Valorisation of sugar beet pulp for the production of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxy valerate)

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Objectives

- Development of Sugar Beet Pulp biorefinery
- Crude enzymes production via solid state fermentation (SSF)
- Sugar Beet Pulp hydrolysate as feedstock for PHB production
- PHBV production with different 3HV monomer content
Poly(3-hydroxybutyrate) (PHB)

The transition to the circular bio-economy era requires technological breakthroughs in sustainable biorefinery development using **crude renewable resources, microbial bioconversions** and recycling of biopolymers

- **Member of polyhydroxyalkanoates (PHAs)**
- **Accumulated in intracellular granules** by Gram+ and – microorganisms
- It is produced **under excess of carbon source and limited nutrient conditions** (N, P, O)
- Serve as a carbon and energy storage

- Similar properties with conventional polymers such as polypropylene and polyethylene.
- It is highly biodegradable, non-toxic, and biocompatible.

- **Encapsulation of medicines for controlled release**
  - Biomedical
  - Packaging
  - Agricultural
  - Industrial

- **Poly(3-hydroxybutyrate) (PHB)**
  - Create difficulties in material processability

- **High degree of crystallinity**
- **Rigidity**
- **Low elongation to break**
Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)

Better physical properties
- its performance can vary when this polymer contains different proportions of the HV monomer
- Impact resistance
- Toughness
- Flexibility
- Low melting point

Due to its superior characteristics, PHBV is more attractive for both biomedical and food packaging applications

PHBV Synthesis
- 3-HV and 3-HB, the monomer units of PHBV are synthesized simultaneously in bacterial cytoplasm via two parallel pathways.
- Levulinic acid acts as a direct precursor that triggers the formation of 3-HV, in addition to 3-HB.

Limited use
- High production cost

The carbon source accounts for 28-50% of the total production cost
One of the most important agricultural crop
Annual production: 253 million t of beets
One of the highest volume vegetable wastes
SBP is the main by-product of sugar industry
10.35 million t/y of SBP was produced in Europe
1 t of sugar beets yields to 50 kg dehydrated SBP

Collection, washing, separation & slicing → Evaporating & boiling → Drying & cooling → Extracting & purifying → Centrifugation → By-products

Sugar beet (7 t) → Sugar Beet Pulp pellets (350 kg) → Molasses (275 kg)

Sugar (1 t) → Parts of the sugar beet crop

By-products:
- Animal feed
- Bioethanol
- Biogas
- Succinic acid
- Lactic acid
- PHAs

Olga Vittou | PhD Candidate
Agricultural University of Athens
## Composition of Sugar beet pulp pellets (% dry basis)

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free sugars</td>
<td>10.9 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>9.0 ± 0.7</td>
<td>1.0 – 8.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.5 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>0.4 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Ash content</td>
<td>3.7 ± 0.6</td>
<td>2.4 – 4.0</td>
</tr>
<tr>
<td>Protein Content (N x 6.25)</td>
<td>9.1 ± 0.1</td>
<td>5.9 - 11.4</td>
</tr>
<tr>
<td>Pectin (uronic acid)</td>
<td>19.1 ± 0.9</td>
<td>13.5 - 22.8</td>
</tr>
<tr>
<td>Lipid Content</td>
<td>0.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Glucan</td>
<td>27.9 ± 4.9</td>
<td>14.0 - 25.5</td>
</tr>
<tr>
<td>Xylan</td>
<td>3.1 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Galactan</td>
<td>5.5 ± 0.2</td>
<td>25.0 -36.6</td>
</tr>
<tr>
<td>Mannan</td>
<td>4.3 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Arabinan</td>
<td>12.0 ± 4.1</td>
<td></td>
</tr>
<tr>
<td>Lignin</td>
<td>2.3 ± 0.3</td>
<td>1.2 - 2.0</td>
</tr>
<tr>
<td>Phenolics (mg GAE)</td>
<td>205.7 ± 22.2</td>
<td></td>
</tr>
</tbody>
</table>

### Reference

- This work
- Literature

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1 cellulose, 2 hemicellulose

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#### SBP Biorefinery

- **Heating at 40°C for 2 h (1:20 w/v)**
- **Free sugars (9.9 g)**
- **Sucrose (9.0 ± 0.7)**
- **Glucose (1.5 ± 0.8)**
- **Fructose (0.4 ± 0.3)**
- **Ash content (3.7 ± 0.6)**
- **Protein Content (N x 6.25) (9.1 ± 0.1)**
- **Pectin (uronic acid) (19.1 ± 0.9)**
- **Lipid Content (0.9)**
- **Glucan (27.9 ± 4.9)**
- **Xylan (3.1 ± 0.2)**
- **Galactan (5.5 ± 0.2)**
- **Mannan (4.3 ± 0.9)**
- **Arabinan (12.0 ± 4.1)**
- **Lignin (2.3 ± 0.3)**
- **Phenolics (mg GAE) (205.7 ± 22.2)**

**REFERENCE**

- This work
- Literature
Production of crude enzymes during solid state fermentations by *Aspergillus awamori* cultivated on a mixture of sugar beet pulp (SBP) & sunflower meal (2:1)

**EXPERIMENTAL DESIGN**
- *Aspergillus awamori*
- SBP free of sugars and pectin & sunflower meal
- Moisture content: 60% and 65%

* U = the amount of enzyme that releases 1 mg of substrate per minute

### Enzyme activities (U/g)

**Phytase, A. awamori**
- 131.7 (60%)
- 120.8 (65%)

**Protease, A. awamori**
- 12.6 (60%)
- 9.7 (65%)

**Xylanase, A. awamori**
- 3.7 (60%)
- 4.5 (65%)

**CMCase, A. awamori**
- 0.8 (60%)
- 1.0 (65%)

**Inorganic Phosphorus**
- 144 mg/L

**Free Amino Nitrogen**
- 76.4 mg/L

**Other sugars and hydrolysate**
- Glucose 67%
- Arabinose 14.4%
- Mannose 14.6%
- Xylose 1.6%
- Hydrolysate 45.5 g/L
### Bioreactors Set up

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Paraburkholderia sacchari DMSZ 17165</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Carbon Source</strong></td>
<td>SBP hydrolysate, Commercial mix sugars 40 g/L, Levulinic Acid</td>
</tr>
<tr>
<td><strong>Inorganic Phosphorus</strong></td>
<td>800(mg/L)</td>
</tr>
<tr>
<td><strong>Working Volume</strong></td>
<td>1 L</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>6.8</td>
</tr>
<tr>
<td><strong>(28% NH₄OH και 2 M HCl)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>30°C</td>
</tr>
<tr>
<td><strong>Agitation</strong></td>
<td>1200 rpm</td>
</tr>
<tr>
<td><strong>Ventilation</strong></td>
<td>2.5 vvm</td>
</tr>
<tr>
<td><strong>Minerals (g/L)</strong></td>
<td>(NH₄)₂SO₄, 4.0 g; KH₂PO₄, 3.0 g; citric acid, 1.7 g; EDTA, 40 mg; trace elements solution, 10 mL; MgSO₄·7H₂O, 1.2 g. <strong>Trace element (g/L):</strong> FeSO₄·7H₂O, 10 g; ZnSO₄·7H₂O, 2.25 g; CuSO₄·5H₂O, 1 g; MnSO₄·4H₂O, 0.5 g; CaCl₂·2H₂O, 2 g; Na₂B₄O₇·10H₂O, 0.23 g; (NH₄)_6Mo₇O₂₄, 0.1 g; 35% HCl 10 mL</td>
</tr>
</tbody>
</table>
Fermentation with SBP hydrolysate for PHB production

- CDW: cell dry weight
- RCW: residual cell weight
- IN: Inorganic nitrogen
- IP: Inorganic phosphorus

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>CDW (g/L)</th>
<th>PHB (%)</th>
<th>PHB g/L</th>
<th>Yield (g PHB / g consumed sugars)</th>
<th>Productivity (g/L/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>38.5</td>
<td>118</td>
<td>41.1</td>
<td>48.54</td>
<td>0.28</td>
<td>1.26</td>
</tr>
</tbody>
</table>
Feeding Strategy with Levulinic Acid supplementation

Case Study 1
- Addition of 8 g Levulinic Acid
- After 24 h of fermentation
- 1 g / h

Case Study 2
- Addition of 13 g Levulinic Acid
- After 24 h of fermentation
- 1 g / 30 min

Case Study 3
- Addition of 22 g Levulinic Acid
- After 24 h of fermentation
- 3 g / h

Case Study 4
- Addition of 28 g Levulinic Acid
- After 24 h of fermentation
- 2 g / 30 min
Fermentations with mixed sugars simulating hydrolysate for PHBV production

<table>
<thead>
<tr>
<th>PHBV (g/L)</th>
<th>3HB (g/L)</th>
<th>3HV (g/L)</th>
<th>Yield (g PHBV/g consumed carbon*)</th>
<th>Productivity (g/L/h)</th>
<th>Levulinic acid consumed (g)</th>
<th>3HV/PHBV</th>
<th>13 g Levulinic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>58.7</td>
<td>49.8</td>
<td>8.86</td>
<td>111</td>
<td>0.29</td>
<td>1.72</td>
<td>13</td>
<td>15%</td>
</tr>
</tbody>
</table>

*Including sugars and Levulinic Acid
Fermentations with mixed sugars simulating hydrolysate for PHBV production

- 10 g Levulinic Acid
- 22 g Levulinic Acid
- 28 g Levulinic Acid
Increasing levulinic acid consumption leads to increased 3HV accumulation

Based on the experimental results presented, the preferred 3HV content can be controlled during fermentation.
Conclusions

- Crude enzymes were produced via solid state fermentation with *Aspergillus awamori*

- SBP hydrolysate was efficiently valorized as carbon source for PHB with a total dry weight of 118 g/L and PHB accumulation of 42% (w/w)

- The total amount of LA consumed demonstrates a significant role in final 3HV accumulation
Thank you for your attention!

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