Bioprocess development for the production of succinic acid from orange peel waste

Maria Patsalou\textsuperscript{1}, Chrysanthi Pateraki\textsuperscript{2}, Marlen Vasquez\textsuperscript{1}, Chryssoula Drouza\textsuperscript{3} and Michalis Koutinas\textsuperscript{1}

\textsuperscript{1}Department of Environmental Science & Technology, Cyprus University of Technology
\textsuperscript{2}Department of Food Science and Human Nutrition, Agricultural University of Athens
\textsuperscript{3}Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology

Limassol, 2016
Citrus fruits

- $88 \times 10^6$ tn worldwide production
- Industrial OPW generation: $15 \times 10^6$ tn/ y
- Oranges: 82 % of the total production
- 50% of fruit is peel waste
- Animal feed
- Disposal in landfills
Orange peel waste

- Peels
- Seeds
- Segment membranes

Composition of peel*

<table>
<thead>
<tr>
<th>Components</th>
<th>% Dry mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble sugar</td>
<td>16.90</td>
</tr>
<tr>
<td>Starch</td>
<td>3.75</td>
</tr>
<tr>
<td>Cellulose</td>
<td>9.21</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>10.50</td>
</tr>
<tr>
<td>Lignin</td>
<td>0.84</td>
</tr>
<tr>
<td>Pectin</td>
<td>42.50</td>
</tr>
<tr>
<td>Ash</td>
<td>3.50</td>
</tr>
<tr>
<td>Fat</td>
<td>1.95</td>
</tr>
<tr>
<td>Protein</td>
<td>6.50</td>
</tr>
<tr>
<td>Others</td>
<td>4.35</td>
</tr>
</tbody>
</table>

Succinic acid

- Di-carboxylic acid

- Important biobased platform chemicals
  - Polybutylene succinate (PBS)
  - Polybutylene succinate-terephthalate (PBST)
  - Polyester polyols
  - Food industry
  - Pharmaceutical industry
  - Production of resins, coatings and pigments

- Chemical technologies
  - Catalytic hydrogenation
  - Paraffin oxidation
  - Electrolytic reduction of maleic acid or anhydride

- High theoretical yield
- Environmental friendly impact
Succinic acid producers

- *Mannheimia succiniciproducens*
- *Anaerobiospirillum succiniciproducens*
- *Basfia succiniciproducens*

- **Actinobacillus succinogenes**
  - Isolated from bovine rumen
  - Capnophilic
  - Mesophilic
  - CO₂
Proposed Flow Diagram

Orange peel waste → Extraction of essential oils → Dryer

Acid hydrolysis → solid

Extraction of pectin → liquid

Enzymes (T. reesei) → Enzyme hydrolysis

Anaerobic digestion → Fermentation of hydrolysate
Aim and Objectives

1. Preliminary study for the development of an OPW bio-refinery to produce succinic acid
   - Release of metal ions after acid hydrolysis and acid/enzyme hydrolysis
   - Dilute-acid hydrolysis conditions
     - SA Fermentations, simple sugars
     - Selection of conditions
     - Presence of HMF in hydrolysates
     - SA Fermentations, dilute-acid hydrolysates
   - Optimal cultivation time for cellulolytic enzyme production by *T. reesei*

2. Selection of conditions
   - Presence of HMF in hydrolysates
   - SA Fermentations, dilute-acid hydrolysates

3. Optimal cultivation time for cellulolytic enzyme production by *T. reesei*
Elemental analysis (ICP-MS) of Hydrolysates

**Acid hydrolysis**

![Graph showing elemental analysis for Acid hydrolysis](image)

**Acid and enzyme hydrolysis**

![Graph showing elemental analysis for Acid and enzyme hydrolysis](image)
Succinic acid production from simple sugars fermentation

Glucose

- Temperature: 37°C
- pH: 7.5
- Initial sugar: 10 gL⁻¹
- Initial MgCO₃: 30 gL⁻¹
- Yeast: 5 gL⁻¹
- CO₂: 0.5 vvm

Fructose

- Temperature: 37°C
- pH: 7.5
- Initial sugar: 10 gL⁻¹
- Initial MgCO₃: 30 gL⁻¹
- Yeast: 5 gL⁻¹
- CO₂: 0.5 vvm
Consumption of each simple sugar, Yields of fermentations

<table>
<thead>
<tr>
<th>Sugar</th>
<th>SA Yield (g_p/g_s)</th>
<th>OA Yield (g_p/g_s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.57</td>
<td>1.26</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.33</td>
<td>1.10</td>
</tr>
<tr>
<td>Galactose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glucose (Bioreactor)</td>
<td>0.66</td>
<td>1.10</td>
</tr>
</tbody>
</table>
Dilute-acid hydrolysis conditions

- 100-120 °C, fructose*
- >120 °C, arabinose and galactose*

- 116°C, 10min, 5%
- 116°C, 20min, 5%
- 109°C, 10min, 5%
- 109°C, 20min, 5%
- 116°C, 10min, 10%
- 116°C, 20min, 10%
- 109°C, 10min, 10%
- 109°C, 20min, 10%

Release of sugar of dilute-acid hydrolysis

NMR analysis

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
<th>Concentration</th>
<th>Y (gts/grm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>109 °C</td>
<td>20 min</td>
<td>10%</td>
<td>0.12</td>
</tr>
<tr>
<td>109 °C</td>
<td>10 min</td>
<td>10%</td>
<td>0.12</td>
</tr>
<tr>
<td>116 °C</td>
<td>20 min</td>
<td>10%</td>
<td>0.12</td>
</tr>
<tr>
<td>116 °C</td>
<td>10 min</td>
<td>10%</td>
<td>0.09</td>
</tr>
<tr>
<td>109 °C</td>
<td>20 min</td>
<td>5%</td>
<td>0.18</td>
</tr>
<tr>
<td>109 °C</td>
<td>10 min</td>
<td>5%</td>
<td>0.19</td>
</tr>
<tr>
<td>116 °C</td>
<td>20 min</td>
<td>5%</td>
<td>0.19</td>
</tr>
<tr>
<td>116 °C</td>
<td>10 min</td>
<td>5%</td>
<td>0.21</td>
</tr>
</tbody>
</table>
Concentration of inhibitors of dilute-acid hydrolysate

Succinic acid production and consumption of total sugars

37°C
pH 7.5
30 gL⁻¹ MgCO₃
5 gL⁻¹ yeast extract
0.5 vvm CO₂
Yields of fermentations of dilute-acid hydrolysate

Organic acids yield (goa/gts)  Succinic acid yield (gsa/gts)

109 °C, 20min, 10%                      0.55  0.88  1.26
109 °C, 10min, 10%                      0.55  0.94  1.17
116 °C, 20min, 10%                      0.47  0.97  1.26
116 °C, 10min, 10%                      0.51  0.88  1.20
109 °C, 20min, 5%                       0.44  0.76  1.17
109 °C, 10min, 5%                       0.44  0.77  1.04
116 °C, 20min, 5%                       0.67  0.88  1.17
116 °C, 10min, 5%                       0.77  0.88  1.04
Cellulase production

- Fermentation *T. reesei*
  - 28 °C, pH 5.5, 180 rpm
  - 40 g L$^{-1}$ wheat bran,
  - 10 g L$^{-1}$ avicel

![Graph showing FPU ml$^{-1}$ vs. Time [d]]
# Succinic acid bio-production

<table>
<thead>
<tr>
<th>Raw material</th>
<th>Nitrogen source</th>
<th>Gas supply, Fermentation, Total volume, Working volume</th>
<th>Succinic acid (g/L)</th>
<th>Y (g&lt;sub&gt;SA&lt;/sub&gt;/g&lt;sub&gt;in&lt;/sub&gt;)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>YE (10)</td>
<td>Anaerobic, fed-batch, bioreactor 2 L - 1.5 L</td>
<td>49.62</td>
<td>0.64</td>
<td>Carvalho et al., 2014</td>
</tr>
<tr>
<td>Wheat hydrolyzate</td>
<td>YE (5) / Vit</td>
<td>Anaerobic, batch, bioreactor 1.8 L, 0.5 L</td>
<td>62.1</td>
<td>1.02</td>
<td>Dorado et al., 2009</td>
</tr>
<tr>
<td>Bread hydrolyzate</td>
<td>BH (200 mg/L FAN)</td>
<td>Anaerobic, batch, bioreactor 2.5 L, n.d.</td>
<td>47.3</td>
<td>nk</td>
<td>Leung et al., 2012</td>
</tr>
<tr>
<td>Cotton stalk hydrolyzate</td>
<td>YE (30) / Urea (2)</td>
<td>Anaerobic, batch SSF, bottles 500 mL, n.d.</td>
<td>63</td>
<td>0.64</td>
<td>Li et al., 2013</td>
</tr>
<tr>
<td>Macroalgal hydrolyzate</td>
<td>YE (16.7)</td>
<td>Anaerobic, batch, bioreactor 3L, 1.5L</td>
<td>33.78</td>
<td>0.63</td>
<td>Morales et al., 2015</td>
</tr>
<tr>
<td>Rapeseed meal</td>
<td>YE (15)</td>
<td>Anaerobic, fed-batch SSF, bioreactor 3 L, 1.2 L</td>
<td>23.4</td>
<td>0.115</td>
<td>Chen et al., 2011</td>
</tr>
<tr>
<td>Whey</td>
<td>YE (5) / Pep (10)</td>
<td>Anaerobic, batch, bioreactor 2.5L, 1.2L</td>
<td>22.2</td>
<td>0.57</td>
<td>Wan et al., 2008</td>
</tr>
<tr>
<td>Acid hydrolysis of OPW</td>
<td>-</td>
<td>Anaerobic, batch (Fibrobacter succinogenes), serum bottles 125ml, 25ml</td>
<td>1.9</td>
<td>0.12</td>
<td>Li et al., 2010</td>
</tr>
<tr>
<td>Dilute-acid hydrolysis of OPW</td>
<td>YE (5)</td>
<td>Anaerobic, batch, bottles 100mL, 100mL</td>
<td>6.17 and 6.13</td>
<td>0.76 and 0.77</td>
<td>Current study</td>
</tr>
</tbody>
</table>
Conclusions

- **Elemental analysis on hydrolysates**
  - $\text{Mg}^{2+}, \text{Ca}^{2+}$

- **Dilute-acid hydrolysis conditions**
  - $109 \, ^\circ\text{C}, 20 \, \text{min}, 5\% \, (\text{w/w}), y=0.76 \, (g_{\text{sa}}/g_{\text{ts}})$
  - $116 \, ^\circ\text{C}, 10 \, \text{min}, 5\% \, (\text{w/w}), y=0.77 \, (g_{\text{sa}}/g_{\text{ts}})$

- **Cellulase production**
  - 5 days incubation
Future Work

- Ultrasound
  - Frequency
  - Duration

- Enzyme hydrolysis
  - Enzyme units
  - Duration
Thank you!