Production and Use of Polyhydroxyalkanoates

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Bioplastics Opportunities for the Future
Final PLASTICE Conference
Slovenj Gradec
22. - 23. 9. 2014
PHA Accumulating Microorganisms

PHB Granules in *Alcaligenes latus*

PHB Granules in *Haloferax mediterranei*
Short chain-length PHAs (scl-PHAs)

P-3HB (scl-PHA)
From Sugars, Alcohols, Fatty acids

\[
\begin{array}{c}
\text{CH}_3 \\
\text{CH}_2 \\
\text{CH} \\
\text{C} \\
\text{O}
\end{array} \quad \text{n}
\]

P-3HB-co-3HV (scl-PHA)
From Sugars, Alcohols, Fatty acids + Precursor (Propionate, Valerate)

\[
\begin{array}{c}
\text{CH}_3 \\
\text{CH}_2 \\
\text{CH} \\
\text{C} \\
\text{O}
\end{array} \quad \text{n}
\]

P-3HB-co-4HB (scl PHA)
From Sugars, Alcohols, Fatty acids + Precursor (γ-butyrolactone, 4-hydroxybutyrate)

\[
\begin{array}{c}
\text{CH}_3 \\
\text{CH}_2 \\
\text{CH} \\
\text{C} \\
\text{O}
\end{array} \quad \text{n}
\]
Variation of properties of PHB by introduction of comonomers or by blending with Ecoflex (BASF) (Dr. Scherzer, BASF)

- Density
- Oxygen barrier
- UV stability
- Temperature resistance
- E-modulus
- Elongation

Comparison between PHB, PHB-copolymers, PHB/Ecoflex-Blend
Properties of PHB / Ecoflex-Blends are close to Polypropylene! (Dr. Scherzer, BASF)
Medium chain-length PHAs (mcl-PHAs)

Mainly produced by the group of "Fluorescent Pseudomonads"

- e.g. *Pseudomonas oleovorans* or *Pseudomonas cepacia*

Long side-chains (up to C12)
- side-chains may be branched or may contain double bonds, halogens, phenolic groups etc.
- in the side-chain
Use of PHAs

PHAs are discussed to be used as

• Packaging materials
• For medical purposes (Implants)
• For various pharmaceutical purposes

! Quality and Costs !

Polymer properties depend on:
- producing strain (scl-, mcl-PHA)
- type of polyester
- raw materials & feeding strategies
- fermentation technology

Polymer costs depend on:
- carbon source (about 50%)
- fermentation technology
- product separation & purification

Microbial strain + Cheap carbon source + Fermentation technology
Important Carbon Sources for Growth and PHA Formation

**Carbohydrates:**
- Molasses and Sucrose
- Starch and Starch Hydrolysates (Maltose)
- Lactose from Whey
- Cellulose hydrolysates (e.g.: Reject fiber wastes from the paper industry after hydrolysis and purification by ion exchange)

**Alcohols:**
- Wastes from biodiesel production: Glycerol, Methanol

**Fats and Oils:**
- Lipids from plant and animal wastes

**Organic Acids:**
- Lactic acid from Solid State Fermentation
SUGAR CANE AS A RAW MATERIAL

- SUGAR CANE
  - CRUSHING
  - EXTRACTION
- RAW JUICE
  - CRYSTALLIZATION
- EXTRACTED BIOMASS (Bagasse)
  - COMBUSTION
    - STEAM
    - ELECTRICAL POWER
    - DOWNSTREAM PROCESSING
      - BIO-PRODUCTS
        - Fine Chemicals
        - scl-PHA
        - Solvents
- RAW MATERIALS FOR BIO-PROCESSES
  - BIO-PROCESS FERMENTATION
View of the PHB Pilot Plant for 50 tons per year
Producing micro-organisms: Cupriavidus necator
<table>
<thead>
<tr>
<th>PROPERTIES</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight (Da)</td>
<td>250,000 – 800,000</td>
</tr>
<tr>
<td>Specific Density at 25 °C (g/cm³)</td>
<td>1.2</td>
</tr>
<tr>
<td>Melting point (°C)</td>
<td>169 - 172</td>
</tr>
<tr>
<td>Glass Transition Temperature (°C)</td>
<td>1 - 5</td>
</tr>
<tr>
<td>Decomposition Temperature (°C)</td>
<td>250</td>
</tr>
<tr>
<td>Crystallinity (%)</td>
<td>70</td>
</tr>
<tr>
<td>Specific Heat (J/kg.°C)</td>
<td>1.42</td>
</tr>
</tbody>
</table>
## Thermomechanical Properties

### PHB Homopolymer, MW (GPC) 450,000 Da

PHB plus „Thermal Stability Packaging"

<table>
<thead>
<tr>
<th>Properties</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile Modulus (Gpa)</td>
<td>2.4</td>
</tr>
<tr>
<td>Tensile Strength at Break (MPa)</td>
<td>33.0</td>
</tr>
<tr>
<td>Elongation to Break (%)</td>
<td>9.0</td>
</tr>
<tr>
<td>Notched Izod Impact (J/m)</td>
<td>26.3</td>
</tr>
<tr>
<td>Crystallinity (%)</td>
<td>51.0</td>
</tr>
<tr>
<td>High Distortion Temperature (°)</td>
<td>75.0 – 80.0</td>
</tr>
<tr>
<td>Melt Flow Index (g/10 min)</td>
<td>5.5 – 11.0</td>
</tr>
</tbody>
</table>
Dairy industry waste is a potential source of biologically-produced polymers with commercial applications in packaging. WHEYPOL is seeking a cost-effective method to tap this abundant and sustainable resource.

http://news.cec.eu.int/comm/research/industrial_technologies/articles/article_805_en.html

Whey production in Europe: 40,420,800 tons/y
Surplus WHEY: 13,462,000 tons/y

Lactose: 619,250 tons /y
205,000 t PHA/y
Direct Conversion of Glucose and Galactose from Hydrolyzed Whey

**High Quality Terpolyester:**
Poly-(73.05\% - 3-HB-co-21.81\% - 3-HV-co-5.14\% - 4-HB)

**Molecular weight:** ca. $1.5 \times 10^6$

**Polydispersity index:** 1.1 – 1.2

**Precursor:** $\gamma$-butyrolactone

**PHA Production from Hydrolyzed Whey with Precursors for 3-HV and 4-HB:**
Composition of PHA [%]
The ANIMPOL PHA Process

The production cycle starts from lipid-rich animal processing waste (1.), transesterification of the lipids towards convertible carbon sources (SFAE and CGP) (2.), microbial conversion of these carbon sources in bioreactors (4.), Accumulation of high shares of PHAs in microbial cells (5.), and processing of isolated PHA towards prototype items.
The ANIMPOL Process: Available quantities of waste lipids from the animal processing industry, and theoretically producible quantities of PHA

- ANIMAL WASTE LIPIDS: 500,000 t/year
  - Transesterification
    - CRUDE GLYCEROL: 50,000 t/year
    - Fatty acid esters (BIODIESEL): 450,000 t/year
      - Separation
        - PHA: 12,000 t/year
        - SATURATED BIODIESEL FRACTION: 50,000 t/year
          - UNSATURATED BIODIESEL FRACTION
            - Excellent 2nd generation Biofuel!
PHA Production with the Saturated Share of Biodiesel (SFAE) and the Crude Glycerol Phase (CGP) from Biodiesel Production
<table>
<thead>
<tr>
<th>Production strain</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cupriavidus necator</strong></td>
<td><em>Cupriavidus necator</em> DSM 545</td>
<td><em>Cupriavidus necator</em> DSM 545</td>
<td><em>Ps. Citronellololis</em> DSM 5033</td>
<td><em>Ps. Chlororaphis</em> DSM 50083</td>
</tr>
<tr>
<td>Carbon source</td>
<td>CGP</td>
<td>SFAE</td>
<td>SFAE</td>
<td>SFAE</td>
</tr>
<tr>
<td>Type of PHA produced</td>
<td>PHB</td>
<td>PHBV</td>
<td>mcl-PHA</td>
<td>mcl-PHA</td>
</tr>
<tr>
<td>$\mu_{\text{max.}}$ [1/h]</td>
<td>0.11</td>
<td>0.17</td>
<td>0.08</td>
<td>0.13</td>
</tr>
<tr>
<td>$q_p$ [g/g h]</td>
<td>0.16</td>
<td>0.19</td>
<td>0.003</td>
<td>0.006</td>
</tr>
<tr>
<td>max. concentration PHA [g/L]</td>
<td>29.5</td>
<td>28.0</td>
<td>3.56</td>
<td>6.44</td>
</tr>
<tr>
<td>Yield biomass / C-source</td>
<td>0.29</td>
<td>0.60</td>
<td>0.59</td>
<td>0.62</td>
</tr>
<tr>
<td>$M_w$ [kDa]</td>
<td>380</td>
<td>306</td>
<td>66</td>
<td>74</td>
</tr>
<tr>
<td>$P_i$</td>
<td>1.28</td>
<td>1.50</td>
<td>1.88</td>
<td>1.93</td>
</tr>
<tr>
<td>$T_m$ [°C]</td>
<td>173.0</td>
<td>169.0</td>
<td>48.6</td>
<td>broad melting range</td>
</tr>
<tr>
<td>$T_g$ [°C]</td>
<td>5.6</td>
<td>4.6</td>
<td>-46.9</td>
<td>-47.0</td>
</tr>
<tr>
<td>$X_c$ [%]</td>
<td>71.2</td>
<td>30.8</td>
<td>12.3</td>
<td>completely amorphous material</td>
</tr>
</tbody>
</table>

$\mu_{\text{max.}}$: max. specific growth rate; $q_p$: specific PHA production rate; $m$: mass fraction PHA in CDM; $M_w$: weight averaged molar mass; $P_i$: polydispersity (dispersity index); $T_m$: melting temperature; $T_g$: glass transition temperature; $X_c$: degree of crystallinity
Discontinuous Fermentation Process for PHA Production

Sterilization

Strain

Shake Flasks

Pre-Reactors 1 + 2

Production Reaktor

C N P

Minerals

Down-Streaming
Continuous Process: Reactor cascade

Feed Stream

Add. Feed Stream

Add. Feed Stream

For $n = 5(+)$:
Plug Flow Characteristics!
Reactor volumes for PHA Production:
PFTR or Reactor Cascade Volume Compared to CSTR Volume

For Cupriavidus necator as a producing strain

V: 11.4% of a CSTR!
Continuous Production in a Reactor Cascade

**Feed**
- Feed
- Growth medium

**Feed Production medium:**
- Individual composition

**Sampling & Controlling:**
- Individually

**Continuous Product Separation & Refining**

**CST Reactor:**
- For production of Biomass

**Sampling & Controlling:**
- Individually

**CST Reactors:**
- For PHA Formation

**Continuous Biomass Separation**
Biomass Concentration in the Fermentors 1 - 5
Feed rates (F), working volumes (V), dilution rates (D) and residence times (RT)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>222.0</td>
<td>1.6</td>
<td>0.139</td>
<td>7.2</td>
</tr>
<tr>
<td>R2</td>
<td>19.6</td>
<td>1.6</td>
<td>0.148</td>
<td>6.7</td>
</tr>
<tr>
<td>R3</td>
<td>22.3</td>
<td>1.7</td>
<td>0.159</td>
<td>6.3</td>
</tr>
<tr>
<td>R4</td>
<td>21.9</td>
<td>1.7</td>
<td>0.167</td>
<td>6.0</td>
</tr>
<tr>
<td>R5</td>
<td>20.5</td>
<td>2.4</td>
<td>0.130</td>
<td>7.7</td>
</tr>
<tr>
<td>Total</td>
<td>9.0</td>
<td></td>
<td></td>
<td>33.9</td>
</tr>
</tbody>
</table>
Polymer Concentration in the Fermentors 1 - 5
# Results

<table>
<thead>
<tr>
<th>Reactor</th>
<th>CDM (g/L)</th>
<th>%PHB</th>
<th>PHB (g/L)</th>
<th>RB (g/L)</th>
<th>$Q_X$ (g/Lh)</th>
<th>$Q_P$ (g/Lh)</th>
<th>$q_P$ (g/gh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>26</td>
<td>4</td>
<td>1.0</td>
<td>25</td>
<td>3.64</td>
<td>0.14</td>
<td>0.005</td>
</tr>
<tr>
<td>R2</td>
<td>42</td>
<td>37</td>
<td>15</td>
<td>27</td>
<td>2.31</td>
<td>2.12</td>
<td>0.080</td>
</tr>
<tr>
<td>R3</td>
<td>59</td>
<td>60</td>
<td>36</td>
<td>24</td>
<td>2.79</td>
<td>3.27</td>
<td>0.139</td>
</tr>
<tr>
<td>R4</td>
<td>71</td>
<td>72</td>
<td>51</td>
<td>20</td>
<td>1.87</td>
<td>2.54</td>
<td>0.130</td>
</tr>
<tr>
<td>R5</td>
<td>81</td>
<td>77</td>
<td>63</td>
<td>19</td>
<td>1.37</td>
<td>1.50</td>
<td>0.081</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>77</td>
<td>63</td>
<td>19</td>
<td>2.39</td>
<td>1.85</td>
<td>0.100</td>
</tr>
</tbody>
</table>

CDM: Cell Dry Mass  
PHB: PHB concentration  
$Q_X$: Volumetric growth rate, production rate  
$Q_P$: Volumetric production rate  
RB: Residual Biomass concentration  
$q_P$: Specific production rate  

%PHB: Intracellular PHB content
Molecular mass and physical properties of the isolated PHB

<table>
<thead>
<tr>
<th>Sample</th>
<th>$M_w$ [kDa]</th>
<th>PDI</th>
<th>$T_m$ [$^\circ$C]</th>
<th>$T_g$ [$^\circ$C]</th>
<th>$X_c$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5</td>
<td>665 ± 1</td>
<td>2.6</td>
<td>178</td>
<td>2.9</td>
<td>68</td>
</tr>
</tbody>
</table>

$M_w$: average weight molecular mass  
$T_m$: melting temperature  
$T_g$: glass transition temperature  
$X_c$: degree of crystallinity  
PDI: polydispersity index
Comparison of multistage PHB production with *C. necator*

<table>
<thead>
<tr>
<th>Reference</th>
<th>Nr. of Reactors</th>
<th>D1 [h⁻¹]</th>
<th>CDW [g/L]</th>
<th>PHB (g/L)</th>
<th>% PHB</th>
<th>Q_P (g/Lh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Du et al., 2001</td>
<td>2</td>
<td>0.075</td>
<td>42</td>
<td>31</td>
<td>72</td>
<td>1.23</td>
</tr>
<tr>
<td>This work</td>
<td>5</td>
<td>0.139</td>
<td>81</td>
<td>63</td>
<td>77</td>
<td>1.85</td>
</tr>
</tbody>
</table>

D1: dilution rate in the first stage; CDW, PHB and %PHB: cell dry weight, PHB concentration, and intracellular PHB content in the system outflow; Q_P is overall volumetric productivity for PHB
Comparison of Industrial PHB Production with Data from the Reactor Cascade

<table>
<thead>
<tr>
<th>Reference</th>
<th>Mode</th>
<th>Process Time [h]</th>
<th>CDM (g/L)</th>
<th>PHB (g/L)</th>
<th>% PHB</th>
<th>Q_p (g/Lh)</th>
<th>q_p (g/gh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonato et al., 2001</td>
<td>Fed-batch</td>
<td>45-50</td>
<td>120-150</td>
<td>72-105</td>
<td>65-70</td>
<td>1.44</td>
<td>0.032</td>
</tr>
<tr>
<td>This work</td>
<td>5-stage continuous</td>
<td>34</td>
<td>81</td>
<td>63</td>
<td>77</td>
<td>1.85</td>
<td>0.100</td>
</tr>
</tbody>
</table>

CDM, PHB and %PHB: cell dry mass, PHB concentration and intracellular PHB content in the system outflow; Q_P: overall volumetric productivity for PHB; q_P: specific PHB production rate
Disconti/Conti (Cascade) PHA Production

Fed Batch:
Biomass propagation: 3 – 4 Pre-reactors, followed by
1 Production reaktor: $V = 150 \text{m}^3$; PHA= 120 kg/m$^3$; 1 Run/ week, 45 weeks/year

ca. 800 t PHA/year

Downstream in process:
1 x per week: Separation of Biomass + PHA from 150m$^3$
• Efficient large scale separator: runs only for 1 day per week!
• Large Holding Tanks for Storing prior to Drying/Extraction
Disconti/Conti (Cascade) PHA Production

Cascade:
- Biomass propagation: 1 Reaktor, followed by a Cascade (ca. 12% of 150m$^3$ = ca. 18 m$^3$); PHA = 120 kg/m$^3$

PHA Production: 5 Reactors of about 3.6 m$^3$ each

ca. 800 t PHA/a

Downstreaming
- Continuously: Separation of Biomass + PHA from ca. 0.9m$^3$/h
  - Small scale Separator, running continuously!
  - Small scale holding tank for storage before Drying/Extraction or continuous downstreaming!
Downstream Processing of Biomass: Extraction

Laboratory: e.g. Soxleth Extractor

Industrial: e.g. Podbielniak Extractor
Next Steps:

1. Addition of Ethanol to the Chloroform – Extract
   Precipitation of PHA; Filtration or Centrifugation

2. Redistillation of the Solvent
   Reuse of the Solvent

COSTS !!!
Downstream Processing by Extraction:
Phase diagram for the System Water – Ethanol - Chloroform
## Downstream Processing:

### Composition of the Phases A and B

<table>
<thead>
<tr>
<th>Phase</th>
<th>Chloroform</th>
<th>Ethanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower Phase (A)</td>
<td>95%</td>
<td>3.7%</td>
<td>1.3%</td>
</tr>
<tr>
<td>Upper Phase (B)</td>
<td>1.0%</td>
<td>18%</td>
<td>81%</td>
</tr>
</tbody>
</table>

Phase A can be used for further extraction without prior distillation!
scl-PHA Extraction with an "scl-PHA non solvent"

Solvent: Aceton
Extraction at 120 °C; Precipitation by mere cooling!
Fats remain in the solvent!
No reduction of the molecular weight!
Conclusions

PHAs will be interesting biopolymesters for the future, if:

- Cheap carbon sources from agricultural surplus and waste materials are used for production:
  
  50% of production costs depend on carbon source

- Modern methods of fermentation technologies replace old type fed batch processes to produce taylor made materials:
  
  continuous high quality PHA production in bioreactor cascades

- New methods for PHA isolation and purification are applied:
  
  Recycling of extraction media; aceton instead of chlorinated solvents
Applications
Applications
Samples of Products: Packaging

Powder containers

Perfume flasks
Samples of Products: Pens
Samples of Products: Automotive Parts, Valve hood

Reinforced by filling with bagasse fibers
Samples of Products: Fibres & Foams
Samples of Products: Thermoforming Sheets
Thank You Very Much for Your Attention!