Cyprus University of Technology

Department of Chemical Engineering

Environmental Bioprocessing Laboratory

4Wealth

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RESEARCH & INNOVATION FOUNDATION

Valorisation of Citrus Processing Waste for Bacterial Cellulose Production through an Integrated Biorefinery Approach

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- Worldwide Citrus Fruits Production: 143 x 10^6 t per year
- Industrial generation of Citrus Peel Waste (CPW): 24 x 10^6 t
- 50 % of the total mass is considered as CPW
  - Peel
  - Membranes
  - Seeds
  - Pulp
- 1 – 17 m^3 Citrus Processing Wastewater (CPWW)/ton proceed fruit
  - Factory cleaning
  - Juice concentration
  - Essential oils extraction

CPW Composition

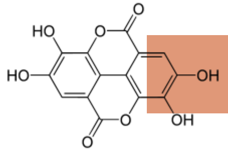
Component	Percentage (% dry basis)
Moisture (% wet basis)	78 – 80
Ash	1.7 – 4.2
Free Sugars	15.0 – 47.8
Protein	1.8 – 9.1
Pectin	14.1 – 42.5
Cellulose	8.1 – 37.1
Hemicellulose	5.7 – 11.1
Lignin	0.6 – 7.2
Essential Oils	0.5 – 4.0
Polyphenols	0.6 – 7.3

**Tsouko et al.,* Renew. Energy, 2020, 160: 944 - 954

CPW Valorisation

Traditional Management Practises:
- Landfilling
- Animal Feed

Essential Oils



Polyphenols

Pectin

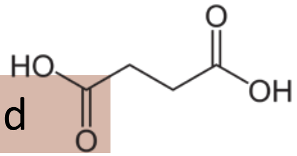
Carotenoids

Extractive Products

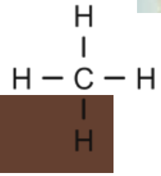
Valorisation Products

Ethanol

Succinic acid



Bacterial Cellulose



Methane

Bacterial Cellulose

- Biopolymer of significant industrial importance
- Produced via fermentation of small-chain sugars via acetic acid bacteria of *Acetobacteraceae*

- Commercial Applications:
  - ✓ Biomedical Industry
  - ✓ Electronics Industry
  - ✓ Food Industry

- Unique properties:
  - ✓ High Purity
  - ✓ High degree of polymerization
  - ✓ High crystallinity
  - ✓ Biodegradability
  - ✓ Biocompatibility
  - ✓ Enhanced mechanical strength
  - ✓ Large water-holding capacity

Drawbacks:

high production cost
low productivity
expensive culture media

Aim and Objectives

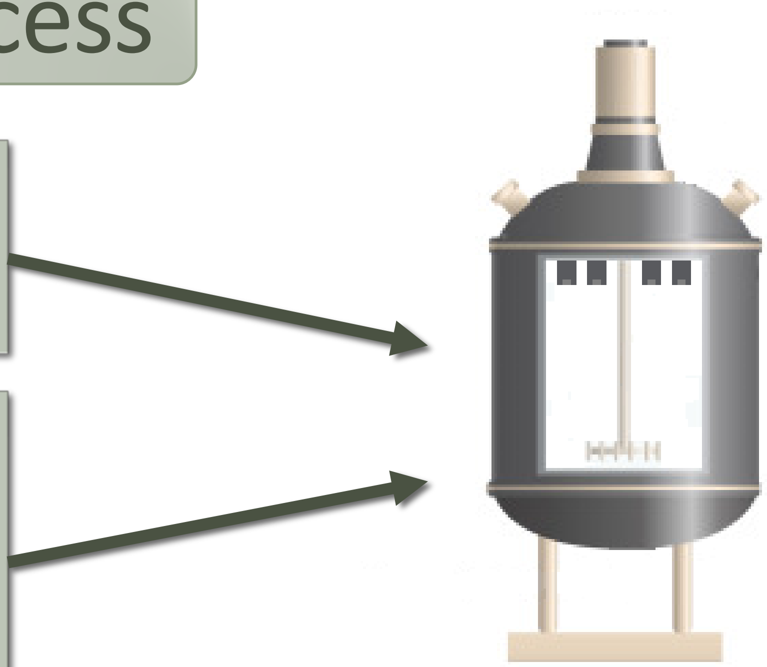
Pre-treatment of OPW using ultrasound-assisted acid hydrolysis for the production of bacterial cellulose

- Optimization of the sono-assisted acid-hydrolysis achieving:
  - High essential oils yield
  - High pectin yield
  - High sugar yield
- Application of the optimal conditions on pilot-scale system

OPW Pre-treatment Process

- Dilute acid-hydrolysis
  - ✓ Hydrolysis of hemicellulose
  - ✓ Pectin Recovery

- Incorporation of ultrasound technology
  - ✓ Disruption of pores
  - ✓ Destruction of hemicellulose structure
  - ✓ Hydrolysis of hemicellulose
  - ✓ Pectin Recovery



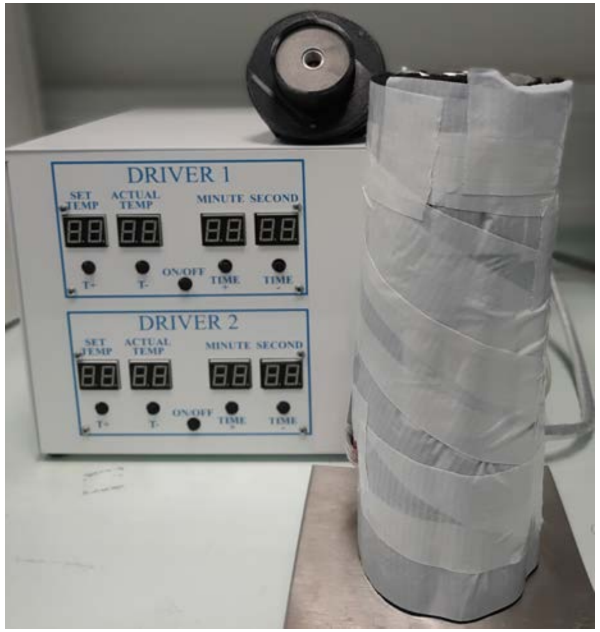
- **Lab – scale system**
- Single-transducer system
- Power: 60 W, Frequency: 40 kHz

Constant temperature: 100 °C

- Response surface methodology (RSM) and Central Composite Design (CCD)
- Quadratic Model
- 20 experiments overall performed in duplicates

Variables	**Levels**		
	-1	0	1
Time (min)	15	30	45
Acid Concentration, v/v%	0.75	1.38	2.00
Solid loading, w/v%	2.50	5.25	8.00

Sonication Systems



- **Lab – scale system**
- Single-transducer system
- Power: 60 W, Frequency: 40 kHz

- **Pilot – scale system**
- Twelve-transducer system
- Power: 60 W, Frequency: 28 kHz

RSM Model

Response: Essential oils

✓ Quadratic model

p-value = 0.0001 - ***significant***

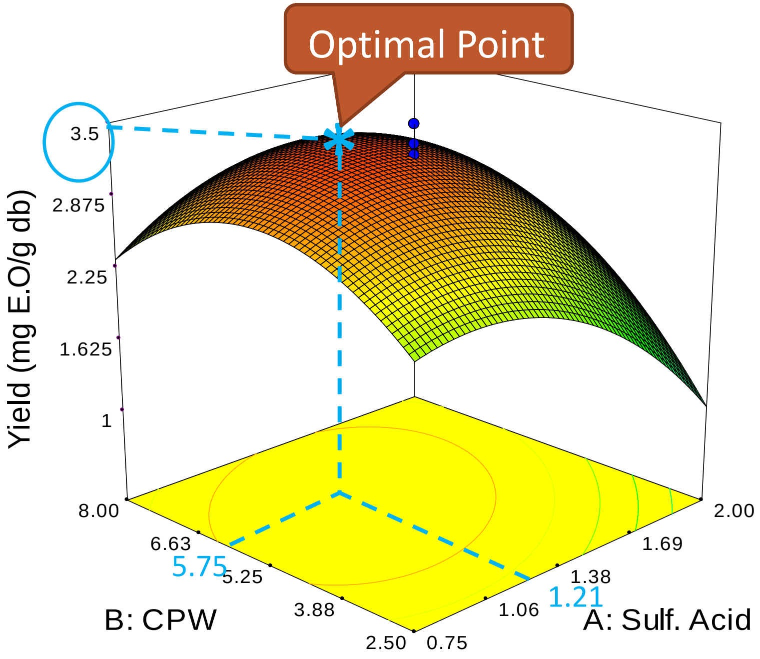
$$\textbf{Yield } (mg_{EO}\, g_{db}^{-1}) = +3.22 - 0.30\mathbf{A} + 0.35\mathbf{B} + \boxed{0.41\mathbf{C}} + 0.36\mathbf{AB} - 0.14\mathbf{AC} - 0.11\mathbf{BC} - 0.55\mathbf{A}^2 - 0.63\mathbf{B}^2 - 0.75\mathbf{C}^2$$

where A – sulfuric acid conc.; B - % solid loading; C – time

Highest effect on response

***Significant factors* (p-value < 0.05):** A, B, C, AB, A^2, B^2, C^2

- Interaction of time with acid conc. (AC) and solid loading (BC) are not significant (p-value > 0.05)


Optimal Point

RSM Model

Response: Pectin

✓ Quadratic model

p-value = 0.0001 - ***significant***

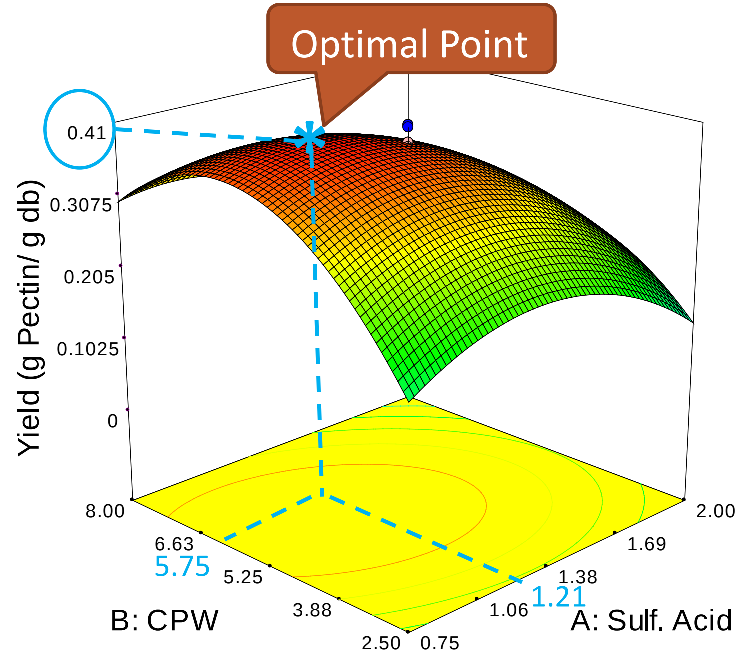
$$\textbf{Yield } (g_{pectin}\, g_{db}^{-1}) = +0.38 - \boxed{0.065\textbf{A}} + 0.021\textbf{B} + 0.060\textbf{C} - 0.044\textbf{AB} - 0.020\textbf{AC} - 0.013\textbf{BC} - 0.082\textbf{A}^2 - 0.13\textbf{B}^2 - 0.071\textbf{C}^2$$

where A – sulfuric acid conc.; B - % solid loading; C – time

Highest effect on response

***Significant factors* (p-value < 0.05):** A, C, A^2, B^2, C^2

- Solid loading is not considered as a significant factor for the response


Optimal Point

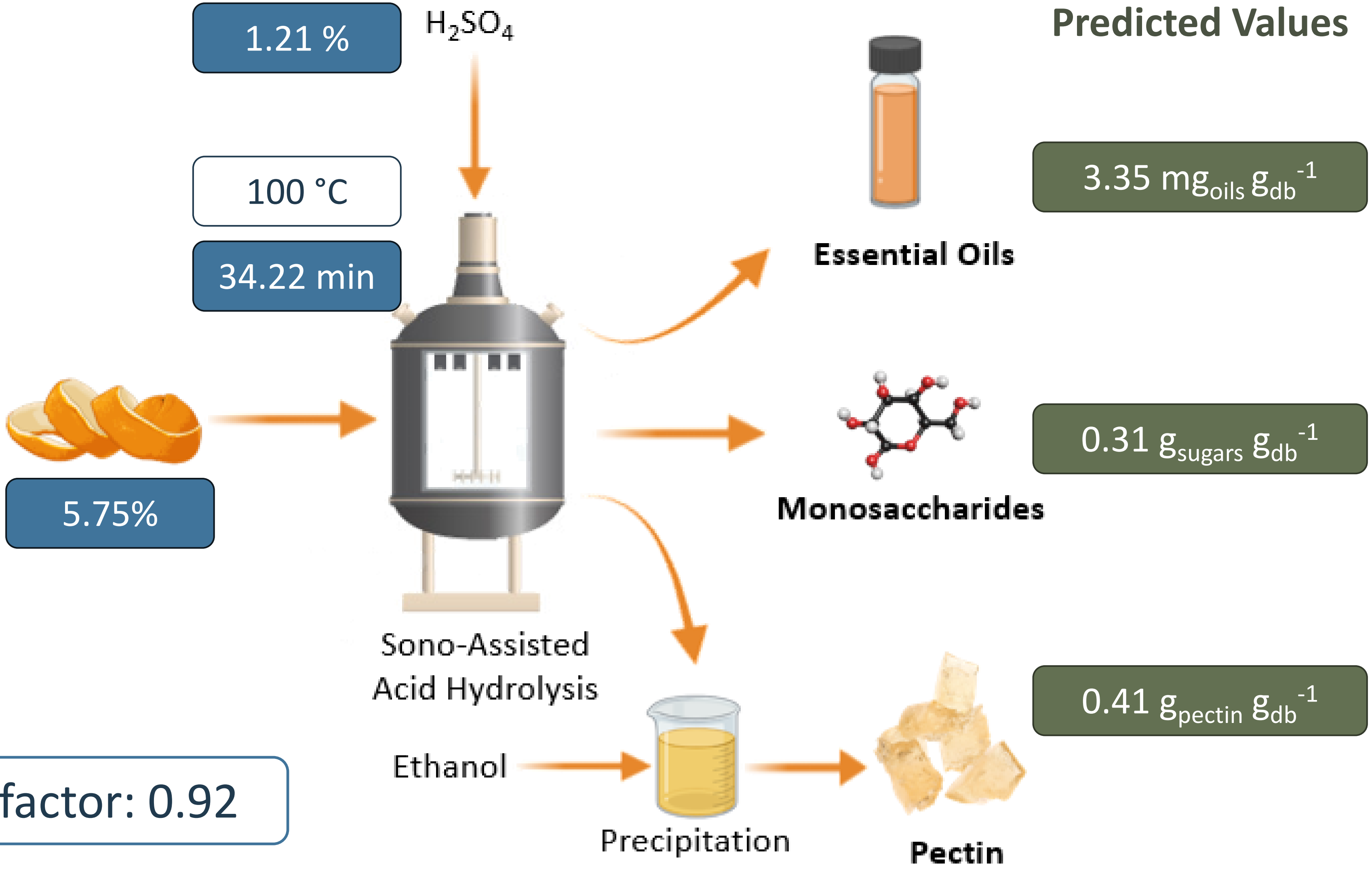
Response: Reducing sugars

✓ Linear model

$$\textbf{Yield } (g_{pectin}\, g_{db}^{-1}) = +\,0.31$$

<u>Shows ***no relationship*** between the studied parameters</u>

Optimal Conditions



1.21 % H_2SO_4

100 °C

34.22 min

5.75%

Sono-Assisted Acid Hydrolysis

Ethanol

Precipitation

Predicted Values

Essential Oils

3.35 $mg_{oils}\ g_{db}^{-1}$

Monosaccharides

0.31 $g_{sugars}\ g_{db}^{-1}$

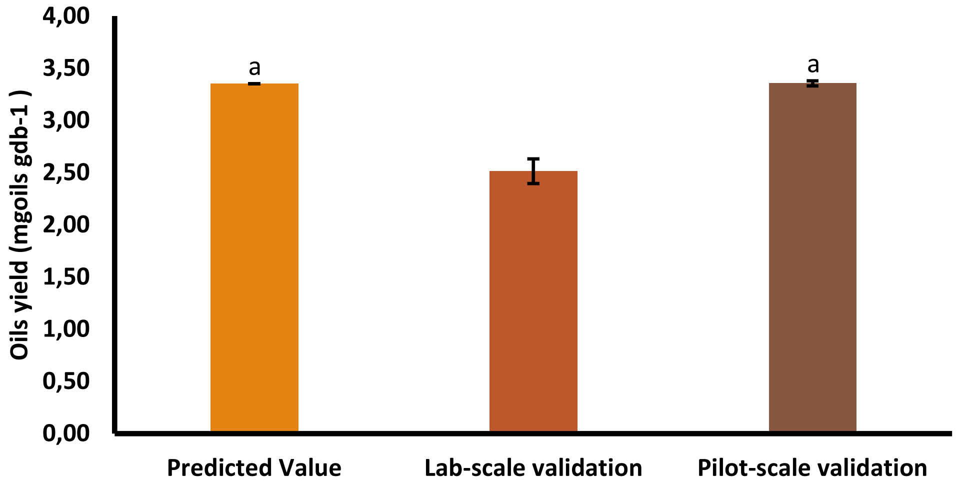
Pectin

0.41 $g_{pectin}\ g_{db}^{-1}$

Desirability factor: 0.92

Validation of the results

- *Essential oils*



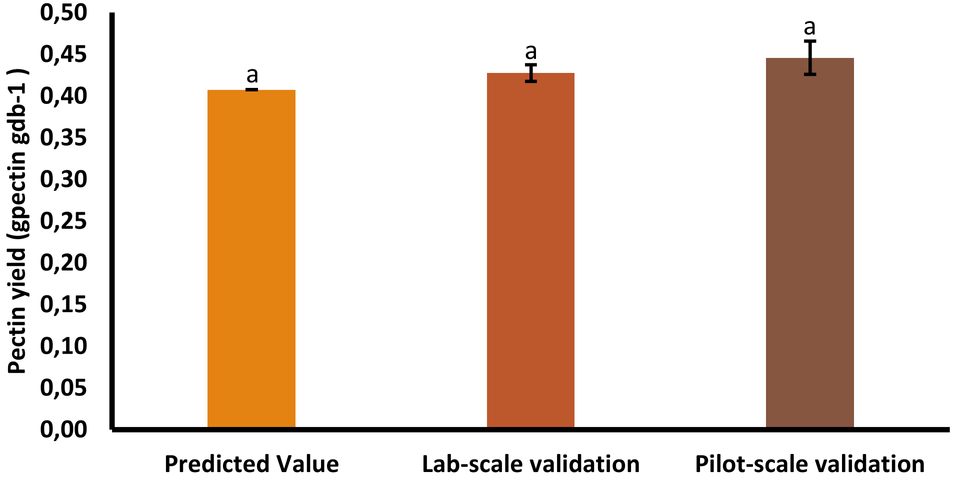
Final extraction: 0.12% (w/w) (wet basis)

❖ **Lower yield achieved on lab-scale system**

❖ **Predicted value achieved on pilot-scale system**

Technology	Yield (%)	Reference
Hydrodistillation	0.66	(Ortiz et al., 2021)
Microwave-assisted extraction	1.16	(Gonzalez-Rivera et al., 2016)
Ultrasound extraction using hexane	0.03	(Khandare et al., 2021)
Sono-assisted acid hydrolysis	0.12	Current study

- *Pectin*



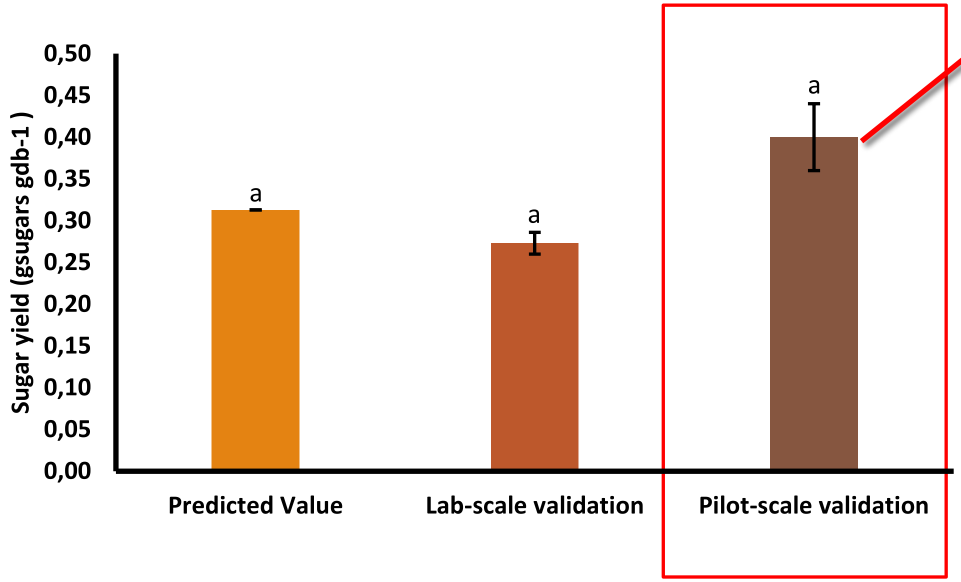
Final extraction: 45% (w/w)

❖ **Similar values achieved on lab-scale and pilot-scale systems**

Substrate	Technology	Conditions	Main results	Reference
Dry OPW	Microwave Hydrodiffusion and Gravity	1:30 solid/liquid ratio pH 2 (0.1 N HNO_3) 500 W, 3 min	24.2%	(Boukroufa et al., 2015)
Dry OPW	Conventional extraction	5% peels, 0.5% H_2SO_4 116 °C, 10 min	30.5%	(Kyriakou et al., 2020)
Dry OPW	Conventional extraction	1:50 solid/liquid ratio pH 2.0 (citric acid) 90 °C, 200 rpm, 160 min	32.6%	(Tsouko et al., 2020)
Dry grapefruit Peel	Ultrasound-assisted extraction	1:50 solid/liquid ratio pH 1.5 (0.1 N HCl) 70 °C, 25 min	17.92%	(Bagherian et al., 2011)
Sour Orange Peel	Ultrasound-assisted extraction	1:20 solid liquid ratio pH 1.5 (citric acid 150 W, 10 min	28.07%	(Hosseini et al., 2019)
Wet OPW	Sono-assisted acid hydrolysis	97 °C, 1.21% H_2SO_4 5.75% solid loading, 34.22 min 60 W, 28 kHz	45%	Current study

Highest Extraction yield!!!

- *Reducing sugars*



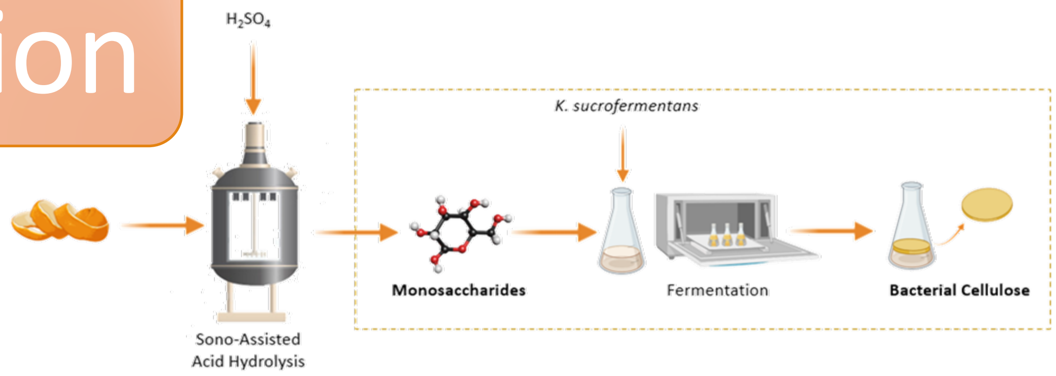
Higher than the predicted value!

Substrate	**Conditions**	**Main results**	**Reference**
Dry citrus peel	121 °C, 1% H_2SO_4 3.87% solid loading, 48.4 min	0.35 $g_{rs}\ g_{db}^{-1}$	(Mathias et al., 2019)
Dry orange peel	121 °C, 0.5% H_2SO_4 12% solid loading, 15 min	0.42 $g_{ts}\ g_{db}^{-1}$	(Oberoi et al., 2010)
Dry OPW	116 °C, 0.5% H_2SO_4 5% solid loading, 10 min	0.21 $g_{ts}\ g_{db}^{-1}$	(Patsalou et al., 2017)
Wet OPW	100 °C, 1.21% H_2SO_4 5.75% solid loading, 34.22 min 60 W, 28 kHz	0.40 $g_{rs}\ g_{db}^{-1}$	(Current study)

	Initial OPW	**After pre-treatment**
Cellulose (%)	21.3	2.6
Hemicellulose (%)	4.8	0.0
Lignin (%)	0.5	21.4

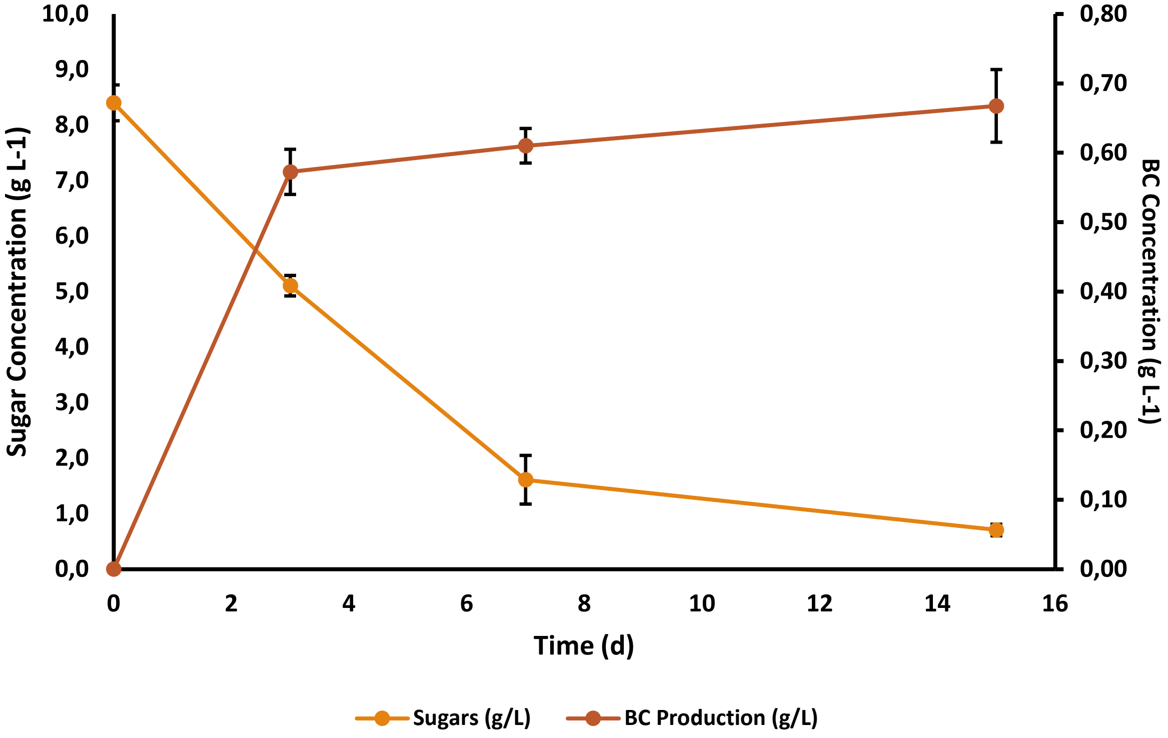
- ✓ 100% hydrolysis of hemicellulose
- ✓ 87.7% degradation of cellulose

Bacterial Cellulose Production



- *K.sucrofermentans* DSM 15973
- T = 30 °C, 150 rpm (for 2 d and then static)
- 15 d fermentation duration

- Initial [Sugars]: 8.4 (g L^{-1})
- Final [BC]: 0.67 (g L^{-1})
- Yield: 0.09 $g_{BC}\ g_{sugar}^{-1}$
- Productivity: 0.04 g L^{-1} d^{-1}



Pre-treatment	Conditions	Culture	Culture Conditions	BC Production	Reference
Dilute Acid Hydrolysis	121 °C, 30 min, 0.5% H_2SO_4, 1:10 solid to liquid ratio	*K. sucrofermentans* DSM 15973	30 °C, pH 6.0, 15 d 20 g L^{-1} initial sugar conc.	1.9 g L^{-1}	(Tsouko et al., 2020)
Dilute Acid Hydrolysis and Enzyme Hydrolysis	Acid Hydrolysis: 121 °C, 30 min, 0.5% H_2SO_4, 1:10 solid to liquid ratio Enzyme Hydrolysis: 50 °C, 150 rpm for 24 h, 0.1 mL Viscozyme $g_{cellulose}^{-1}$ 25 U b-glucosidase $g_{cellulose}^{-1}$ at 15 g L^{-1} total solids			4.4 g L^{-1}	
Acid Hydrolysis	100 °C, 2 h, 0.6 M H_2SO_4, liquid/solid ratio 10 mL g^{-1}	*K. hansenii* GA2016	28 -32 °C, under static conditions, 21 d fermentation 30 g L^{-1} initial sugar conc.	2.33 g $100g_{OPW}^{-1}$	(Güzel and Akpınar, 2019)
Enzyme Hydrolysis	1589.41 $U_{cellulases}\ g_{sludge}^{-1}$ 31.75 $U_{pectinase}\ g_{sludge}^{-1}$ 100 mM acetate buffer pH 4.75 50 °C, 500 rpm, 5.28 h (also performed without buffer)	*G. xylinus*	30 °C, static, 8 d 20 g L^{-1} initial sugar conc. 0.5% Yeast extract + 0.5% Peptone 20 g L^{-1} initial sugar conc.	4.11$\pm$0.15 g L^{-1} 0.83$\pm$0.23 g L^{-1} (without buffer)	(Kuo et al., 2019)
Enzyme Hydrolysis	50 °C, pH 5.0, 6 h 0.3 $g_{multiple\ enzymes}$ 100 mL^{-1}	*Komagataeibacter xylinus* CICC No. 10529	30 °C, static, 8 d 0.4% Yeast extract + 0.4% Peptone + 0.8% (v/v) ethanol 34 g L^{-1} initial sugar conc. Batch mode	5.7$\pm$0.7 g L^{-1}	(Fan et al., 2016)
Acid Hydrolysis	1:2 solid/liquid ratio 90 °C, 1 h, 1N H_2SO_4	*Komagataeibacter europaeus* SGP37	30 °C, static, 16 d Fed-batch fermentation 62.54 g L^{-1} initial sugar conc.	4.2 g L^{-1}	(Dubey et al., 2018)
Sono-assisted dilute acid hydrolysis	28 kHz, 500 W, 34.22 min, 100 °C, 1.21% H_2SO_4 and 5.75% w/v solid loading	*Komagataeibacter sucrofermentans* DSM 15973	30 °C, 150 rpm (for 2 d and then static), pH 6.0, 15 d 8.4 g L^{-1} initial sugar conc 0.5% Peptone + 0.5% Yeast extract, 0.27% Na_2HPO_4 + 0.115% citric acid	5.8 g $100g_{OPW}^{-1}$ **Highest yield!!**	Current Study

Processing Wastewater

Three streams:

- ✓ Heating/Cooling
- ✓ Juice vacuum concentration
- ✓ Essential Oils Extraction

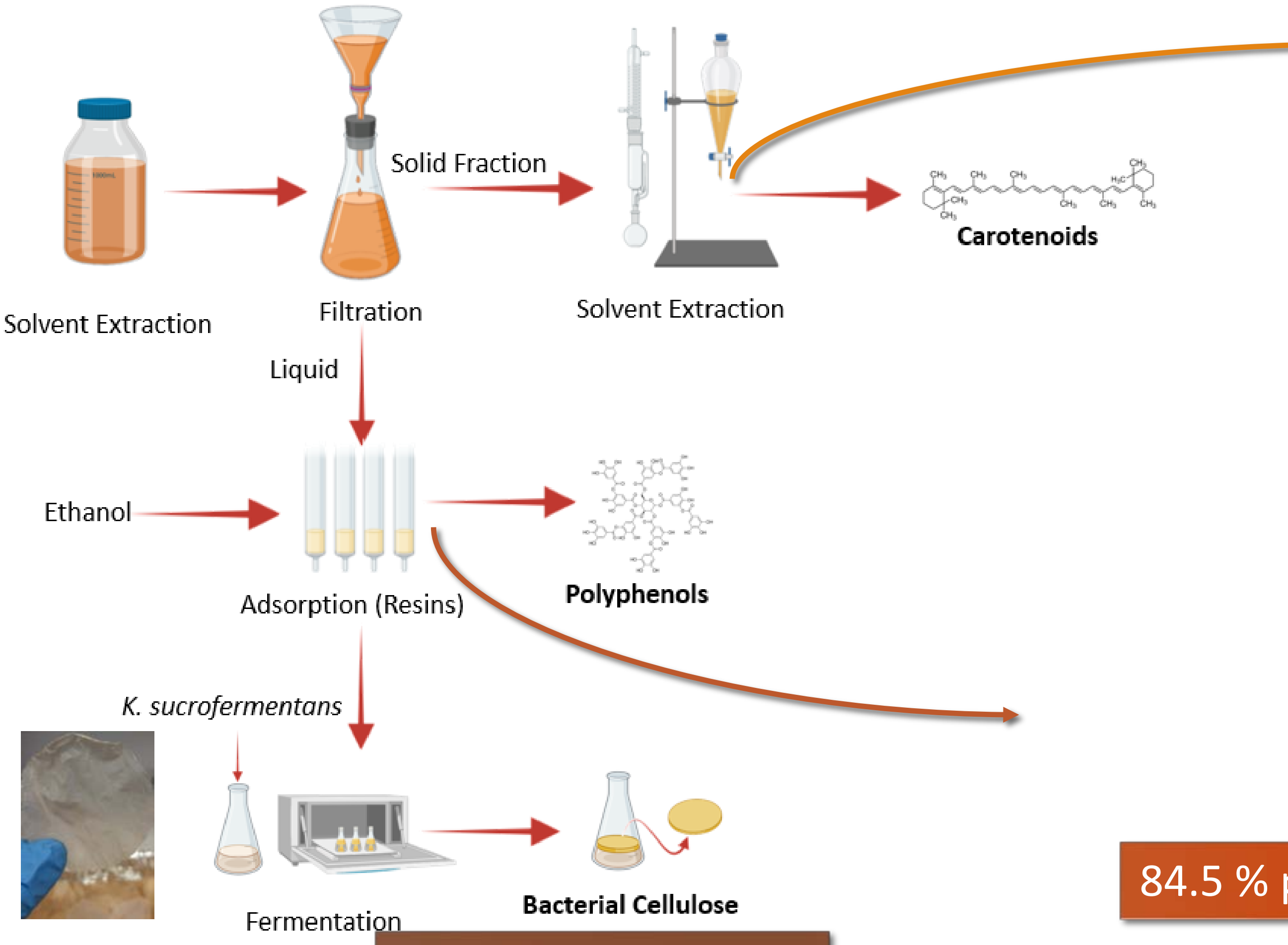
COD value < 0.6 g L^{-1}

Sugars, phenols and essential oils *were not detected*

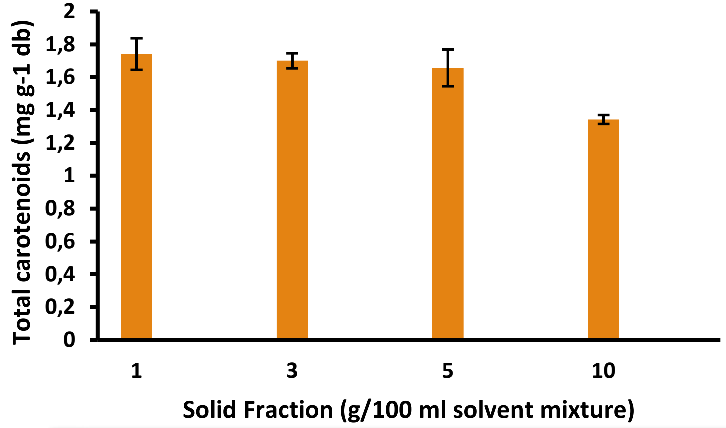
CPWW Composition

Component	**Values**
COD (g L^{-1})	104.6
Total sugars ($g_{sucrose}$ L^{-1})	73.6
Total Phosphorus (g L^{-1})	0.7
Amino Nitrogen ($mg_{glycine}$ L^{-1})	95.0
Total Phenols ($g_{GA\ eq.}$ L^{-1})	1.4
d-limonene (mg L^{-1})	196.4
Total Solids (%)	6.7
pH-value	3.9

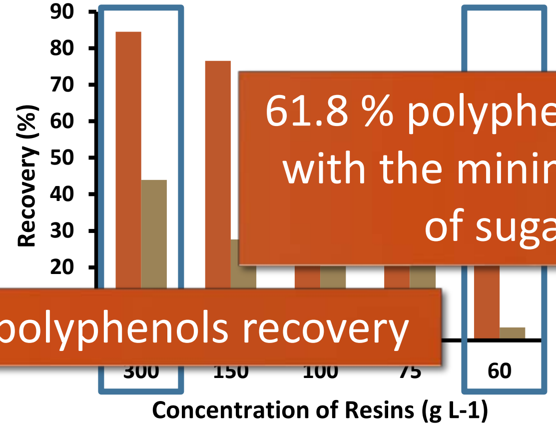
CPWW - Biorefinery



Production: 6.5 g L^{-1}



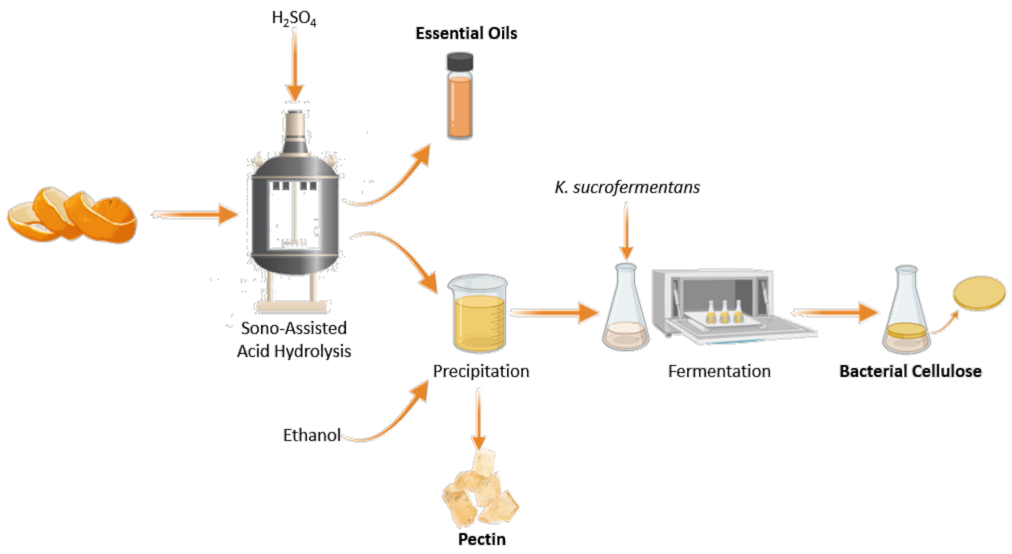
1.74 mg carotenoids per g db



61.8 % polyphenols recovery with the minimum amount of sugar loss

84.5 % polyphenols recovery

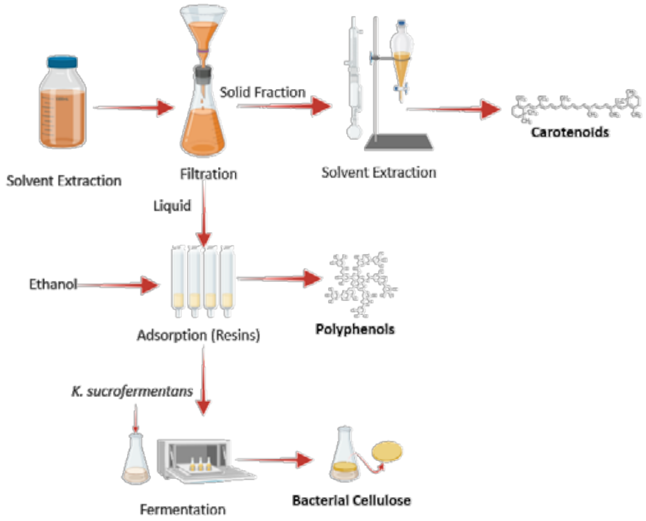
CPW Biorefinery



- ✓ Applicability of the model in pilot-scale system
- ✓ Recovery of the whole amount of pectin
- ✓ Recovery of high amount of sugars
- ✓ Recovery of essential oils through a short solventless process
- ✓ High BC Production yield

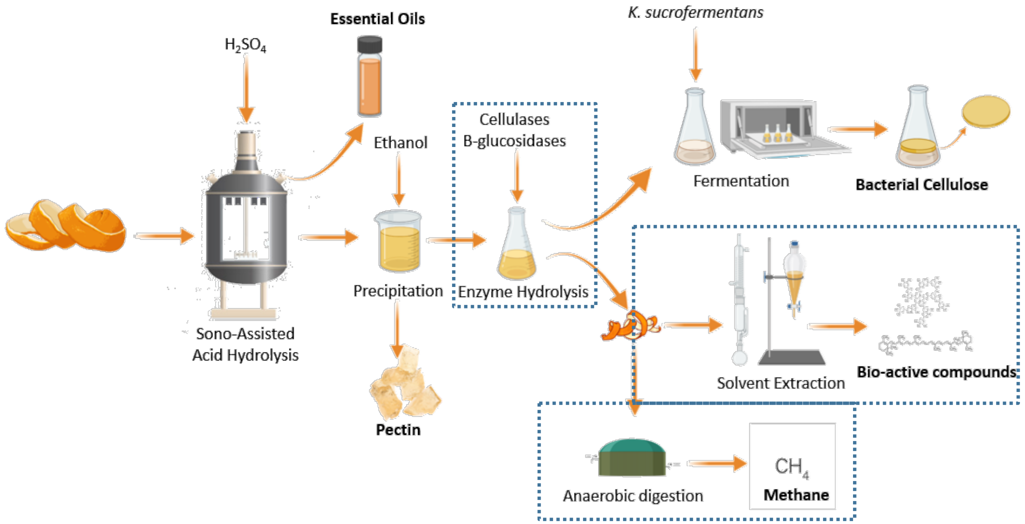
CPWW Biorefinery

- ✓ CPWW a valuable feedstock for bacterial cellulose production
- ✓ High composition of bioactive compounds



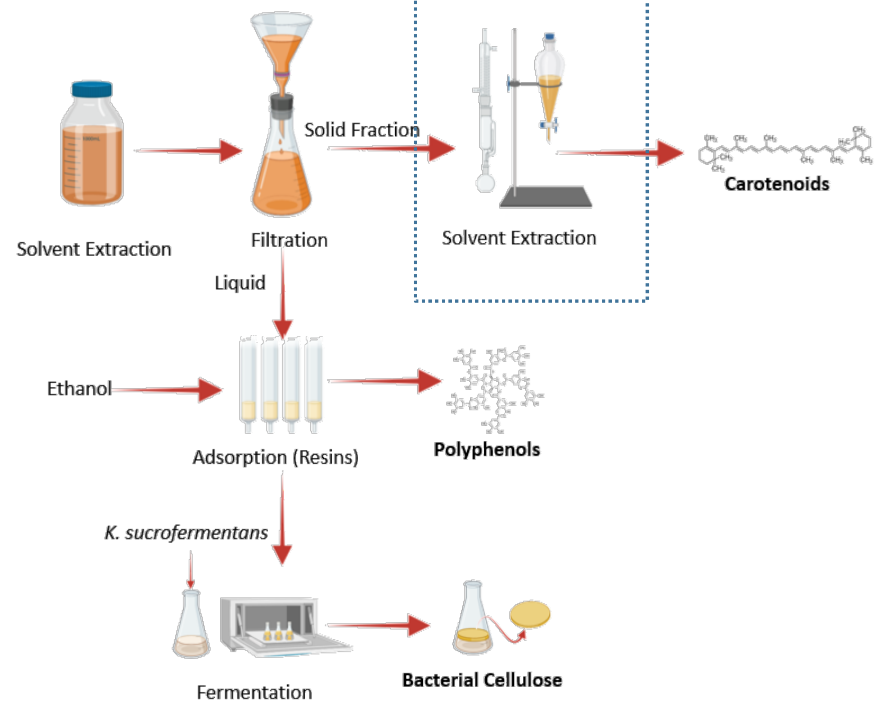
Future Work

Peel-based Biorefinery



- Enzyme Hydrolysis of cellulose
- Extraction of bioactive compounds
- Methane production using the biorefinery residues

Wastewater-based Biorefinery



- Examination of food-grade solvents for bioactive compounds extraction


thank you