Section II. Developments in biorefinery: feedstocks, processes and products (I)

Production of bio-based chemicals and polymers from industrial waste and by-product streams

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Main research interests

- Biorefinery development using agri-industrial waste and by-product streams
- Separation of value-added co-products
- Bioprocess development using entirely renewable resources for the production of platform chemicals, biopolymers and microbial lipids
- Biorefinery and bioprocess design including techno-economic evaluation and life cycle assessment

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Chem Soc Rev 2014, 43, 2587-2627

Valorization of industrial waste and by-product streams via fermentation for the production of chemicals and biopolymers

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Approach on industrial waste/by-product biorefinery development: The case of food waste

Food Wastes → Upstream Processing

Enzymatic Hydrolysis

Solid State Fermentation

Fungi

Fermentation

Filtration
L-L Extraction
Distillation
Crystallisation
Drying

Downstream processing

Bio-based products

PHB

Platform chemicals

Bacteria

Cellulos

Microbial oil

Carotenoids
Biorefinery/bioprocess design and techno-economic evaluation

Bioreactor total installed capacity (m³)

Bioreactor total installed capacity (L)

Fixed Capital Investment per L installed bioreactor capacity ($/L)

Koutinas et al. 2016. Bioresource Technology 204:55-64
Biorefinery development based on the valorisation of biodiesel industry by-products for the production of antioxidant-rich fraction, protein isolate and poly(3-hydroxybutyrate)
Current biodiesel production process from oilseeds

- **Biodiesel**
  - Transesterification
  - **Crude glycerol** (35-50% glycerol)
  - Purification to more than 85%
  - Animal feed or other low value applications
  - **Oil**
    - Screw press and solvent extraction
    - **Oilseeds**
      - **Hull**
      - **Crush**
  - **Seed residues**
    - Sunflower meal (by-product 2) 42,000 t/y
  - **Animal feed**
  - **Sunflower seeds** 70,000 t/y
  - **Oil**
    - 28,000 t/y
  - **Glycerol** (by-product 1) 2,912 t/y
  - **Biodiesel**
    - 28,140 t/y
The Bioeconomy in Transition Gela Workshop

Apostolis Koutinas, Agricultural University of Athens, Greece

Base-case processing scenario

Sunflower seed

Sunflower meal

Mechanical pressing and hexane extraction

Sunflower meal

Solid state fermentation

Aspergillus oryzae

Enzymatic hydrolysis

Nutrient-rich supplement

Crude glycerol

Carbon source

Biodiesel

Transesterification

Poly(3-hydroxybutyrate)
PHB production from whole sunflower meal hydrolysate (SFM) and crude glycerol

Fermentation time (h)

Glycerol, TDW, PHB (g/L)

FAN, IP (mg/L)

PHB production stage

FAN (△)
In. Phosphorus (■)
Glycerol (□)
Total Dry Weight (▲)
PHB (●)

Microbial growth stage

Crude glycerol
Sunflower meal hydrolysate

Polyhydroxybutyrate

Purification of PHAs using commercial enzymes

**Process I**

- Sunflower seed
  - Sunflower meal
  - Oil
  - Crude glycerol

**Solid state fermentation** (Aspergillus oryzae)

- Biodiesel
- Enzymatic hydrolysis
  - Nutrient-rich supplement
  - Levulinic acid production

**Remaining lignocellulose-rich solids**

- Heat treatment to deactivate enzymes

**Microbial Bioconversion**

- non-PHA cell lysis with commercial enzymes

- PHB or P(3HB-co-3HV)

**Commercial Pancreatin**

- Removal of cell debris, colour and water
Advanced sunflower-based biorefinery

1. **Sunflower seeds**
   - Partial Dehulling
   - Partly dehulled or undehulled sunflower meal

2. **Oil**
   - Mechanical pressing and hexane extraction
   - Antioxidants

3. **Protein-rich fraction**
   - Aqueous extraction
   - Liquid fraction
   - Remaining stream

4. **Residual streams**
   - Crude enzymes
   - Remaining solids

5. **Enzymatic hydrolysis**
   - Nutrient supplement

6. **Crude glycerol**
   - Transesterification

7. **Protein isolate**
   - Microbial fermentation

8. **PHB Production**
   - Crude lignocellulose-rich fraction
   - Solid state fermentation
PHB fermentation using the hydrolysate produced when all residual streams are employed

This medium is efficient for enhanced PHB production

Kachrimanidou et al. 2015. Ind. Crops Prod. 71:106–113

25 - 27 May 2017, Gela, Italy
Integration of multipurpose usage of crude enzymes produced on-site

Process II

Sunflower seed

- Sunflower meal → Aqueous extraction → Protein-rich fraction → Antioxidants and Protein isolate or hydrolysate
- Fibre-rich fraction → Solid state fermentation

Sunflower meal

- Aqueous extraction → Heat treatment to deactivate enzymes
- Enzymatic hydrolysis → Remaining solids → Solid state fermentation
- Nutrient-rich supplement

Oil

- Crude glycerol → Microbial Bioconversion
- Biodiesel
- Crude glycerol

Sunflower meal

- Aqueous extraction → Enzymatic hydrolysis
- Nutrient-rich supplement
- Remaining lignocellulose-rich solids

Crude glycerol

- Microbial Bioconversion
- Heat treatment to deactivate enzymes
- non-PHA cell lysis with crude on-site enzymes

Levulinic acid production

Remaining solids

- Antioxidants and Protein isolate or hydrolysate
- Remaining lignocellulose-rich solids

Levulinic acid

- Production

PHB or P(3HB-co-3HV)

- Remaining lignocellulose-rich solids
- Removal of cell debris, colour and water
Evaluation of hydrolyzed solution of bacterial cells as fermentation substrate for PHB production

Cupriavidus necator DSM 7237

FAN: 329 mg/L
Glycerol: 20 g/L
TDW: 5.75 g/L
PHB content: 20%

The lysate from enzymatic non-PHB cell lysis could be used as fermentation feedstock supplemented with phosphate salts.
## Profitability assessment of different processing scenarios

<table>
<thead>
<tr>
<th>Process type</th>
<th>Current practice</th>
<th>Net Present Value 10 year (million $)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Current practice</strong></td>
<td>- utilisation of SFM and crude glycerol as animal feed</td>
<td>31.5</td>
</tr>
<tr>
<td><strong>Process I</strong></td>
<td>- PHB production from crude glycerol and part of SFM - SFM utilisation as animal feed</td>
<td>Lower than 31.5</td>
</tr>
<tr>
<td><strong>Process II</strong> (low range of market prices for biorefinery products)</td>
<td>- fractionation of SFM to produce protein isolate and antioxidants - PHB production from crude glycerol and remaining SFM fractions</td>
<td>Lower than 31.5</td>
</tr>
<tr>
<td><strong>Process III</strong> (high range of market prices for biorefinery products)</td>
<td>- fractionation of SFM to produce protein isolate and antioxidants - PHB production from crude glycerol and remaining SFM fractions</td>
<td>Higher than 31.5</td>
</tr>
</tbody>
</table>
NPV range estimated based on low and high market prices for the protein isolate (1,250 – 2,500 $/t) and antioxidants (6 – 15 $/kg)

- **Low NPV value** estimated when the protein isolate and antioxidants market prices are 1,250 $/t and 6 $/kg, respectively
  - Lower than 31.5 million $
- **High NPV value** estimated when the protein isolate and antioxidants market prices are 2,500 $/t and 15 $/kg, respectively
  - Higher than 31.5 million $

**Current Practice**
NPV = 31.5 million $
Biorefinery development based on the valorisation of spent sulphite liquor for the production of lignosulphonates, succinic acid and antioxidant-rich fraction
Spent sulphite liquor (SSL) production in the sulphite wood pulping process

<table>
<thead>
<tr>
<th>SSL Characterisation</th>
<th>Value</th>
<th>St Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Density (g/mL)</td>
<td>1.277</td>
<td>0.007</td>
</tr>
<tr>
<td>Viscosity (cP)</td>
<td>552</td>
<td>167</td>
</tr>
<tr>
<td>Dry Matter (g-DM/L)</td>
<td>816.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Lignosulphonates (g/L)</td>
<td>458.8</td>
<td>2.7</td>
</tr>
<tr>
<td>Ash % (g/g-DM)</td>
<td>8.62</td>
<td>0.55</td>
</tr>
<tr>
<td>Phenolics % (g/g-DM)</td>
<td>1.55</td>
<td>0.04</td>
</tr>
<tr>
<td>Carbohydrates (g/L)</td>
<td>176.41</td>
<td></td>
</tr>
<tr>
<td>Xylose (g/L)</td>
<td>128.08</td>
<td>0.59</td>
</tr>
<tr>
<td>Galactose (g/L)</td>
<td>21.47</td>
<td>5.50</td>
</tr>
<tr>
<td>Glucose (g/L)</td>
<td>19.27</td>
<td>0.39</td>
</tr>
<tr>
<td>Mannose (g/L)</td>
<td>7.41</td>
<td>1.30</td>
</tr>
<tr>
<td>Arabinose (g/L)</td>
<td>0.18</td>
<td>0.05</td>
</tr>
<tr>
<td>Acetic Acid (g/L)</td>
<td>6.91</td>
<td>0.49</td>
</tr>
</tbody>
</table>
Integrated biorefinery based on current pulp and paper mills

- Phenolic Extract
- Lignosulphonates
- SSL
- Fermentation
- Nanofiltration
- Phenolic extraction with ethyl acetate
- Fireproof biopolymers
- Polybutylene succinate
- Succinic acid production
- Succinic acid separation and purification

Bioprocess optimisation
Techno-economic evaluation
Life Cycle Analysis
Effect of SSL and extracted lignosulphonate (LS) concentration on succinic acid production

- Actinobacillus succinogenes
- Basfia succiniciproducens

Inhibition begins at specific LS Concentration (g/L) for each species.
Pretreatment of spent sulphite liquor (SSL)

SSL → Nanofiltration → Phenolic extraction with ethyl acetate → Fermentation
Fed-batch fermentations in bench-top bioreactor

**Pure mixed sugars**

- **Final SA concentration**: 34.2 g/L
- **Yield**: 0.65 g/g
- **Productivity**: 0.59 g/L/h

**Nanofiltrated SSL**

- **Final SA concentration**: 33.8 g/L
- **Yield**: 0.58 g/g
- **Productivity**: 0.48 g/L/h
Continuous cultures for the production of succinic acid from SSL

**Observations**

- Succinic acid concentration varied between 20-25 g/L when synthetic xylose was consumed.
- Xylose accumulation was observed when dilution rate was increased.
- The optimum dilution rate observed was 0.04 h⁻¹ when pretreated SSL was used.
- The maximum succinic acid concentration achieved was 20.7 g/L at dilution rate 0.04 h⁻¹.
Direct crystallization scheme for succinic acid purification

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield (%)</th>
<th>Purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic fermentation broth</td>
<td>76</td>
<td>98</td>
</tr>
<tr>
<td>Actual fermentation broth</td>
<td>up to 80</td>
<td>up to 99.7</td>
</tr>
</tbody>
</table>

- **Fermentation Broth**
- **Centrifugation**
- **Activated carbon**
  - **Acidification**
  - **Ion-Exchange Resins**
- **Evaporation**
- **Crystallization**
- **Drying**
- **Succinic acid crystals**
Cost evaluation of bio-based succinic acid production

Succinic acid production via fermentation using spent sulphite liquor as substrate

Two different fermentation strategies
- Continuous
- Fed-batch

Three production capacities
- 5000 (t/y)
- 30000 (t/y)
- 100000 (t/y)
Succinic acid production flow diagram

- **Nutrients**
- **Spent Sulphite liquor**
- **Nano filtration**
- **Sterilization**
- **Inoculum**
- **Fermentation**
- **Direct Crystallization**
- **Succinic acid crystals**
Cost evaluation of bio-based succinic acid production

Succinic acid total production cost

<table>
<thead>
<tr>
<th>Capacity (t/y)</th>
<th>Continuous process</th>
<th>Fed-batch process</th>
</tr>
</thead>
<tbody>
<tr>
<td>5000</td>
<td>3.92</td>
<td>4.34</td>
</tr>
<tr>
<td>3000</td>
<td>3.56</td>
<td>4.19</td>
</tr>
<tr>
<td>10000</td>
<td>3.37</td>
<td>4.08</td>
</tr>
</tbody>
</table>

% contribution of individual costs on Total Production Cost

<table>
<thead>
<tr>
<th>Cost Item</th>
<th>Contribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCL</td>
<td>22.56</td>
</tr>
<tr>
<td>Raw material</td>
<td>67.65</td>
</tr>
<tr>
<td>Quality control</td>
<td>9.77</td>
</tr>
</tbody>
</table>

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BRIGIT
Thank you for your attention