Chemo-enzymatic synthesis routes for the production of bio-based chemicals from sugar and waste residues

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São Paulo, 09.11.2017
Change of resources of the ORGANO-chemical industry
Nature’s Chemical Diversity for the Diversity of Chemical Products

Synthesis gas
CO, H₂ → Alkanes, Alkenes

Biomass
(C₁₃H₂₁O₁₀)ₙ

Carbohydrates

Lignin

Fatty acids

Amino acids

Terpenoids
Nature's Chemical Diversity for the Diversity of Chemical Products

Biomass
\((C_{13}H_{21}O_{10})_n\)

- Carbohydrates
- Lignin
- Fatty acids
- Amino acids
- Terpene

Diols
Phenols
Bifunctional alkanes
Cycles
Monoterpene based building blocks

- α-Pinene
- β-Pinene
- 3-Carene
- Camphor

Accumulation of terpenes in the cellulose production

<table>
<thead>
<tr>
<th>Cellulose and wood pulp production</th>
<th>Amount of cellulose [t/a]</th>
<th>Amount of terpenes [t/a]</th>
<th>Amount of α-Pinen [t/a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zellstoff Stendal</td>
<td>670.000</td>
<td>3.000*</td>
<td>1.800</td>
</tr>
<tr>
<td>Germany</td>
<td>2.700.000</td>
<td>11.000</td>
<td>6.600</td>
</tr>
<tr>
<td>Europe</td>
<td>31.000.000</td>
<td>155.000</td>
<td>93.000</td>
</tr>
<tr>
<td>World</td>
<td>130.000.000</td>
<td>650.000</td>
<td>390.000</td>
</tr>
</tbody>
</table>

Limonen
Terpenes for cyclic Co-Monomers in High Performance Polymers

=> Polyamides

- Linear chains, made of Diamines and Dicarboxylic acids or amino-carboxylic acids
- World market 25 Billion USD, annual growth rate 4 % (2017)
- Generally high durability and strength
- Application as automotive applications, carpets, shoes, textiles etc.

Typical production using fossil based cyclohexanone:

Cyclohexanone $\rightarrow$ $\varepsilon$-Caprolactam $\rightarrow$ Polyamide 6

Adjustment of properties using plasticers!
Cyclic Co-Monomers for High Performance Polymers

- $M_N = 22\,000$ g/mol; $M_W = 38\,000$ g/mol; $T_g$: ca. 110 - 120°C; no melting point up to 350°C
- Light weight and highly stable
- Highly shock-resistant (even after cooling with liquid nitrogen)
- Transparent
1. High selectivity of enzyme catalysis

2. High activity and robustness of chemical catalysis

M. Hofer, H. Strittmatter, V. Sieber ChemCatChem 2013, 3351
http://faculty.sites.uci.edu/poulos/cytochrome-p450/
**Challenge**: Sustainable process => sustainable solvents

**Actions:**
(i) substitution of hazardous solvents by ones with better environmental, health & safety properties
(ii) use of “bio-solvents”, i.e. solvents produced with renewable resources
(iii) substitution of organic solvents with supercritical fluids
(iv) substitution of organic solvents with ionic liquids that show low vapour pressure

=> Sugar based Deep Eutectic Solvents (DES)
Sugar based solvents

=> Deep Eutectic Solvents (DES)

→ a class of ionic liquids
→ prepared by mixing two components:
  → quaternary ammonium salt (such as choline chloride ChCl)
  → hydrogen bond donor (HBD) (alcohol, amide, carboxylic acid)

- **Sorbitol**
- **Glucose**
- **Fructose**

→ polar and non-volatile
→ of natural origin
→ capable of biological degradation
→ easy product removal (dissolution in water)

Synthetic Enzyme Cascades for carbohydrate conversion

**Challenges:**
- Compatible Enzymes/Catalysts
- Promiscuity for reduced number of enzymes
- Thermodynamics: All the way downhill
- Cheap and stable cofactors

**Example:** Ethanol, Isobutanol, Butandiol
A novel and artificial glycolytic pathway…

Glycolysis

10 Enzymes
2 Cofactors
A novel and artificial glycolytic pathway...

 Artificial Glycolysis

„Promiscuity for enzyme reduction“

Guterl JK et al., 2012 ChemSusChem 5(11), 2165
In vitro production of alcohols

Ethanol – 6 Enzymes

Isobutanol – 8 Enzymes

Glucose concentration was doubled to allow for direct comparison.

Guterl JK et al., ChemSusChem 2012
Advantage of *in vitro* production for product isolation

Product formation in the presence of isobutanol

Guterl JK *et al.*, 2012 *ChemSusChem* 5(11), 2165
### Profitability – Enzyme costs for chemicals production

<table>
<thead>
<tr>
<th>Assumption</th>
<th>Currently</th>
<th>Slight optimization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of enzymes</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Cost of enzymes</td>
<td>1000 €/kg</td>
<td>200 €/kg</td>
</tr>
<tr>
<td>Average activity per enzyme</td>
<td>8 u/mg</td>
<td>35 u/mg</td>
</tr>
<tr>
<td>Activity for all enzymes</td>
<td>1 u/mg</td>
<td>5 u/mg</td>
</tr>
<tr>
<td>Process life time for enzyme</td>
<td>10 h</td>
<td>100 h</td>
</tr>
</tbody>
</table>

| Calculation                                      |            |                    |
| Amount Isobutanol/ Enzyme                       | 54 kg/kg   | 2.7 to/kg          |
| Amount Enzyme / Isobutanol                      | 18.5 g/kg  | 0.37 g/kg          |
| Cost of enzyme for Isobutanol                   | 18.5 €/kg  | 0.07 €/kg          |

Enzyme Engineering for improved Enzymes

- Promiscous
- Easy production
- High Activity
- High Stability
The power of synthetic cascades

4-step formation of 1,4 butanediol from glucose

5 enzymes for the formation of α-ketoglutarate from uronic acids

Cascade for the release of monomers from lignin

Pick et al., *Microbial Biotechnology*, 8, 633–643
Reiter et al. *Green Chem.*, 2013, 15, 1373
Polysaccharides as Biogenic Polymers

**Linear homopolymers**

- **Cellulose**
  - *- OH
  - *- OH
  - *- OH
  - *- OH

- **Amylose**
  - *- OH
  - *- OH
  - *- OH
  - *- OH

**Branched homopolymers**

- **Amylopectin**
  - *- OH
  - *- OH
  - *- OH

**Linear block polymers**

- **Alginat**
  - *- OH

**Monomers**

- **β-Glucose**
- **α-Glucose**
- **α-D-Mannuronic acid**
- **α-L-Guluronin acid**
Exopolysaccharides as Biogenic Polymers

- Secreted by the microorganisms
  - Good & Bad Biofilms

Complex microbial Polymers

- Ca. 6000 Polysaccharide forming microorganisms have been described*

- Ca. 15 - 20 (Exo)polysaccharides are used commercially

* Bacterial Carbohydrate Structure DataBase
Automated High-Throughput Analysis
=> from sample to monomer composition within one day

Part 1: Automated Screening
- Säurebildung
- Viskosität
- Polymerbildung
- Restglukosegehalt
- Gesamtzuckergehalt
- Weitere Module

Part 2: Carbohydrate Fingerprint
- Pyruvat Substituenten
- Monomerzusammensetzung:
  - Pentosen, Hexosen
  - Deoxy-, amino-Zucker
  - Uronsäuren
  - Di- und Trimere
  - Zuckermodifikationen: z.B. Pyruvateketal
  - Selten Zucker
  - ....

Example: New Polymer for Cosmetics

Polymer eines *Paenibacillus* Isolats

- High productivity (0.25 g L\(^{-1}\) h\(^{-1}\))
- High viscosity (35 Pa \(\cdot\) s, shear-rate of 1 s\(^{-1}\), Conc. 1 % w/w of Polymer)
- Good film formation
- Further application e.g. in lubrication
- Properties can be tailored by fermentation conditions:

Components:
Glucose, mannose, galactose, glucuronic acid, pyruvate

Example: Incorporation of Polysaccharides into mortar

Aluminum mortar forms Layered Double Hydroxide (LDH)

Incorporation in Zn-Al-LDH layers

Control

With Polysaccharides

Going for a new generation of scientists to enable bioeconomy

Interdisciplinary study programs with elements of **natural sciences, engineering and economics**

Bachelor- and Master study courses (year of start)

- Chemical Biotechnology (2017/2019)
- Bioeconomy (2018/2020)
- Biogenic Materials (2019/2019)
- Renewable Resources (2013/2009)
TU München, Campus Straubing for Biotechnology and Sustainability
Straubing as Center of biogenic resources
Acknowledgement

Chair for Chemistry of Biogenic Resources
Technische Universität München

Dr. Jochen Schmid
Dr. Broder Rühmann
Dr. Jan Guterl
Dr. Jörg Carsten
Sumanth Rangathan

Claudia Nowak
Barbara Beer
André Pick
Marius Rütering

Fraunhofer Institute branch BioCat

Dr. Fabian Steffler
Dr. Harald Strittmatter
Dr. Michael Hofer

Collaborators

Prof. Bettina Siebers
Prof. Gary Schenk
Prof. Thomas Brück
Prof. Bernhard Rieger

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Federal Ministry of Education and Research
Bundesministerium für Ernährung und Landwirtschaft

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