Citrus peel waste valorization through a biorefinery strategy for the production of succinic acid, ethanol, methane and fertilizer

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6th International Conference on Sustainable Solid Waste Management
From Oil Refineries to Bio Refineries

Oil Refinery:
- Fuels
- Solvents
- Chemical intermediates
- Plastics
- Fibers
- Specialty chemicals
- Oils

Biorefinery:
- Fuels
- Solvents
- Chemical intermediates
- Plastics
- Natural fibers
- Specialty chemicals
- Oils
- Food

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Citrus Fruits

- 121 x 10^6 tons global citrus production
- 25 x 10^6 tons citrus peel waste

- 50% of the fruit is peel waste
  - Peels
  - Seeds
  - Segment membranes
Current Practice

- Animal feed
- Disposal in landfills

Composition of peel

42.50% pectin
16.90% soluble sugars
10.50% hemicellulose
9.21% cellulose
20.89% others

0.5% essential oils


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Fermentation Products

Succinic acid
- Di-carboxylic acid
- Important biobased platform chemicals
- High theoretical yield
- Environmental friendly impact
- *Actinobacillus succinogenes*

Ethanol
- Biofuel
- *Pichia kudriavzevii KVMP10*
- *Kluyveromyces marxianus*
- *Saccharomyces cerevisiae*
Valorization of CPW

Extraction Products

Essential oils
- Antimicrobial agent
- Food
- Medicines
- Flavorings

Pectin
- Food industries
- Pharmaceutical industries

Side Products

Methane
- biofuel

Fertilizer

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Process Flow Sheet of CPW Biorefinery

Citrus peel waste → Extraction of essential oils

Dryer

Acid hydrolysis → Anaerobic digestion → Methane

→ Extraction of pectin

→ Fertilizer

Fermentation of hydrolyzate → Succinic acid → Ethanol

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CPW BioRefinery

**Succinic acid**
- Nitrogen sources
- Vitamins

**Ethanol**
- Conditions of acid hydrolysis
- Nitrogen source
- Enzyme hydrolysis

**Methane**
- Solid biorefinery residues
- Raw CPW
- Dry CPW

**Fertilizer**
- Solid biorefinery residues
- Evaluation of lettuce seedling production

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Succinic acid

- 116 °C, 10 min
- 0.5% H₂SO₄
- 5% dry raw material

*Actinobacillus succinogenes*

37 °C, 0.5 vvm CO₂
30 g L⁻¹ MgCO₃

No nitrogen source added
- Ammonium sulfate
- Yeast extract
- Corn steep liquor

Supplementation of vitamins
- Yeast extract
- Corn steep liquor

*Patsalou et al., J Clean Prod., 2017; 166: 706-716*

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<table>
<thead>
<tr>
<th>Raw material</th>
<th>Nitrogen source</th>
<th>Fermentation</th>
<th>Succinic acid (g L⁻¹)</th>
<th>Yield (gₐₔ g⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>YE (10 g L⁻¹)</td>
<td>Fed-batch, bioreactor</td>
<td>49.6</td>
<td>0.64</td>
<td>Carvalho et al., 2014</td>
</tr>
<tr>
<td>Wheat hydrolyzate</td>
<td>YE (5 g L⁻¹)/Vit</td>
<td>Batch, bioreactor</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⁻¹ PAN)</td>
<td>Batch, bioreactor</td>
<td>47.3</td>
<td>n.d.</td>
<td>Leung et al., 2012</td>
</tr>
<tr>
<td>Cotton stalk hydrolyzate</td>
<td>YE (30 g L⁻¹)/Urea (2 g L⁻¹)</td>
<td>Batch SSF³, shake flasks</td>
<td>63.0</td>
<td>0.64</td>
<td>Li et al., 2013</td>
</tr>
<tr>
<td>Macroalgal hydrolyzate</td>
<td>YE (16.7 g L⁻¹)</td>
<td>Batch, bioreactor</td>
<td>33.8</td>
<td>0.63</td>
<td>Morales et al., 2015</td>
</tr>
<tr>
<td>Rapeseed meal</td>
<td>YE (15 g L⁻¹)</td>
<td>Fed-batch SSF³, bioreactor</td>
<td>23.4</td>
<td>0.115</td>
<td>Chen et al., 2011</td>
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<tr>
<td>Whey</td>
<td>YE (5 g L⁻¹)/Pep (10 g L⁻¹)</td>
<td>Batch, bioreactor</td>
<td>22.2</td>
<td>0.57</td>
<td>Wan et al., 2008</td>
</tr>
<tr>
<td>CPW hydrolyzate</td>
<td>YE (5 g L⁻¹)</td>
<td>Batch, shake flasks</td>
<td>8.3</td>
<td>0.70</td>
<td>Current study</td>
</tr>
</tbody>
</table>
Ethanol

- 5% dry raw material
- 0.5% H₂SO₄
- 108 °C, 116 °C, 125 °C
- 10 min & 20 min

Pichia kudriavzevii KVMP10

- Kluyveromyces marxianus
- Saccharomyces cerevisiae

42 °C

Concentration of ethanol (g L⁻¹)

- STILLAGE RECYCLING: 30.7
- ACID AND ENZYME HYDROLYSIS:
  - Ethanol: 9.21
  - Total reducing sugars: 0.42 g ethanol g⁻¹
- ACID HYDROLYSIS:
  - Ethanol: 5.83
  - Total reducing sugars: 0.48 g ethanol g⁻¹
<table>
<thead>
<tr>
<th>Raw material</th>
<th>Pretreatment method</th>
<th>Process conditions</th>
<th>Micro-organism</th>
<th>Total initial sugar content</th>
<th>Ethanol concentration</th>
<th>Ethanol productivity [g l(^{-1} \text{ h}^{-1})]</th>
<th>Yield [g ethanol g(_{\text{glc}})</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Orange peel hydrolysate</td>
<td>nd</td>
<td>SF</td>
<td>S. cerevisiae</td>
<td>nd</td>
<td>40-45 (g l(^{-1}))</td>
<td>0.82-0.90</td>
<td>nd</td>
<td>Grohmann et al. (1994)</td>
</tr>
<tr>
<td>Orange peel hydrolysate</td>
<td>nd</td>
<td>SF</td>
<td>E. coli</td>
<td>111 (g l(^{-1}))</td>
<td>35-35 (g l(^{-1}))</td>
<td>0.42-0.80</td>
<td>nd</td>
<td>Grohmann et al. (1996)</td>
</tr>
<tr>
<td>Orange peel hydrolysate</td>
<td>nd</td>
<td>SF, 37°C</td>
<td>K. marxianus</td>
<td>90.6 (g l(^{-1}))</td>
<td>37 (g l(^{-1}))</td>
<td>0.51</td>
<td>0.44</td>
<td>Wilkins et al. (2007a)</td>
</tr>
<tr>
<td>Orange peel hydrolysate</td>
<td>nd</td>
<td>SF, 37°C</td>
<td>S. cerevisiae</td>
<td>90.6 (g l(^{-1}))</td>
<td>41 (g l(^{-1}))</td>
<td>0.55</td>
<td>0.45</td>
<td>Wilkins et al. (2007a)</td>
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<tr>
<td>Citrus peel waste</td>
<td>Steam expolision</td>
<td>SSF, 37°C, 0.08% e.o.</td>
<td>S. cerevisiae</td>
<td>0.31 (g g(^{-1}) dry raw material)</td>
<td>39.03 (g l(^{-1}))</td>
<td>1.62</td>
<td>0.43</td>
<td>Wilkins et al. (2002b)</td>
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<tr>
<td>Orange peel hydrolysate</td>
<td>nd</td>
<td>SF, 37°C, 0.05% e.o.</td>
<td>Z. mobilis</td>
<td>90.6 (g l(^{-1}))</td>
<td>43.5 (g l(^{-1}))</td>
<td>0.60</td>
<td>0.48</td>
<td>Wilkins (2009)</td>
</tr>
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<td>Mandarin waste and banana peels</td>
<td>Steam depressurization</td>
<td>SSF, 30°C</td>
<td>S. cerevisiae and P. tannophilus</td>
<td>0.17 (g g(^{-1}) dry raw material)</td>
<td>26.84 (g l(^{-1}))</td>
<td>0.55</td>
<td>0.42</td>
<td>Sharma et al. (2007)</td>
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<tr>
<td>Orange peel hydrolysate</td>
<td>Two stage acid hydrolysis</td>
<td>SF, 34°C</td>
<td>S. cerevisiae</td>
<td>27.54 (g l(^{-1}))</td>
<td>30.33 (g l(^{-1}))</td>
<td>3.37</td>
<td>0.46</td>
<td>Oberoi et al. (2010)</td>
</tr>
<tr>
<td>Mandarin waste</td>
<td>Hydrothermal sterilization</td>
<td>SSF, 37°C</td>
<td>S. cerevisiae</td>
<td>74 (g l(^{-1}))</td>
<td>42 (g l(^{-1}))</td>
<td>3.50</td>
<td>0.48</td>
<td>Oberoi et al. (2011)</td>
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<tr>
<td>Citrus waste</td>
<td>Dilute-acid hydrolysis and pectin recovery</td>
<td>SSF, 30°C</td>
<td>S. cerevisiae</td>
<td>32.97 (g l(^{-1}))</td>
<td>39.64 (g l(^{-1}))</td>
<td>nd</td>
<td>0.43</td>
<td>Pourbafani et al. (2010)</td>
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<td>Mandarin waste</td>
<td>Enzyme hydrolysis</td>
<td>SSF, 40°C</td>
<td>P. kudriavzevi</td>
<td>64 (g l(^{-1}))</td>
<td>33.87 (g l(^{-1}))</td>
<td>2.82</td>
<td>0.67</td>
<td>Sandhu et al. (2012)</td>
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<tr>
<td>Mandarin waste</td>
<td>Steam expolision</td>
<td>SSF, 37°C</td>
<td>S. cerevisiae</td>
<td>nd</td>
<td>60 (g l(^{-1}))</td>
<td>nd</td>
<td>0.43</td>
<td>Boluda-Aguilar et al. (2010)</td>
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<tr>
<td>Lemon peel waste</td>
<td>Steam expolision</td>
<td>SSF, 37°C</td>
<td>S. cerevisiae</td>
<td>nd</td>
<td>67.83 (g l(^{-1}))</td>
<td>nd</td>
<td>nd</td>
<td>Boluda-Aguilar and Lopez-Gomez (2013)</td>
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<tr>
<td>Orange peel waste</td>
<td>Enzyme hydrolysis</td>
<td>Aer, 32°C</td>
<td>M. indicus</td>
<td>39 (g l(^{-1}))</td>
<td>15 (g l(^{-1}))</td>
<td>0.62</td>
<td>0.39</td>
<td>Lennarsson et al. (2012)</td>
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<td>Orange peel waste hydrolysate</td>
<td>nd</td>
<td>SF, Aer, 32°C</td>
<td>Rhizopus sp.</td>
<td>50 (g l(^{-1}))</td>
<td>nd</td>
<td>nd</td>
<td>0.37</td>
<td>Lennarsson et al. (2012)</td>
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<tr>
<td>Orange peel waste hydrolysate</td>
<td>nd</td>
<td>SF, Aer, 32°C</td>
<td>M. indicus</td>
<td>50 (g l(^{-1}))</td>
<td>nd</td>
<td>nd</td>
<td>0.41</td>
<td>Lennarsson et al. (2012)</td>
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<tr>
<td>Orange peel waste hydrolysate</td>
<td>nd</td>
<td>SF, Aer, 32°C</td>
<td>M. indicus</td>
<td>50 (g l(^{-1}))</td>
<td>nd</td>
<td>nd</td>
<td>0.39</td>
<td>Lennarsson et al. (2012)</td>
</tr>
<tr>
<td>Orange peel waste hydrolysate</td>
<td>Pepping and enzyme hydrolysis</td>
<td>SF, 30°C</td>
<td>S. cerevisiae</td>
<td>0.63 (g g(^{-1}) raw material)</td>
<td>46.2 (g l(^{-1}))</td>
<td>3.85</td>
<td>0.91</td>
<td>Choi et al. (2013)</td>
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<tr>
<td>Orange peel hydrolysate</td>
<td>nd</td>
<td>SF, 30°C</td>
<td>P. kudriavzevi</td>
<td>101 (g l(^{-1}))</td>
<td>25 (g l(^{-1}))</td>
<td>1.08</td>
<td>nd</td>
<td>This study</td>
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<tr>
<td>Orange peel hydrolysate</td>
<td>nd</td>
<td>SF, 42°C</td>
<td>P. kudriavzevi</td>
<td>101 (g l(^{-1}))</td>
<td>54 (g l(^{-1}))</td>
<td>2.25</td>
<td>nd</td>
<td>This study</td>
</tr>
</tbody>
</table>
Methane

- Solid biorefinery residues
- Raw CPW
- Dry CPW

Mesophilic conditions
6 g L⁻¹ volatile solids
<table>
<thead>
<tr>
<th>Raw material</th>
<th>CH\textsubscript{4} (ml g\textsubscript{VS}^{-1})</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus sinensis (dried peels)</td>
<td>400</td>
<td>Gunaseelan et al., 2004</td>
</tr>
<tr>
<td>Citrus waste (steam distilled)</td>
<td>125</td>
<td>Martin et al., 2010</td>
</tr>
<tr>
<td>Orange peel hydrolysate and solid residue</td>
<td>363</td>
<td>Pourbafrani et al., 2010</td>
</tr>
<tr>
<td>Citrus waste (steam explosion)</td>
<td>500</td>
<td>Forgcs et al., 2011</td>
</tr>
<tr>
<td>Citrus waste</td>
<td>50</td>
<td>Forgcs et al., 2011</td>
</tr>
<tr>
<td>Orange peel</td>
<td>267</td>
<td>Sanjaya et al., 2016</td>
</tr>
<tr>
<td>Mandores</td>
<td>342</td>
<td>Current study</td>
</tr>
<tr>
<td>Mandores (hydrolysed)</td>
<td>349</td>
<td>Current study</td>
</tr>
</tbody>
</table>

**Biogas (ml g\textsubscript{VS}^{-1})**

- **Raw material:**
  - 35 days: 102.03, 35 days: 141.41, 112 days: 156.51

- **Dry raw material:**
  - 35 days: 67.78, 75.99, 112 days: 83.77

- **Hydrolysed:**
  - 35 days: 54.59, 71.85, 112 days: 89.35

**Methane (ml g\textsubscript{grm}^{-1})**

- **Raw material:**
  - 35 days: 42.95, 71 days: 42.06, 112 days: 45.30

- **Dry raw material:**
  - 35 days: 42.80, 71 days: 50.85, 112 days: 49.88

- **Hydrolysed:**
  - 35 days: 72.21, 71 days: 77.56, 112 days: 84.45
### Fertilizer

- Applied as substrate for lettuce

<table>
<thead>
<tr>
<th></th>
<th>0%</th>
<th>1%</th>
<th>2.5%</th>
<th>5%</th>
<th>10%</th>
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</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
<td>5.59 ± 0.0318a</td>
<td>5.10 ± 0.0289b</td>
<td>4.85 ± 0.0289c</td>
<td>4.03 ± 0.0231d</td>
<td>3.31 ± 0.0203e</td>
</tr>
<tr>
<td><strong>EC (μS cm⁻¹)</strong></td>
<td>1215 ± 7.10e</td>
<td>1374.1 ± 8.03d</td>
<td>2085.9 ± 12.15c</td>
<td>2778.9 ± 16.19b</td>
<td>3970.9 ± 23.15a</td>
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<tr>
<td><strong>Organic matter %</strong></td>
<td>97.87 ± 0.372ab</td>
<td>98.57 ± 0.248a</td>
<td>98.18 ± 0.003ab</td>
<td>97.84 ± 0.003b</td>
<td>97.99 ± 0.121ab</td>
</tr>
<tr>
<td><strong>Organic C %</strong></td>
<td>56.77 ± 0.217ab</td>
<td>57.18 ± 0.147a</td>
<td>56.95 ± 0.000ab</td>
<td>56.75 ± 0.000b</td>
<td>56.84 ± 0.069ab</td>
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<tr>
<td><strong>Total N (g kg⁻¹)</strong></td>
<td>4.60 ± 0.087d</td>
<td>6.02 ± 0.318b</td>
<td>5.13 ± 0.044cd</td>
<td>5.55 ± 0.193ab</td>
<td>6.65 ± 0.017a</td>
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<td><strong>K (g kg⁻¹)</strong></td>
<td>0.62 ± 0.003e</td>
<td>0.69 ± 0.015d</td>
<td>0.76 ± 0.002c</td>
<td>0.83 ± 0.025b</td>
<td>1.10 ± 0.003a</td>
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<tr>
<td><strong>P (g kg⁻¹)</strong></td>
<td>0.402 ± 0.0364b</td>
<td>0.357 ± 0.0165b</td>
<td>0.396 ± 0.0069b</td>
<td>0.414 ± 0.0147ab</td>
<td>0.473 ± 0.0040a</td>
</tr>
<tr>
<td><strong>Na (g kg⁻¹)</strong></td>
<td>0.172 ± 0.0012b</td>
<td>0.182 ± 0.0038b</td>
<td>0.179 ± 0.0009b</td>
<td>0.180 ± 0.0121b</td>
<td>0.215 ± 0.0090a</td>
</tr>
<tr>
<td><strong>Total porosity % (v/v)</strong></td>
<td>85.4 ± 0.84bc</td>
<td>82.3 ± 1.06c</td>
<td>82.7 ± 0.10c</td>
<td>85.9 ± 1.59ab</td>
<td>88.8 ± 0.31a</td>
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<td><strong>Air filled porosity % (v/v)</strong></td>
<td>11.8 ±1.01bc</td>
<td>9.3 ± 0.21d</td>
<td>10.6 ± 0.20cd</td>
<td>12.7 ± 0.61ab</td>
<td>14.2 ± 0.70a</td>
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<td><strong>Container capacity % (v/v)</strong></td>
<td>73.6 ± 0.17ab</td>
<td>73.0 ± 0.85ab</td>
<td>72.1 ± 0.29b</td>
<td>73.3 ± 0.97ab</td>
<td>74.6 ± 0.39a</td>
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<tr>
<td><strong>Bulk density (g cm⁻³)</strong></td>
<td>25.0 ± 0.33a</td>
<td>24.5 ± 0.16a</td>
<td>23.6 ± 0.26b</td>
<td>24.5 ±0.24bc</td>
<td>25.2 ± 0.28a</td>
</tr>
</tbody>
</table>

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**Diagram:**
- Citrus peel waste
- Extraction of essential oils
- Dryer
- Anaerobic digestion
- Methane
- Fertilizer
- Succinic acid
- Ethanol

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Fertilizer

In vitro

[Graph showing germination percentage over days for different concentrations of fertilizer]

In vivo

[Photos of plants grown in different conditions labeled with control and fertilizer concentrations]
BioRefinery

**Succinic acid**
- Simultaneous Saccharification and Fermentation
- Fed-Batch fermentations

**Ethanol**
- Optimal Conditions: 116 °C, 10 min
- *Pichia kudriavzevii* KVMP10, 30.70 g L⁻¹ with the application of stillage recycling

**Methane**
- Dry CPW
- Adaptation of sludge

**Fertilizer**
- Enhance plant growth

**Technoeconomical analysis**
Thank you 😊