protonation as present at pH 7.5
Biocatalysis & Crystallization

"salt pair": 'simplified' binary separation process

patent WO 002019096973 A1;
Method for preparing amines from carbonyl compounds by transaminase reaction under salt precipitation
Biocatalysis & Crystallization

ternary phase diagrams diastereomeric salt behavior

"salt pair": 'simplified' binary separation process

in situ-product crystallization (ISPC)
Biocatalysis & Crystallization

Which R?

**product amine salt**

**donor amine salt**

patent WO 002019096973 A1;
Method for preparing amines from carbonyl compounds by transaminase reaction under salt precipitation

Computational prediction?

Crystal lattice energy

Not available & screening required

Solvation enthalpy

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Biocatalysis & Crystallization

exemplary amines and carboxylic acids

 donor amines

exemplary product amines

patent WO 002019096973 A1;
Method for preparing amines from carbonyl compounds by transaminase reaction under salt precipitation
acids, 1st screening (part A)
Biocatalysis & Crystallization

acids, 1\textsuperscript{st} screening (part B)
Biocatalysis & Crystallization

acids, 1st screening (part C)

and more ...
### Biocatalysis & Crystallization

**Exemplary Donor Amines**

<table>
<thead>
<tr>
<th>Donor Amine</th>
<th>Concentration (mM)</th>
<th>isopropylamine</th>
<th>rac-1-phenylethylamine</th>
<th>2-butyramine</th>
<th>LD-alanine</th>
<th>L-alanine</th>
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**Exemplary Product Amines**

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<tr>
<th>Product Amine</th>
<th>Concentration (mM)</th>
<th>(S)-1-PEA 2a</th>
<th>(S)-3CI-1-PEA 2b</th>
<th>(R)-3CI-1-PEA 2c</th>
<th>(R)-3F-1-PEA 2d</th>
<th>(R)-4F-1-PEA 2e</th>
<th>(S)-3MeO-1-PEA 2f</th>
<th>(R)-4MeO-1-PEA 2g</th>
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<tbody>
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</table>

**Comparison of Salt Pairs**

- No precipitation
- Few crystals
- Medium precipitation
- Significant precipitation

**Donor Amine Salt**

- $\text{NH}_3$
- R-COO

**Product Amine Salt**

- $\text{NH}_3$
- R-COO

AfATA, amine transaminase from *Aspergillus fumigates* (ECS-ATA01)
GzATA, amine transaminase from *Gibberella zeae* (ECS-ATA02)
NfATA, amine transaminase from *Neosartorya fischeri* (ECS-ATA03)
AoATA, amine transaminase from *Aspergillus oryzae* (ECS-ATA04)
AtATA, amine transaminase from *Aspergillus terreus* (ECS-ATA05)
MvATA, amine transaminase from *Mycobacterium vanbaalenii* (ECS-ATA07)
SpATA, amine transaminase from *Silicibacter pomeroyi* (ECS-ATA08)

Biocatalysis & Crystallization

low solubility
ca. 20 mM @ pH 7.5

3,3-diphenylpropionic acid
3DPPA

conversion / %
(product amine in solution + salt)
reaction time / h
in situ-product crystallization

ECS-ATA08, 100 mM acetophenone, 250 mM isopropylamine

applicability for other substrates

Conditions: 15 mg·mL⁻¹ lyophilized cells (SpATA), 100 mM substrate, 250 mM isopropylamine, 125 mM 3DPPA, 30 °C non-seeded process

<table>
<thead>
<tr>
<th>substrate</th>
<th>R¹</th>
<th>x</th>
<th>conversion reference [%]</th>
<th>conversion ISPC [%]</th>
<th>e.e.(S) [%]</th>
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<td>36</td>
<td>96</td>
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</table>

Continuous amine production (solid to solid reaction)

Biocatalysis & Crystallization

3 equilibria in a steady state (1 reaction and 2 solubilities)

Biocatalysis & Crystallization

partial acetone removal is sufficient

stoichiometric process

91.5 % isolated yield
>99 % purity

carboxylic acid can be recycled

Biocatalysis & Crystallization

J. Neuburger et al. in preparation

Towards >1 mol · L⁻¹ (ca. 30 wt%) no process control required
>95 % isolated yield
>99 % purity
@ gram scale

80% 71%
11% 0%

Product concentration / mM

Reaction time / h

Substrate addition intermittent filtrations

800
600
400
200

0
0 48 96 144
Summary

- Transaminase process with ISPC concept successfully established
- Different substrates converted
- Screening of acids necessary (other amines may require other acids) → commercial screening plates in preparation
Acknowledgements

Jan von Langermann
Marco Tänzler
Dennis Hülseweke
Jan Neuburger

Enzymicals Team

Funding
Thank you for your attention