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

M. Patsalou, E. Protopapa, S. Stavrinou, A. Chrysargyris, N. Tzortzakis, I. Vyrides, M. Koutinas

[1]Department of Environmental Science & Technology, Cyprus University of Technology

[2]Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology

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Environmental Bioprocessing Laboratory

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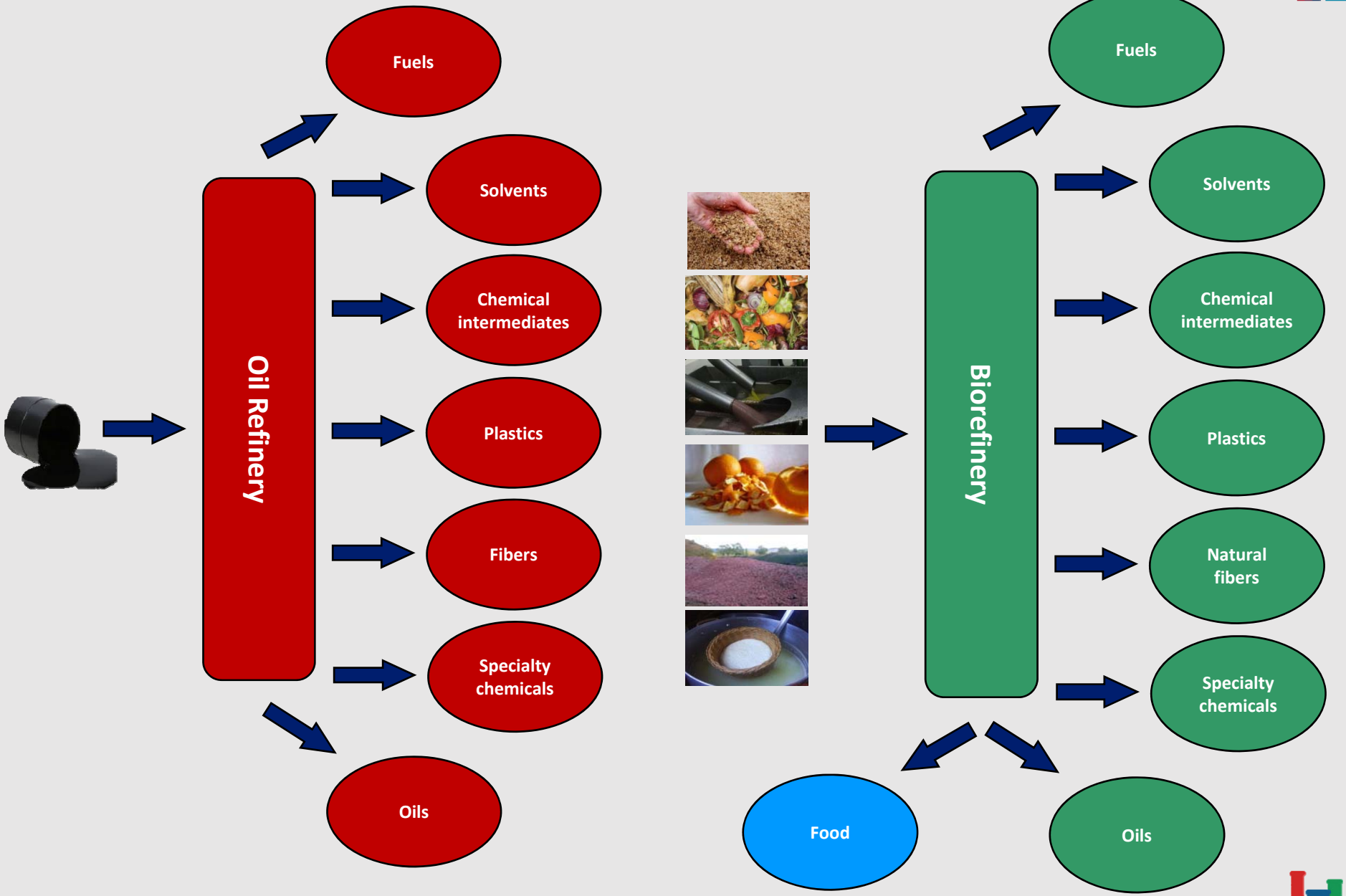
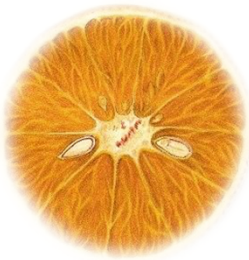
From Oil Refineries to Bio Refineries



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**

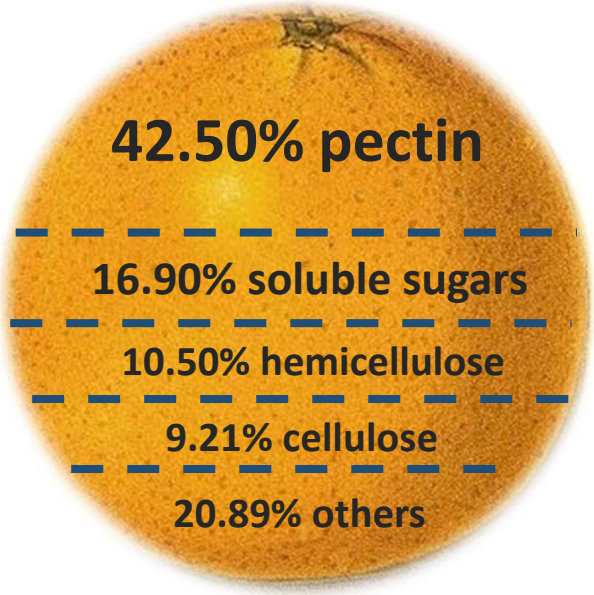


Citrus Peel Waste

Current Practice

- Animal feed
- Disposal in landfills

Composition of peel



Components	% Dry mass
Starch	3.75
Lignin	0.84
Ash	3.50
Fat	1.95
Protein	6.50
Others	4.35

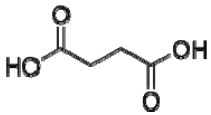
➢0.5% essential oils

*Lopez *et al.*, Crit Rev Biotechnol. , 2010; 30: 63-69

Valorization of CPW

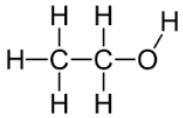
Fermentation Products

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

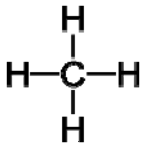
Pectin

- Food industries
- Pharmaceutical industries

Side Products

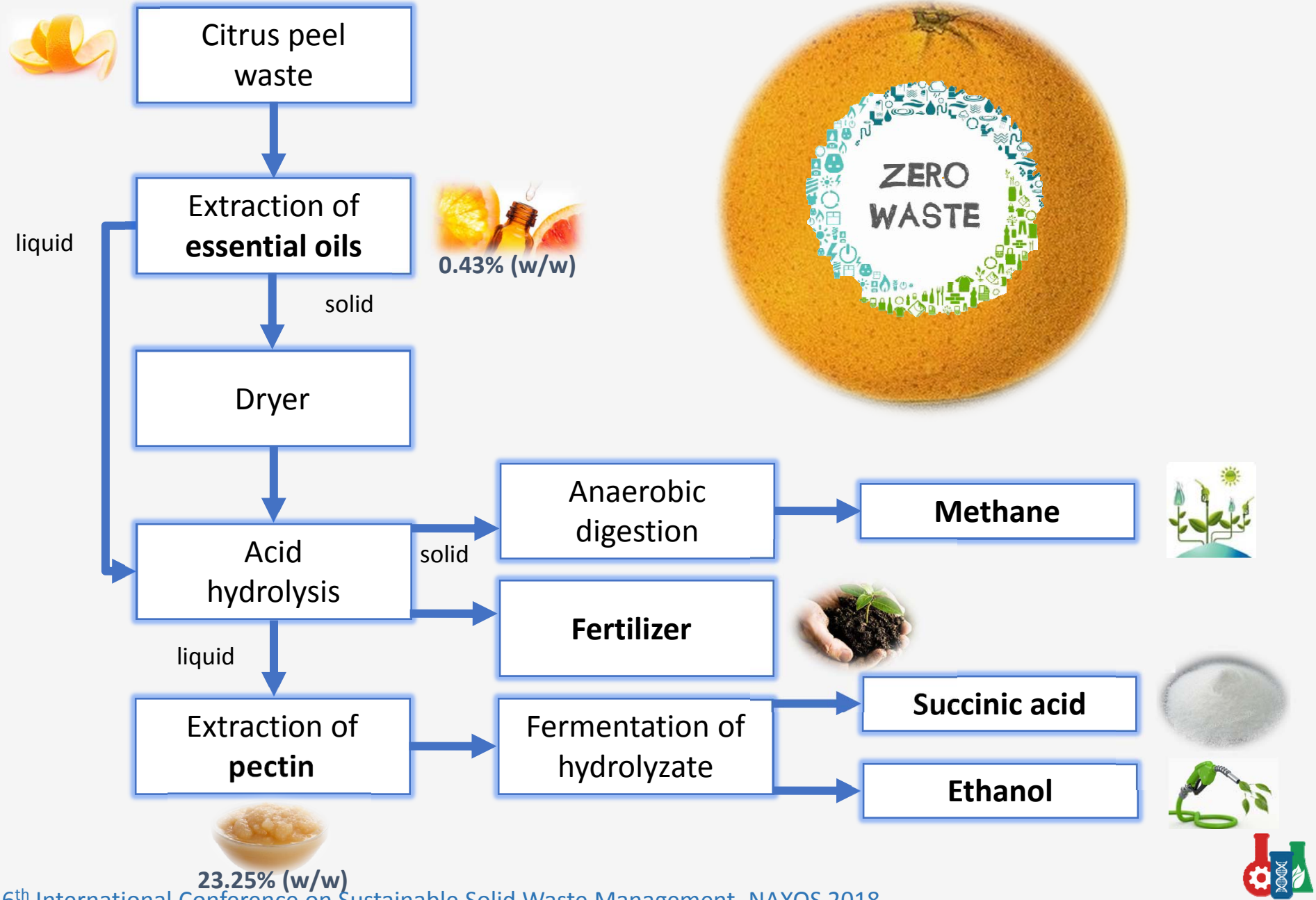
Methane

- biofuel



Fertilizer

Process Flow Sheet of CPW Biorefinery



CPW BioRefinery

Succinic acid

- Nitrogen sources
- Vitamins

Ethanol

- Conditions of acid hydrolysis
- Nitrogen source
- Enzyme hydrolysis

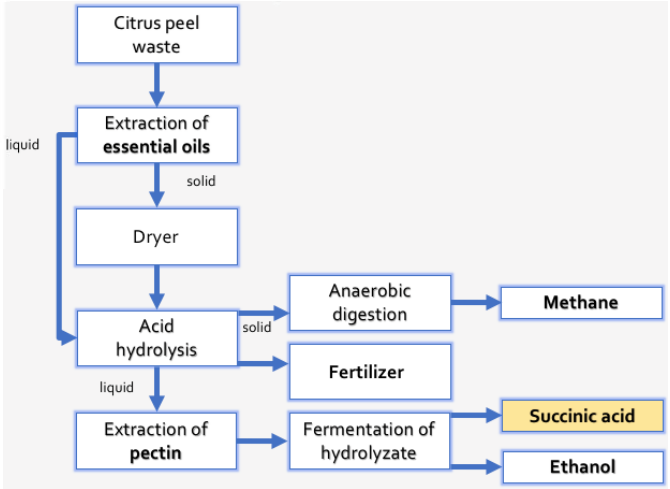
Methane

- Solid biorefinery residues
- Raw CPW
- Dry CPW

Fertilizer

- Solid biorefinery residues
- Evaluation of lettuce seedling production

Succinic acid



- 116 °C , 10 min
- 0.5% H_2SO_4
- 5% dry raw material

Actinobacillus succinogenes

37 °C, 0.5 vvm CO_2

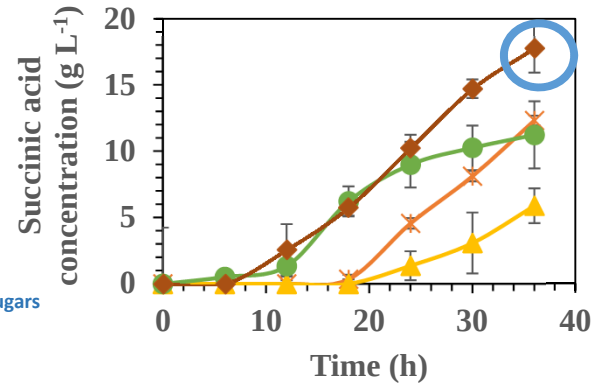
30 g L^{-1} $MgCO_3$

No nitrogen source added

Ammonium sulfate

Yeast extract — 0.48 $g_{ethanol}$ $g^{-1}_{total\ reducing\ sugars}$

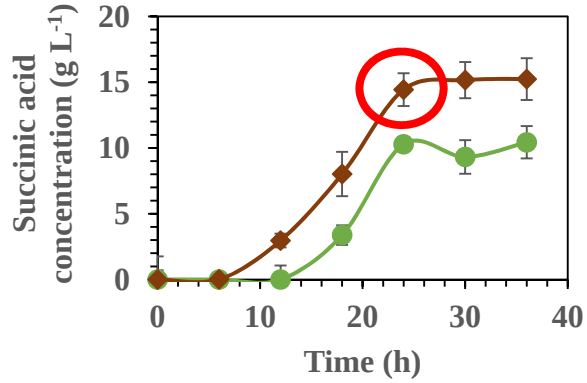
Corn steep liquor — 0.49 $g_{ethanol}$ $g^{-1}_{total\ reducing\ sugars}$



Supplementation of vitamins

Yeast extract

Corn steep liquor



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

Raw material	Nitrogen source	Fermentation	Succinic acid (g L^{-1})	Yield (g_{sa} g_{tsc}^{-1})	Reference
Glycerol	YE (10 g L^{-1})	Fed-batch, bioreactor	49.6	0.64	Carvalho et al., 2014
Wheat hydrolyzate	YE (5 g L^{-1})/Vit	Batch, bioreactor	62.1	1.02	Dorado et al., 2009
Bread hydrolyzate	BH (200 mg L^{-1} FAN)	Batch, bioreactor	47.3	n.d.	Leung et al., 2012
Cotton stalk hydrolyzate	YE (30 g L^{-1})/Urea (2 g L^{-1})	Batch SSF[a], shake flasks	63.0	0.64	Li et al., 2013
Macroalgal hydrolyzate	YE (16.7 g L^{-1})	Batch, bioreactor	33.8	0.63	Morales et al., 2015
Rapeseed meal	YE (15 g L^{-1})	Fed-batch SSF[a], bioreactor	23.4	0.115	Chen et al., 2011
Whey	YE (5 g L^{-1})/Pep (10 g L^{-1})	Batch, bioreactor	22.2	0.57	Wan et al., 2008
CPW hydrolyzate	YE (5 g L^{-1})	Batch, shake flasks	8.3	0.70	Current study

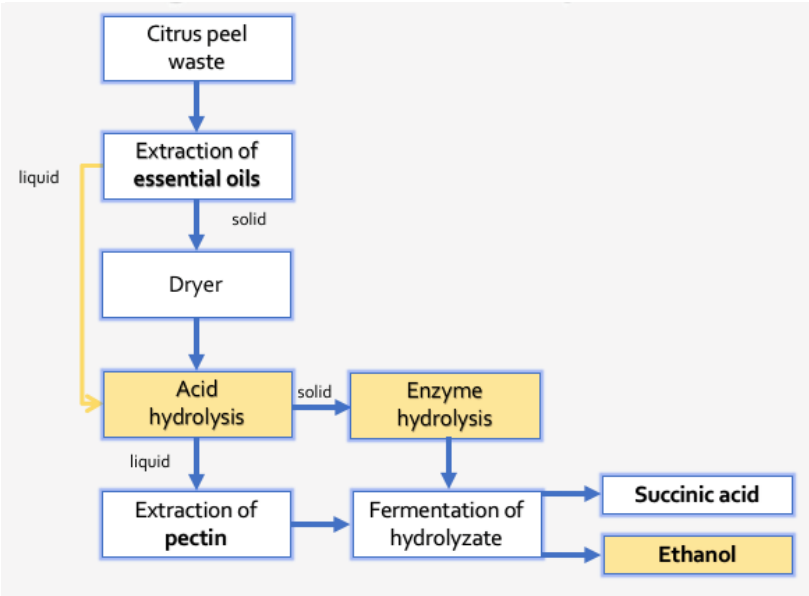
Ethanol

- 5% dry raw material
- 0.5% H_2SO_4
- 108 °C, 116 °C, 125 °C
- 10 min & 20 min

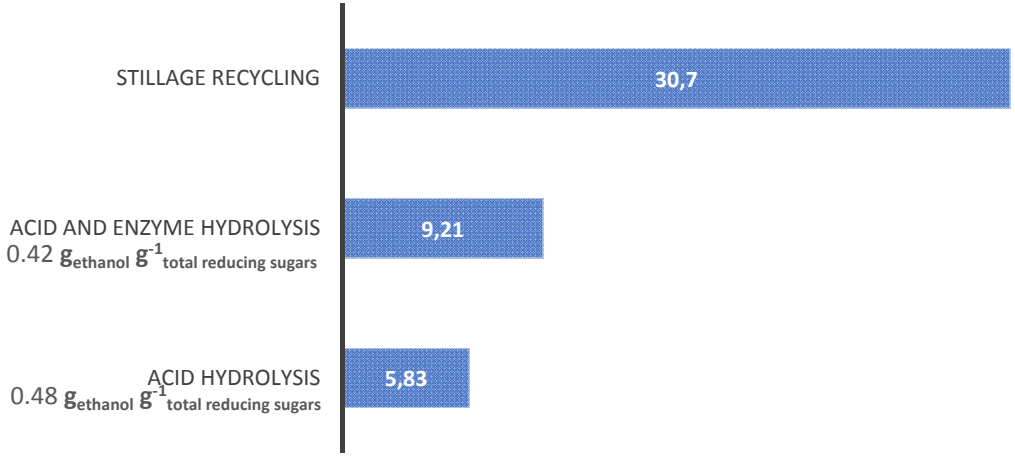
Pichia kudriavzevii KVMP10

- *Kluyveromyces marxianus*
- *Saccharomyces cerevisiae*

42 °C

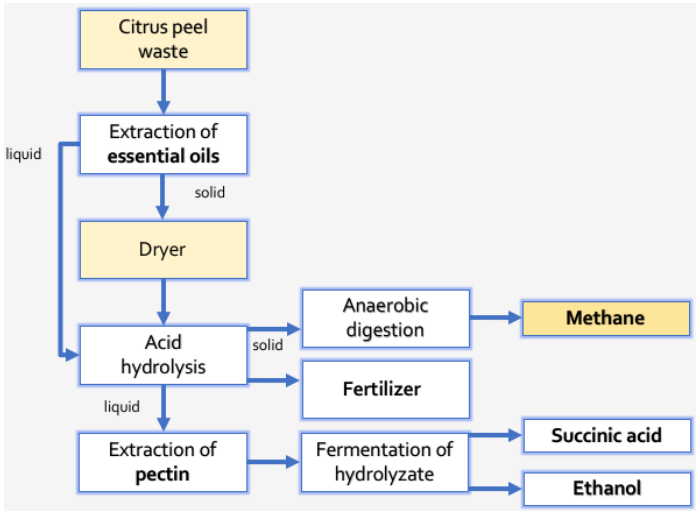


Concentration of ethanol (g L^{-1})



Raw material	Pretreatment method	Process conditions	Micro-organism	Total initial sugar content	Ethanol concentration	Ethanol productivity [g l^{-1} h^{-1}]	Yield [$g_{ethanol}$ g_{sugars}^{-1}]	Reference
Orange peel hydrolysate	nd	SF	*S. cerevisiae*	nd	40–45 (g l^{-1})	0·82–0·90	nd	Grohmann *et al.* (1994)
Orange peel hydrolysate	nd	SF	*E. coli*	111 (g l^{-1})	35–38 (g l^{-1})	0·42–0·80	nd	Grohmann *et al.* (1996)
Orange peel hydrolysate	nd	SF, 37°C	*K. marxianus*	90·6 (g l^{-1})	37 (g l^{-1})	0·51	0·44	Wilkins *et al.* (2007a)
Orange peel hydrolysate	nd	SF, 37°C	*S. cerevisiae*	90·6 (g l^{-1})	41 (g l^{-1})	0·56	0·45	Wilkins *et al.* (2007a)
Citrus peel waste	Steam explosion	SSF, 37°C, 0·08% e.o.	*S. cerevisiae*	0·31 (g g^{-1} dry raw material)	39·03 (g l^{-1})	1·62	0·43	Wilkins *et al.* (2007b)
Orange peel hydrolysate	nd	SF, 37°C, 0·05% e.o.	*Z. mobilis*	90·6 (g l^{-1})	43·5 (g l^{-1})	0·60	0·48	Wilkins (2009)
Mandarin waste and banana peels	Steam depressurization	SSF, 30°C	*S. cerevisiae* and *P. tannophilus*	0·17 (g g^{-1} dry raw material)	26·84 (g l^{-1})	0·55	0·42	Sharma *et al.* (2007)
Orange peel hydrolysate	Two stage acid hydrolysis	SF, 34°C	*S. cerevisiae*	27·54 (g l^{-1})	30·33 (g l^{-1})	3·37	0·46	Oberoi *et al.* (2010)
Mandarin waste	Hydrothermal sterilization	SSF, 37°C	*S. cerevisiae*	74 (g l^{-1})	42 (g l^{-1})	3·50	0·48	Oberoi *et al.* (2011)
Citrus waste	Dilute-acid hydrolysis and pectin recovery	SF, 30°C	*S. cerevisiae*	32·97 (g l^{-1})	39·64 (l tn wet raw material^{-1}), 14·17 (g l^{-1})	nd	0·43	Pourbafrani *et al.* (2010)
Mandarin waste	Enzyme hydrolysis	SSF, 40°C	*P. kudriavzevii*	64 (g l^{-1})	33·87 (g l^{-1})	2·82	0·67	Sandhu *et al.* (2012)
Mandarin waste	Steam explosion	SSF, 37°C	*S. cerevisiae*	nd	60 (l tn raw material^{-1})	nd	nd	Boluda-Aguilar *et al.* (2010)
Lemon peel waste	Steam explosion	SSF, 37°C	*S. cerevisiae*	nd	67·83 (l tn raw material^{-1})	nd	nd	Boluda-Aguilar and Lopez-Gomez (2013)
Orange peel waste	Enzyme hydrolysis	Aer, 32°C	*M. indicus*	39 (g l^{-1})	15 (g l^{-1})	0·62	0·39	Lennartsson *et al.* (2012)
Orange peel waste hydrolysate	nd	SF, Aer, 32°C	*Rhizopus sp.*	50 (g l^{-1})	nd	nd	0·37	Lennartsson *et al.* (2012)
Orange peel waste hydrolysate	nd	SF, Aer, 32°C	*M. indicus*	50 (g l^{-1})	nd	nd	0·41	Lennartsson *et al.* (2012)
Orange peel waste hydrolysate	nd	SF, An, 32°C	*Rhizopus sp.*	50 (g l^{-1})	nd	nd	0·39	Lennartsson *et al.* (2012)
Orange peel waste hydrolysate	nd	SF, An, 32°C	*M. indicus*	50 (g l^{-1})	nd	nd	0·43	Lennartsson *et al.* (2012)
Mandarin peel waste	Popping and enzyme hydrolysis	SF, 30°C	*S. cerevisiae*	0·63 (g g^{-1} raw material)	46·2 (g l^{-1})	3·85	0·91	Choi *et al.* (2013)
Orange peel hydrolysate	nd	SF, 30°C	*P. kudriavzevii* KVMP10	101 (g l^{-1})	25 (g l^{-1})	1·08	nd	This study
Orange peel hydrolysate	nd	SF, 42°C	*P. kudriavzevii* KVMP10	101 (g l^{-1})	54 (g l^{-1})	2·25	nd	This study

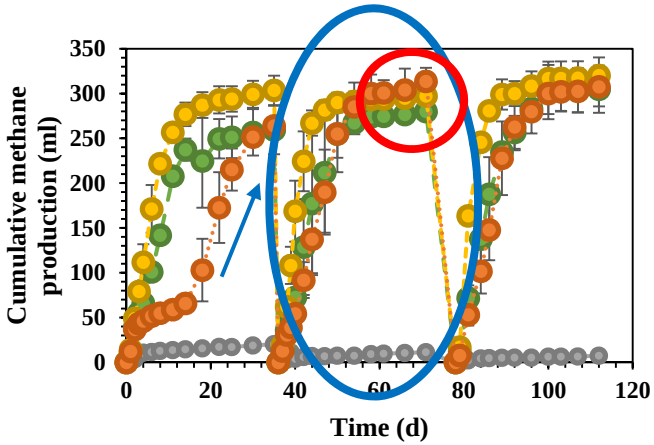
Methane

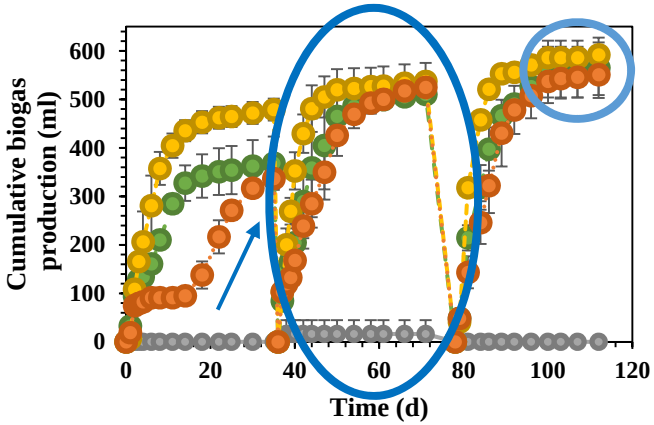


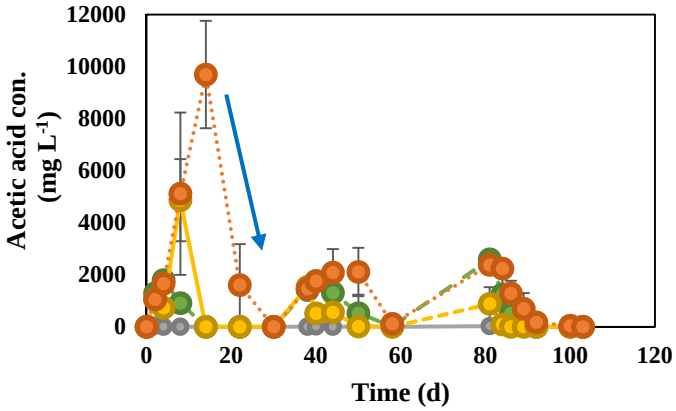
- Solid biorefinery residues
- Raw CPW
- Dry CPW

Mesophilic conditions

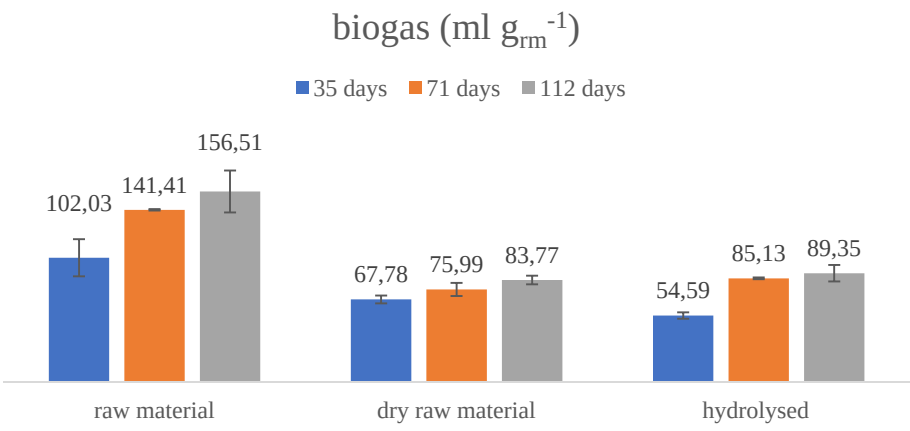
6 g L^{-1} volatile solids

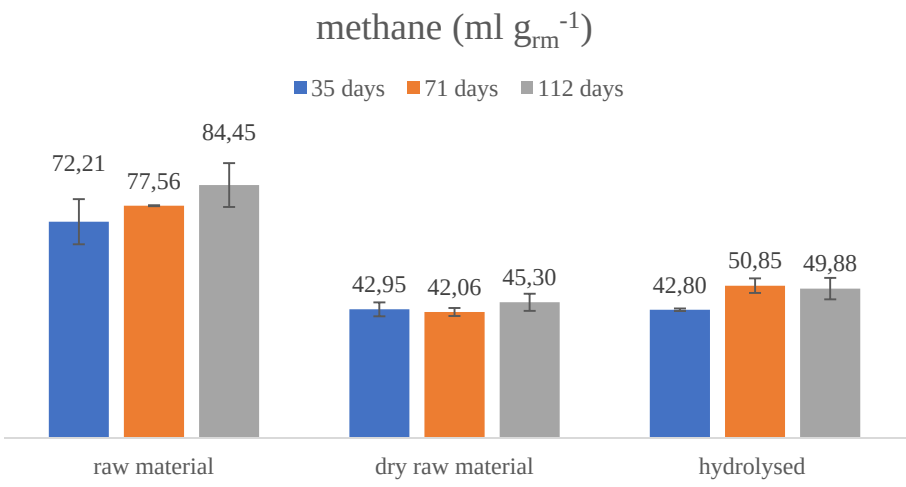






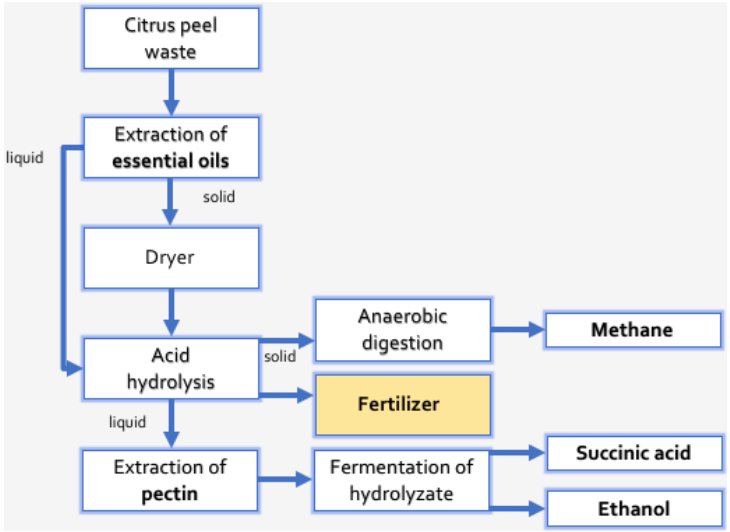
Raw material	CH_4 (ml g_{VS}^{-1})	Reference
Citrus sinensis (dried peels)	400	Gunaseelan et al., 2004
Citrus waste (steam distilled)	125	Martin et al., 2010
Orange peel hydrolysate and solid residue	363	Pourbafrani et al., 2010
Citrus waste (steam explosion)	500	Forgcs et al., 2011
Citrus waste	50	Forgcs et al., 2011
Orange peel	267	Sanjaya et al., 2016
Mandores	**342**	**Current study**
Mandores (hydrolysed)	**349**	**Current study**





Fertilizer

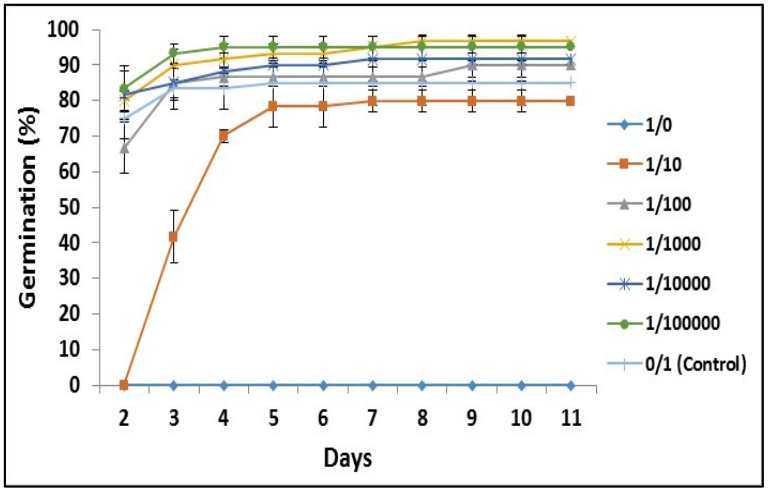
- Applied as substrate for lettuce

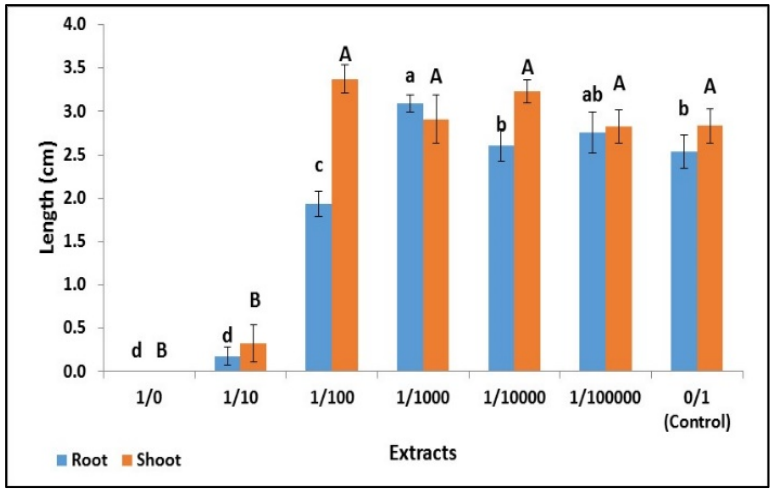


	0%	1%	2.5%	5%	10%
pH	5.59 ± 0.0318a[Y]	5.10 ± 0.0289b	4.85 ± 0.0289c	4.03 ± 0.0231d	3.31 ± 0.0203e
EC (μS cm^{-1})	1215 ± 7.10e	1374.1 ± 8.03d	2085.9 ± 12.15c	2778.9 ±16.19b	3970.9 ± 23.15a
Organic matter %	97.87 ± 0.372ab	98.57 ± 0.248a	98.18 ± 0.003ab	97.84 ± 0.003b	97.99 ± 0.121ab
Organic C%	56.77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

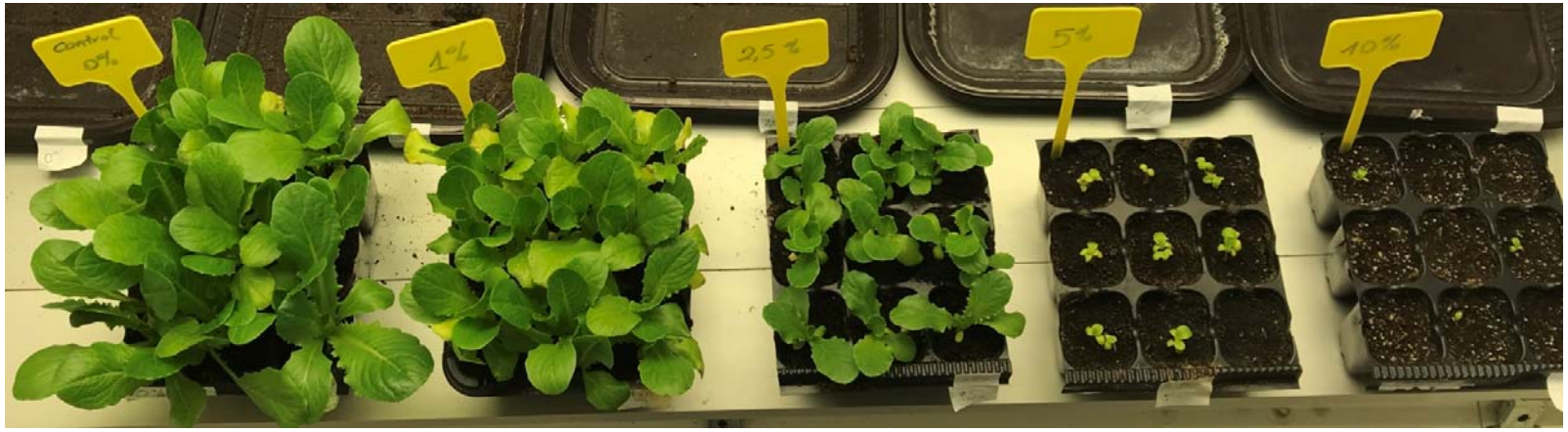
Fertilizer

In vitro





In vivo



BioRefinery

Succinic acid

- 14.43 g L^{-1}, 24 h hydrolysis and fermentation
- Simultaneous saccharifications and supplementation of vitamins and corn steep liquor
- Fed-Batch fermentations

Ethanol

- Optimal Conditions: 116 ^{o}C, 10 min
- *Pichia kudriavzevii* KVMP10, 30.70 g L^{-1} with the application of stillage recycling

Methane

- Dry CPW
- Adaptation of sludge

Fertilizer

- Stimulant plant growth
- pH adjustment

Technoeconomical analysis

Thank you ☺