Development of a citrus processing waste-based biorefinery for production of high-added value commodities

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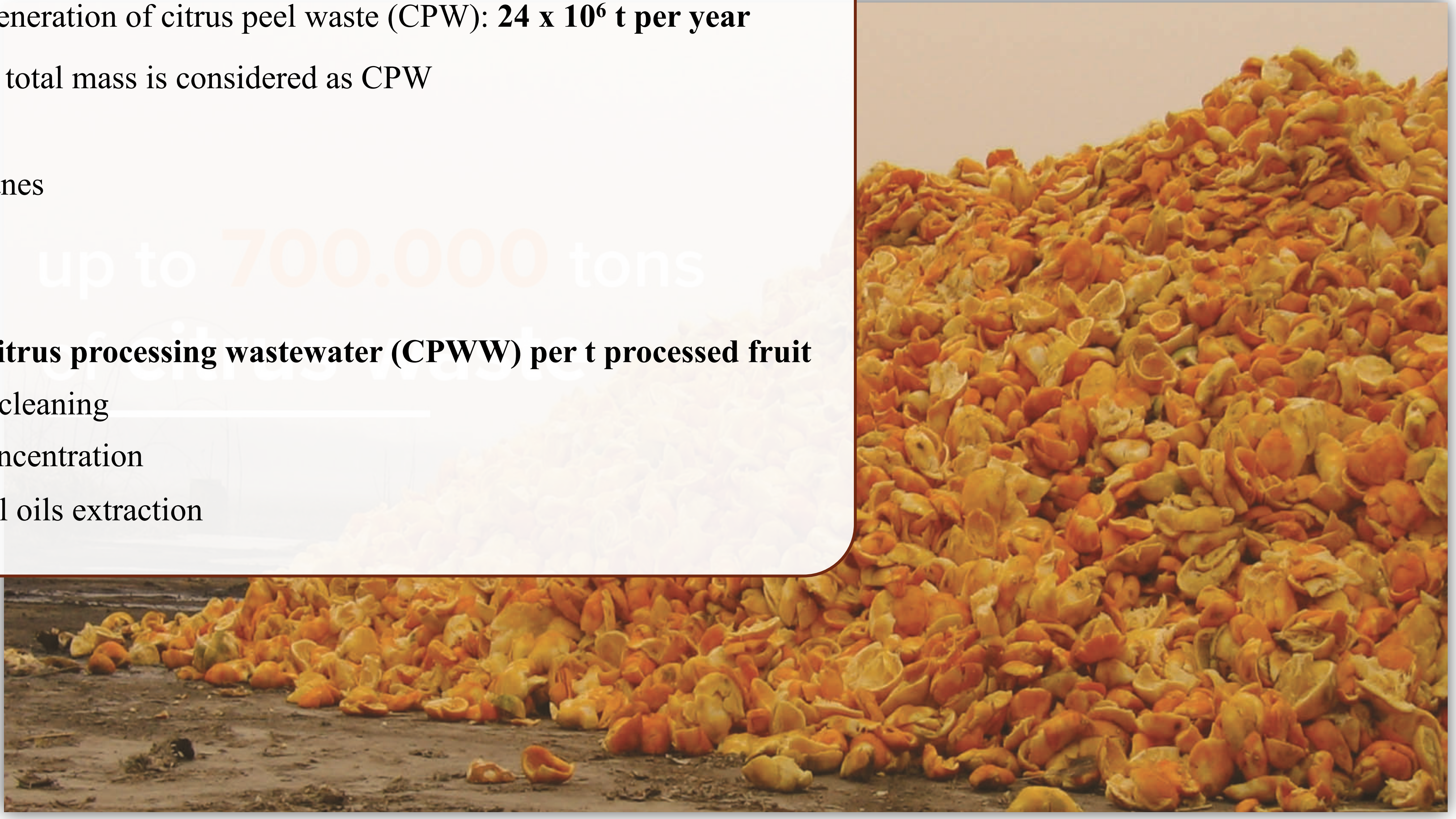
- Worldwide citrus fruits production: **143 x 10^6 t per year**
- Industrial generation of citrus peel waste (CPW): **24 x 10^6 t per year**
- 50 % of the total mass is considered as CPW
    - Peel
    - Membranes
    - Seeds
    - Pulp
- **1 – 17 m^3 citrus processing wastewater (CPWW) per t processed fruit**
    - Factory cleaning
    - Juice concentration
    - Essential oils extraction



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  - Factory cleaning
  - Juice concentration
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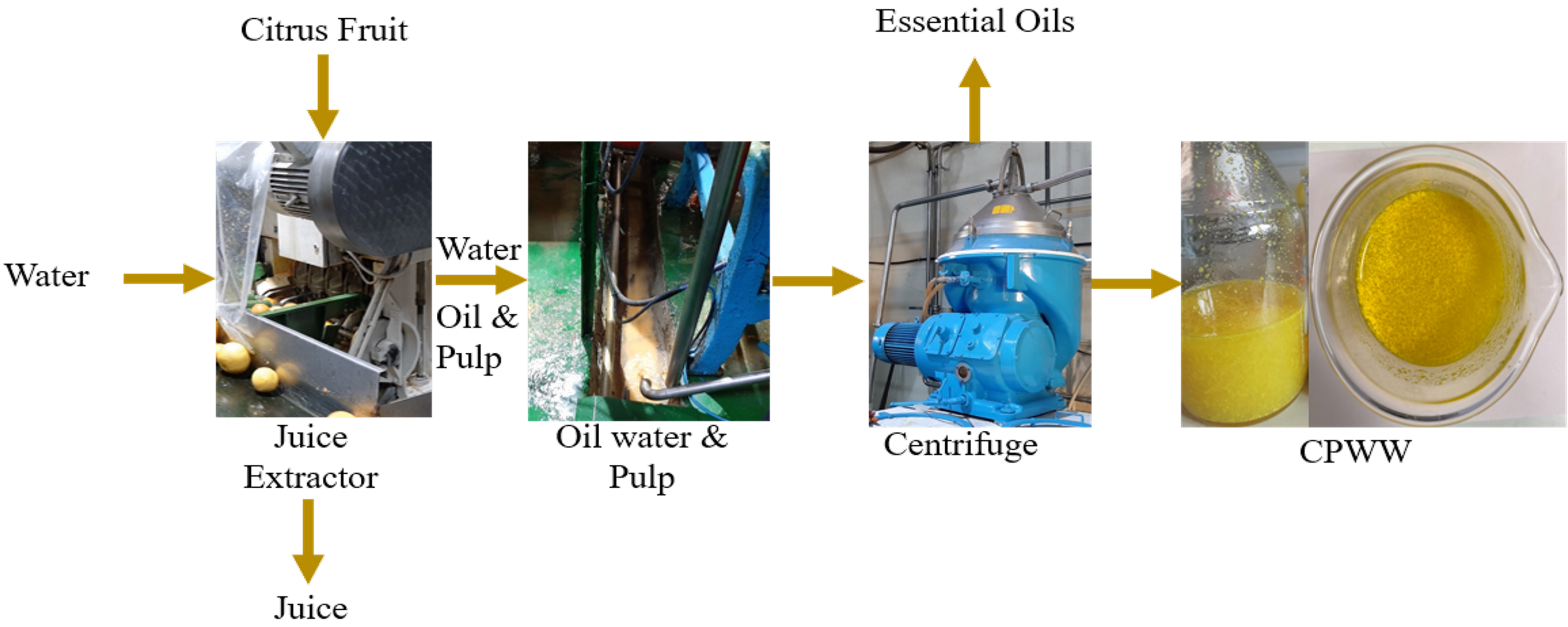
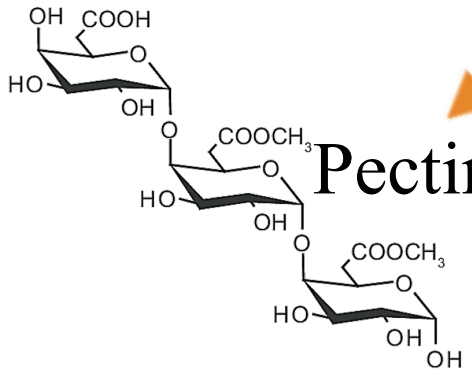


Previous CPWW Exploitation Studies



Pectin

Bio-hydrogen

Bio-methane

- All studies aimed to reduce the COD content

Essential Oils

- Antioxidants
- Preservative
- Flavoring
- Anti-microbial properties

- Antimicrobial agents
- Foods
- Medicine
- Green solvent
- Platform chemical

Polyphenols

Antioxidant Characteristics:
- Anticancer
- Antiviral
- Anti-inflammatory properties

- Antimicrobial agents
- Foods
- Medicine
- Cosmetics

Carotenoids

- Antioxidants
- Pro-vitamin A activity (β-carotene)

- Foods
- Medicine
- Cosmetics

Bacterial Cellulose

- High crystallinity
- Biodegradability
- High degree of polymerization
- High water-holding capacity
- Enhanced Mechanical strength
- High Purity

- Food Industry
- Electronics Industry
- Biomedical Industry

Bacterial Cellulose

Properties

- High crystallinity
- Biodegradability
- High degree of polymerization
- High water-holding capacity
- Enhanced Mechanical strength
- High Purity

Drawbacks

- COST — High production cost
- % — Low productivity
- Extensive cultivation time

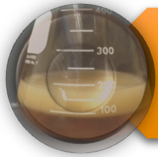
Aim and Objectives

Biorefinery development for manufacture of value-added extractable products and bacterial cellulose using citrus processing wastewater

Recovery of essential oils

Assessment of different organic solvents for carotenoids extraction

Assessment of different adsorption materials for polyphenols recovery



Production of bacterial cellulose

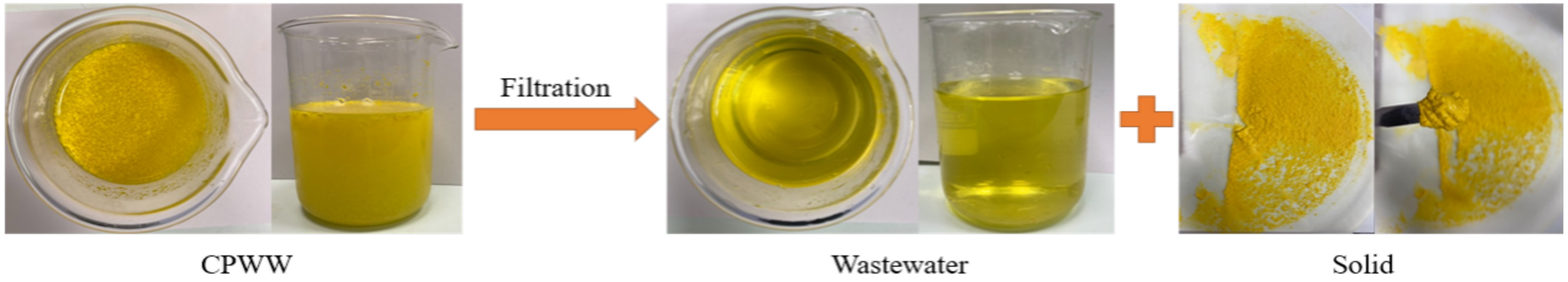
CPWW Composition

Three streams:

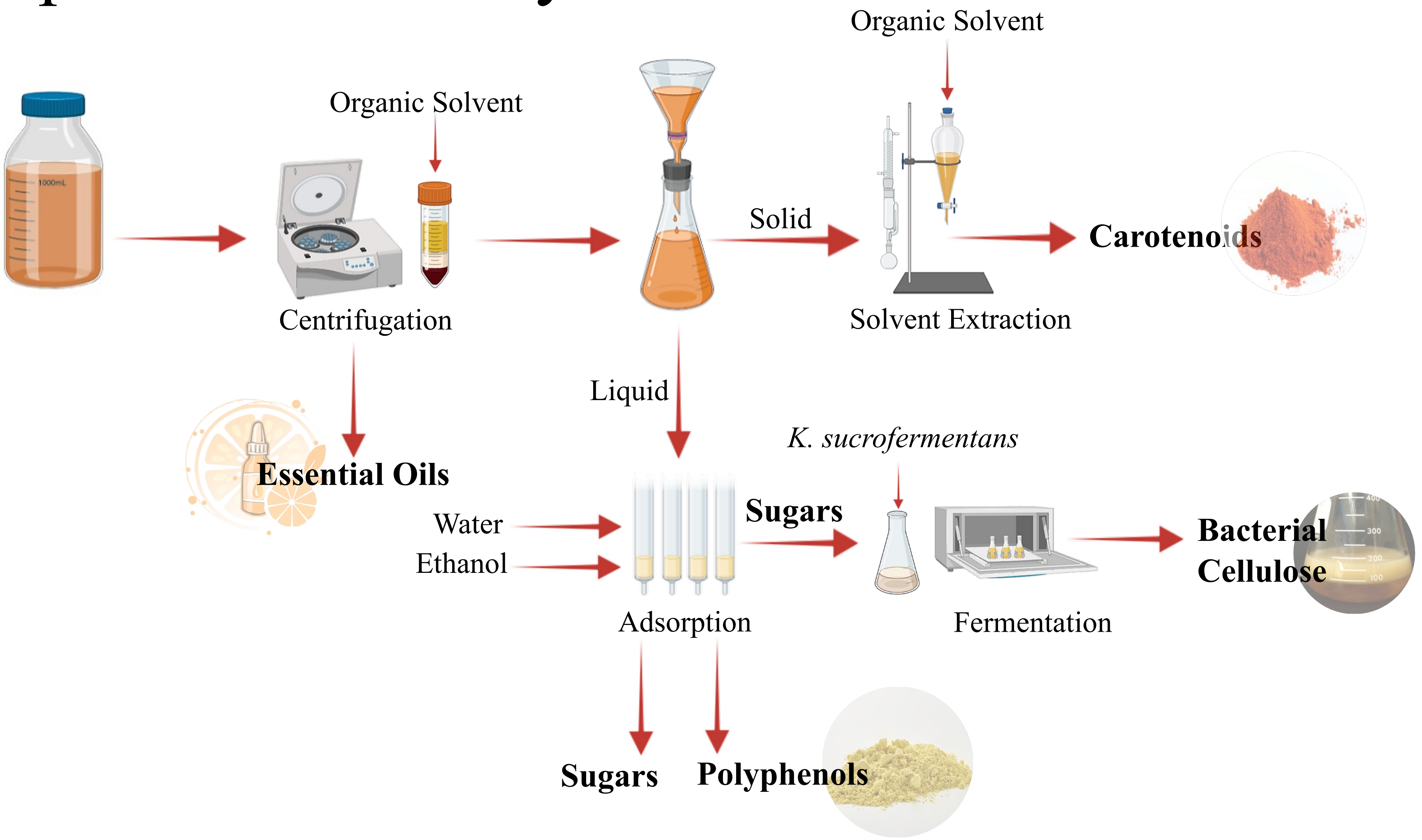
- Heating/Cooling
- Juice vacuum concentration
- Essential Oils Extraction

COD content < 0.6 g L^{-1}
Sugars and bioactive compounds ***were not detected***

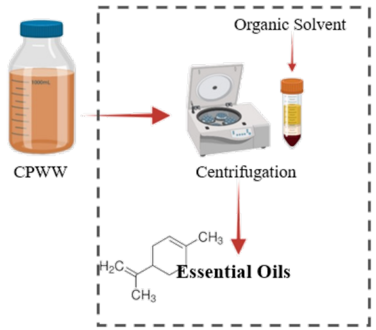
	Valencia, Mandora, Lemon, Grapefruit wastewater	**Current Study**
COD [g L^{-1}]	87.2 – 104.6	104.6
Reducing sugars [g L^{-1}]	33.0 – 92.7	48.0
Free Amino Nitrogen [$mg_{glycine}$ L^{-1}]	59.7 – 108.9	95.0
Total Phenolic Content [$g_{GA\ eq.}$ L^{-1}]	1.1 – 2.1	0.8
Essential Oils [mg L^{-1}]	196.4 – 637.8	450.1
Total Solids [%]	5.6 – 6.7	5.5
pH-value	3.6 – 4.1	3.9

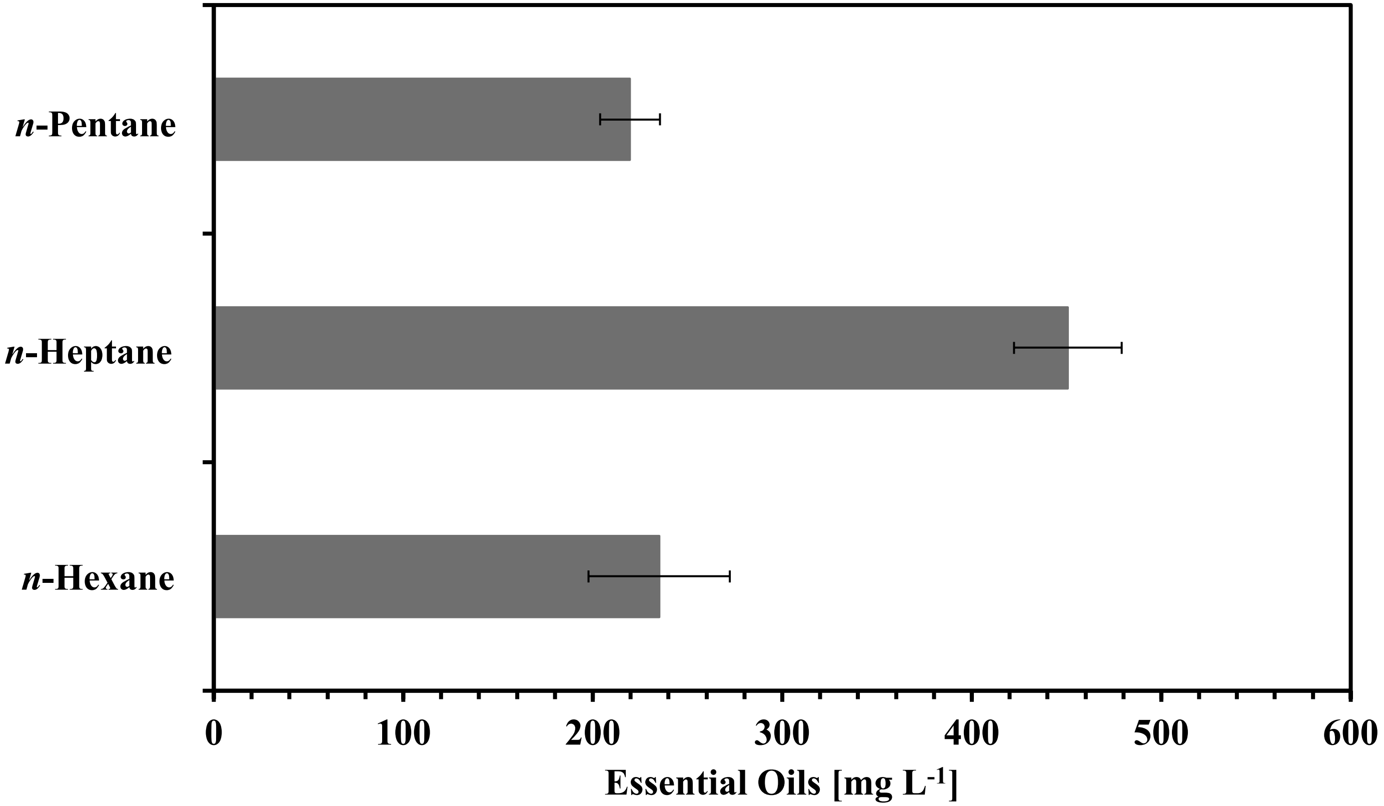


Proposed Biorefinery Process



Essential Oils Extraction

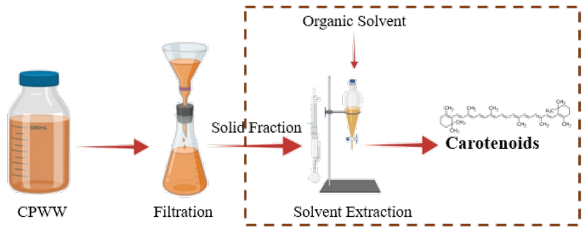


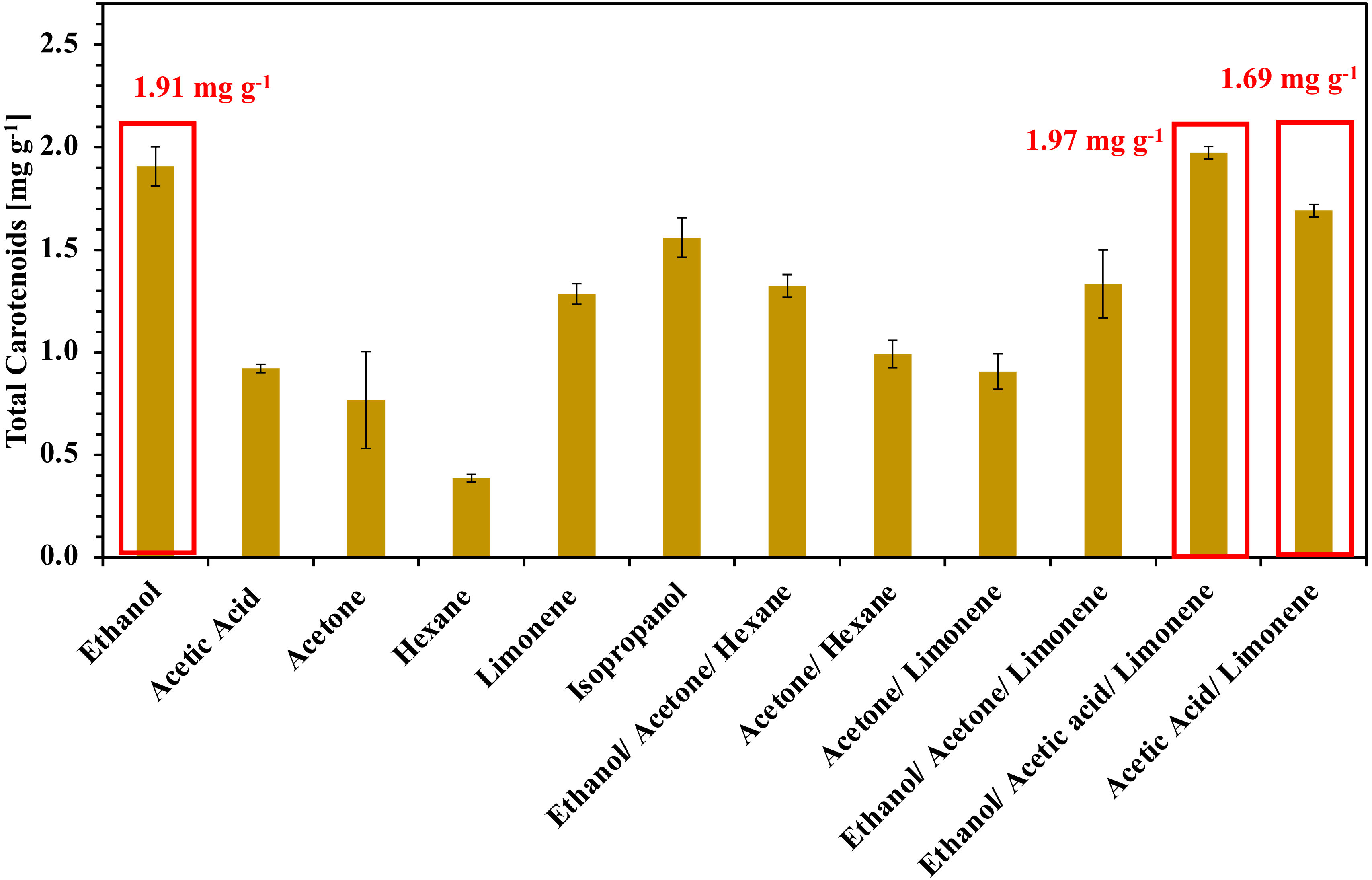


Experimental Conditions:

- 3500 rpm
- 25 °C
- 30 min
- 5:1 CPWW-to-solvent

Carotenoids Extraction



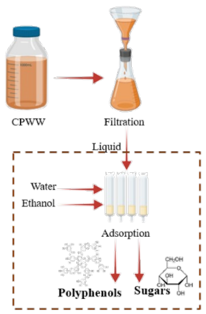


Experimental Conditions:

- 100 rpm
- 35 °C
- 35 min
- 2% solid loading

Feedstock	Extraction method	Solvent	Production	Ref.
Orange peel	Ultrasound-assisted extraction	50%, v/v Ethanol	0.63 mg β-carotene/100 g dry basis	Montero-Calderon et al., 2019
Kinnow mandarin peel	Ultrasound-assisted extraction	Ethanol	2.97 mg lutein/100 g dry basis	Saini et al., 2020
Mandarin peel	Solid-liquid extraction	Ethanol/Acetone/Hexane (25/25/50 v/v)	27 mg/100 mg dry basis	Barman et al., 2020
Mandarin peel	Supercritical fluid extraction	CO_2-acetone (7%)	0.39 mg β-carotene/100 g dry basis	Tsitsagi et al., 2018
Mandarin peel	High Voltage electric discharges	N/A	0.369 mg β-carotene/100 ml of extract	Buniowska et al., 2015
Orange Peel	Ultrasound-assisted extraction	Limonene	11.25 mg β-carotene/100 g dry basis	Boukroufa et al., 2017
CPWW solid	Solvent extraction	Ethanol	191 mg β-carotene/100 g dry basis	Current study
		Ethanol/Acetic acid/Limonene (25/25/50 v/v)	197 mg β-carotene/100 g dry basis	

Polyphenols Recovery

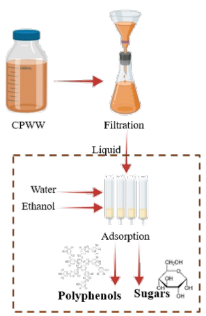


✓ Assessment of different adsorption materials and different concentrations

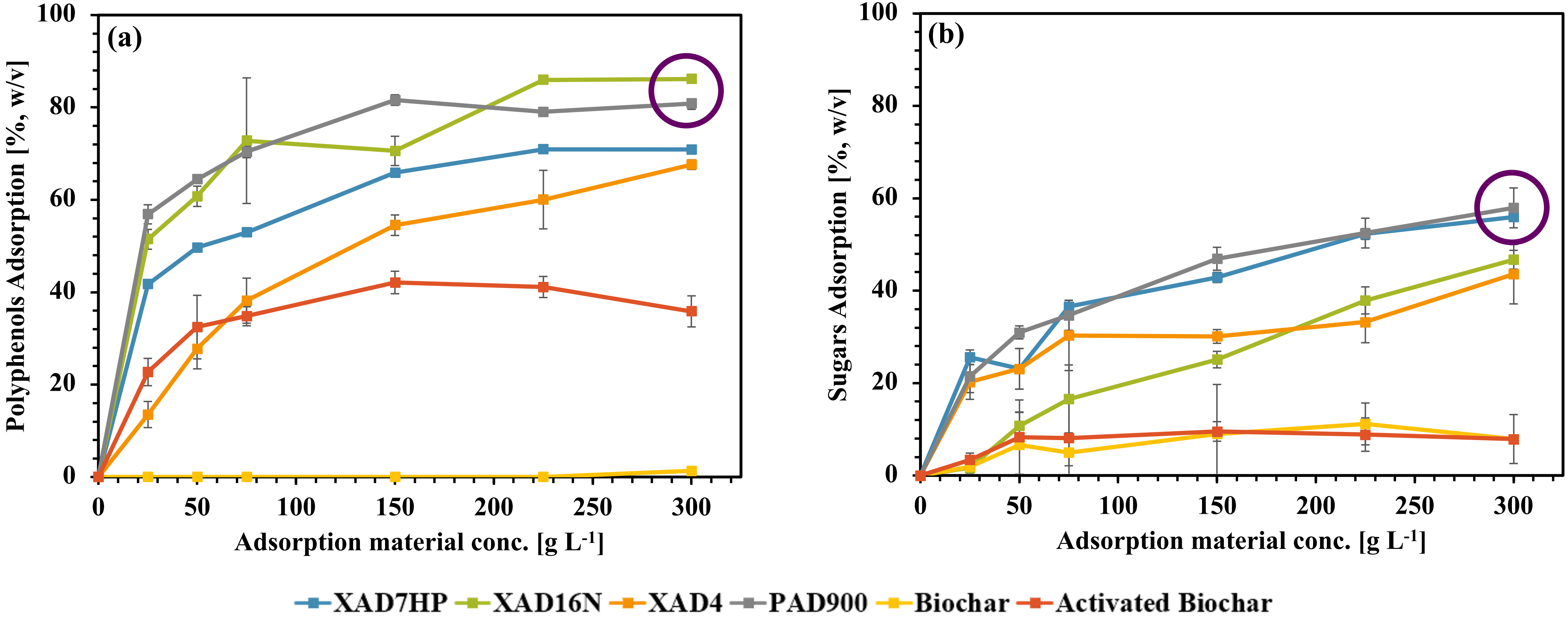
Adsorption material	**Polarity**	**Particle size diameter [mm]**	**Surface area [m^2 g^{-1}]**	**Pore size [nm]**
Amberlite XAD4	non-polar	0.56 - 0.71	750	10
Amberlite XAD16N	non-polar	0.56 - 0.71	800	20
Amberlite XAD7HP	moderate polar	0.56 - 0.71	380	30-40
PuroSorb PAD900	non-polar	0.35 – 1.20	850	15-30
Biochar*	non-polar	n.d.	n.d.	n.d.
Activated Biochar**	non-polar	n.d.	n.d.	n.d.

*Pistachio shells pyrolyzed at 500 °C for 3 min (Kyriakou et al., 2020)
**Pistachio Biochar Activation performed using 1 M KOH for 24 h at 25 °C

Polyphenols Adsorption



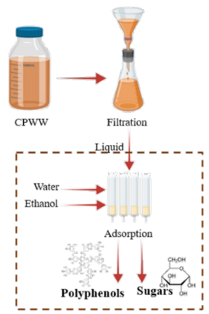
Batch Experiments, 25 °C, 100 rpm, 1 h, conical flasks 50 ml CPWW



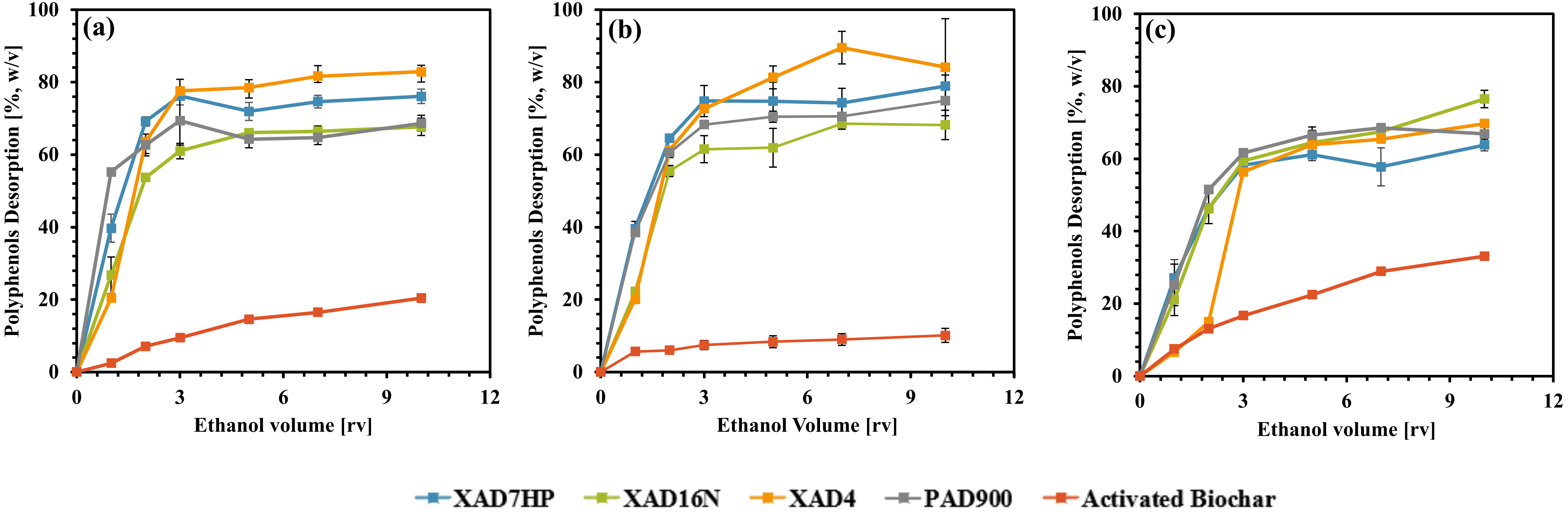
(a) Polyphenols (b) Sugars

Polyphenols Desorption

Desorption Experiments: 10 g of each adsorption material in columns

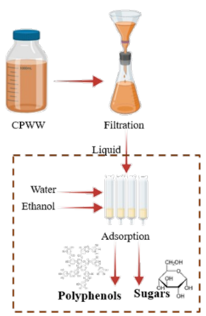


1 resin volume [rv] = 10 ml



(a) 5 rv h^{-1} (b) 10 rv h^{-1} (c) 15 rv h^{-1}

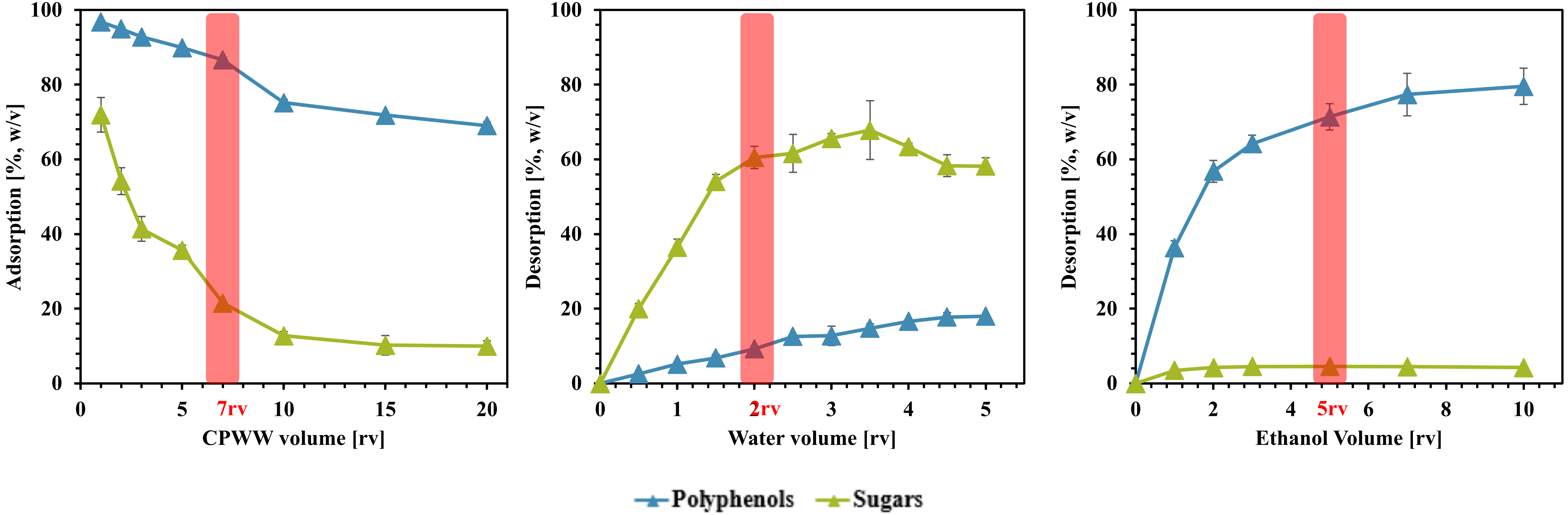
Polyphenols Desorption



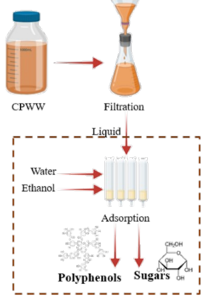
Desorption Experiments: 10 g of each adsorption material in columns

Chosen resin: Pursorb PAD900

1 resin volume [rv] = 10 ml



Polyphenols Desorption



Desorption Experiments: 10 g of each adsorption material in columns

1 resin volume [rv] = 10 ml

100% Polyphenols
100% Sugars

7 rv CPWW

10 rv h^{-1}

82.9% Polyphenols
27.5% Sugars

1 rv

17.1% Polyphenols
72.5% Sugars

2 rv H_2O

10 rv h^{-1}

73.6% Polyphenols
13.6% Sugars

1 rv

9.3% Polyphenols
13.8% Sugars

5 rv Ethanol

10 rv h^{-1}

16.7% Polyphenols
13.0% Sugars

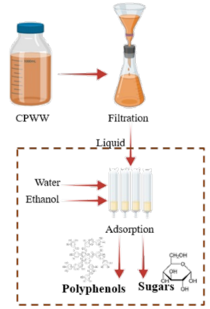
1 rv

56.9% Polyphenols
0.62% Sugars

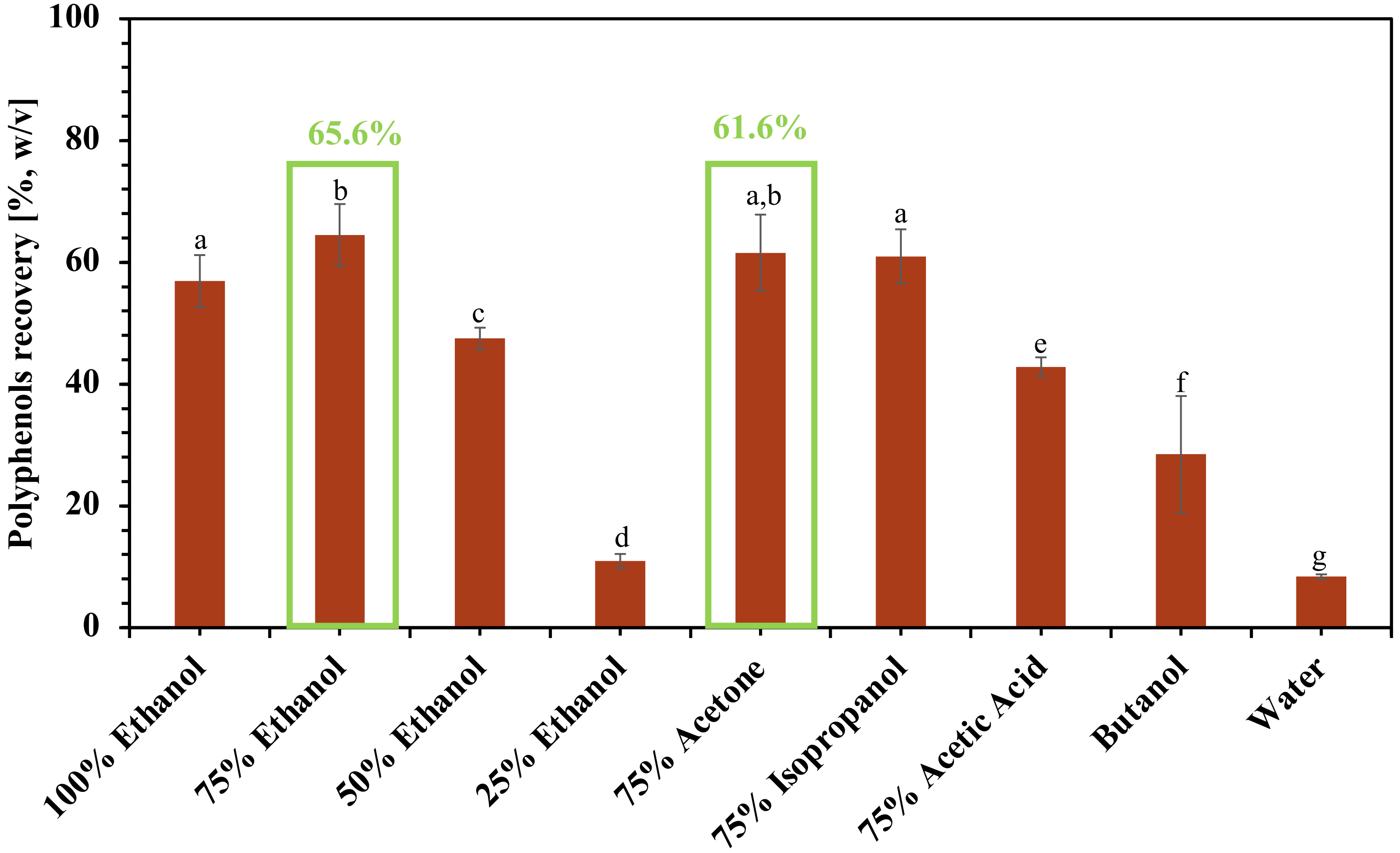
~80% Desorption!

Polyphenols Desorption

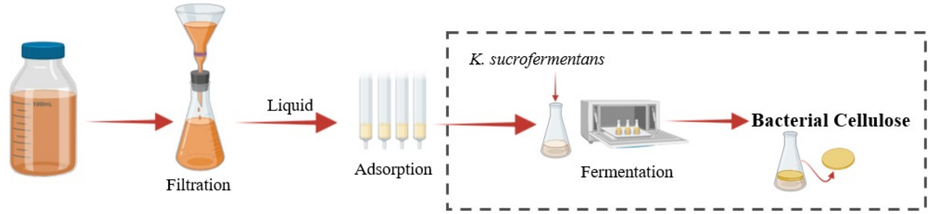
Desorption Experiments: 10 g of each adsorption material in columns



1 resin volume [rv] = 10 ml



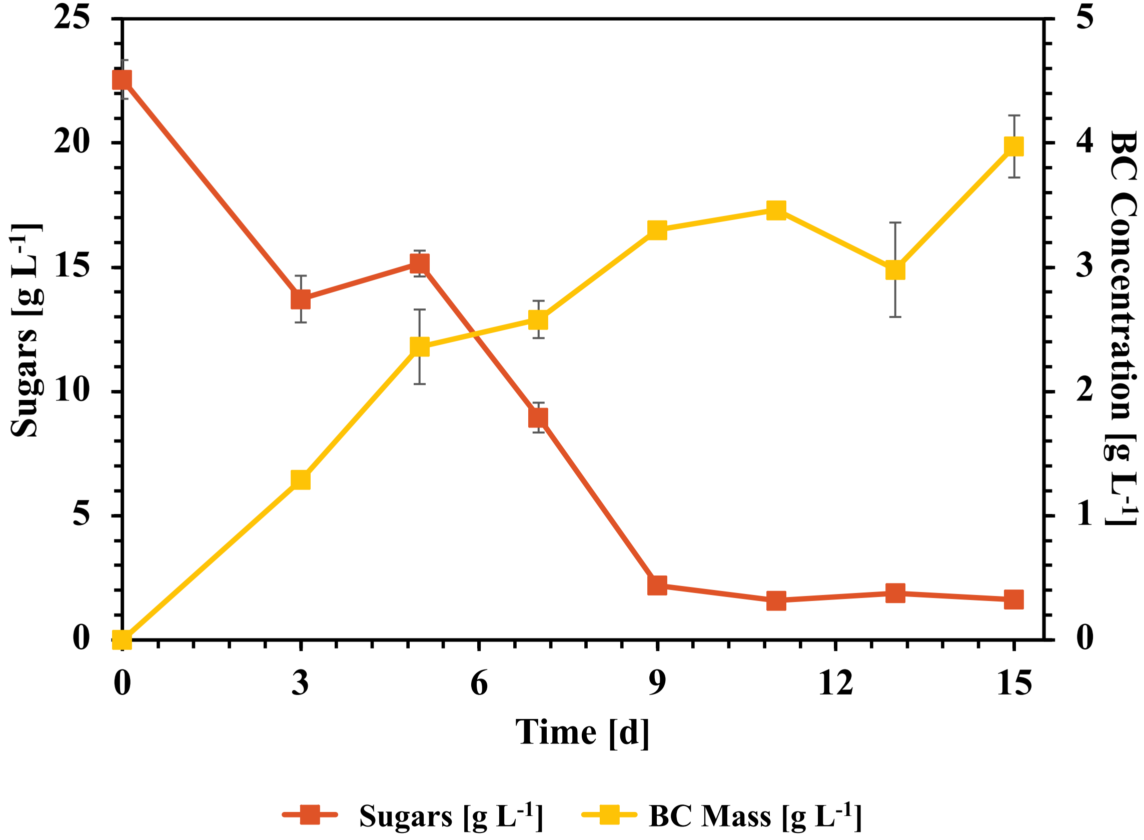
Bacterial Cellulose Production



Experimental Conditions:

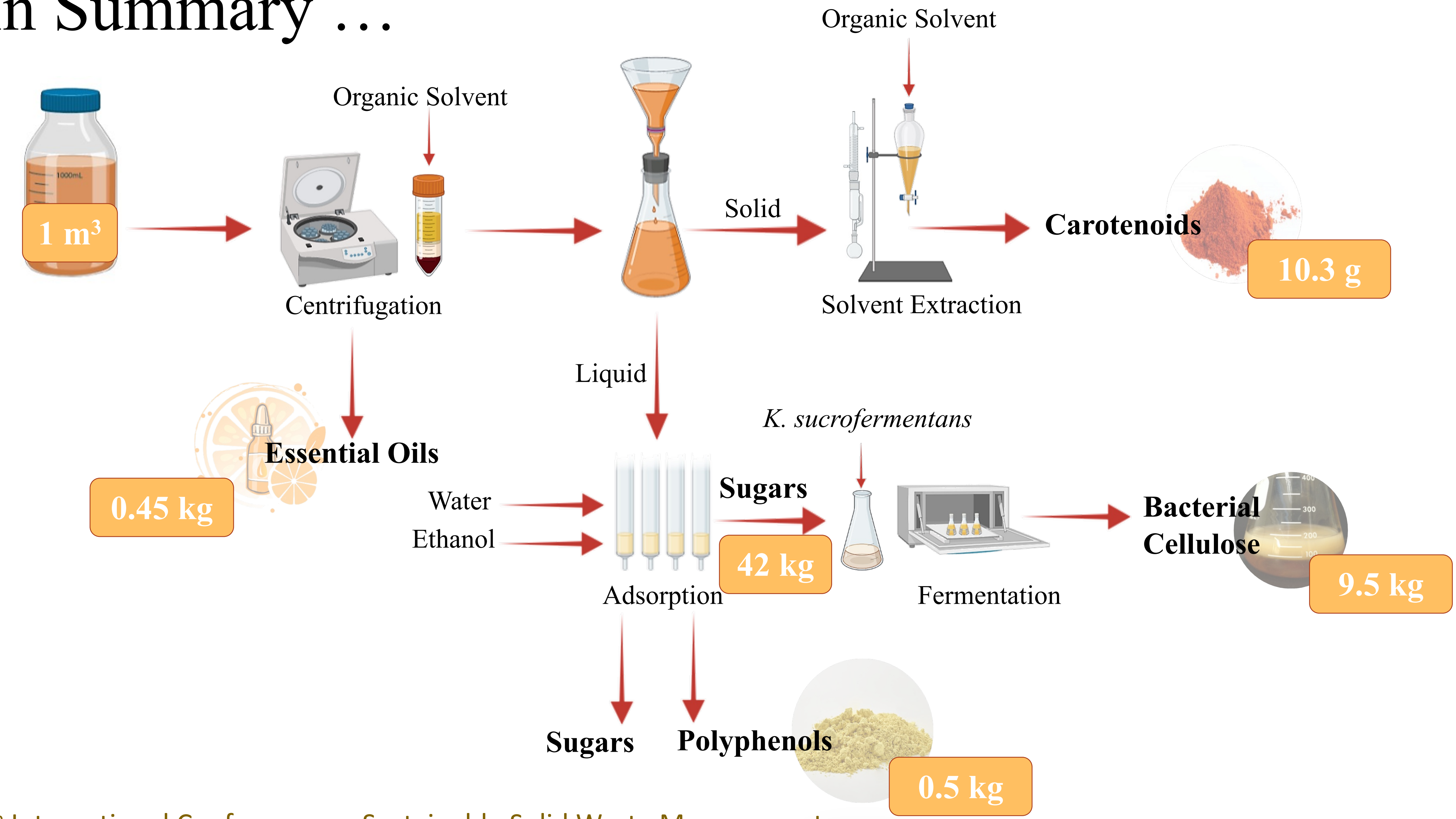
- *K. sucrofermentans* DSM 15973
- T = 30 °C, 150 rpm (for 2 d then static)
- 15 d fermentation duration
- **Initial sugars conc.: 22.5 g L^{-1}**

✓**Final BC conc.: 3.9 g L^{-1}**
✓**Yield: 0.19 $g_{BC}\ g_{sugar}^{-1}$**
✓**Productivity: 0.39 g L^{-1} d^{-1}**



Industrial Waste	Additional Nutrient	Culture	BC Conc. [g L^{-1}]	BC Yield [g_{BC} g^{-1}]	Ref.
Citrus peel fluid	Acetate buffer	*G. xylinus*	2.3	0.12	Kuo et al., 2019
Sugar beet molasses	Null	*G. xylinus* PTCC 1734	4.6	0.25	Salari et al., 2019
Cheese whey	Null		3.6	0.14	
Sweet lime pulp	Null	*K. europaeus* SGP37	6.3	0.19	Dubey et al., 2018
Sugarcane molasse	Null	*K. rhaeticus*	1.9	0.04	Machado et al., 2018
Ripe dates	Yeast extract, peptone, Na_2HPO_4, citric acid	*A. xylinum* 0416	5.8	0.19	Lotfiman et al., 2018
Pecan nutshell	Yeast extract, peptone, ethanol	*G. entanii*	2.8	0.07	Dorame-Miranda et al., 2019
CPWW	Yeast extract, peptone, Na_2HPO_4, citric acid	*K. sucrofermentans* DSM 15973	3.9	0.19	Current Study

In Summary …

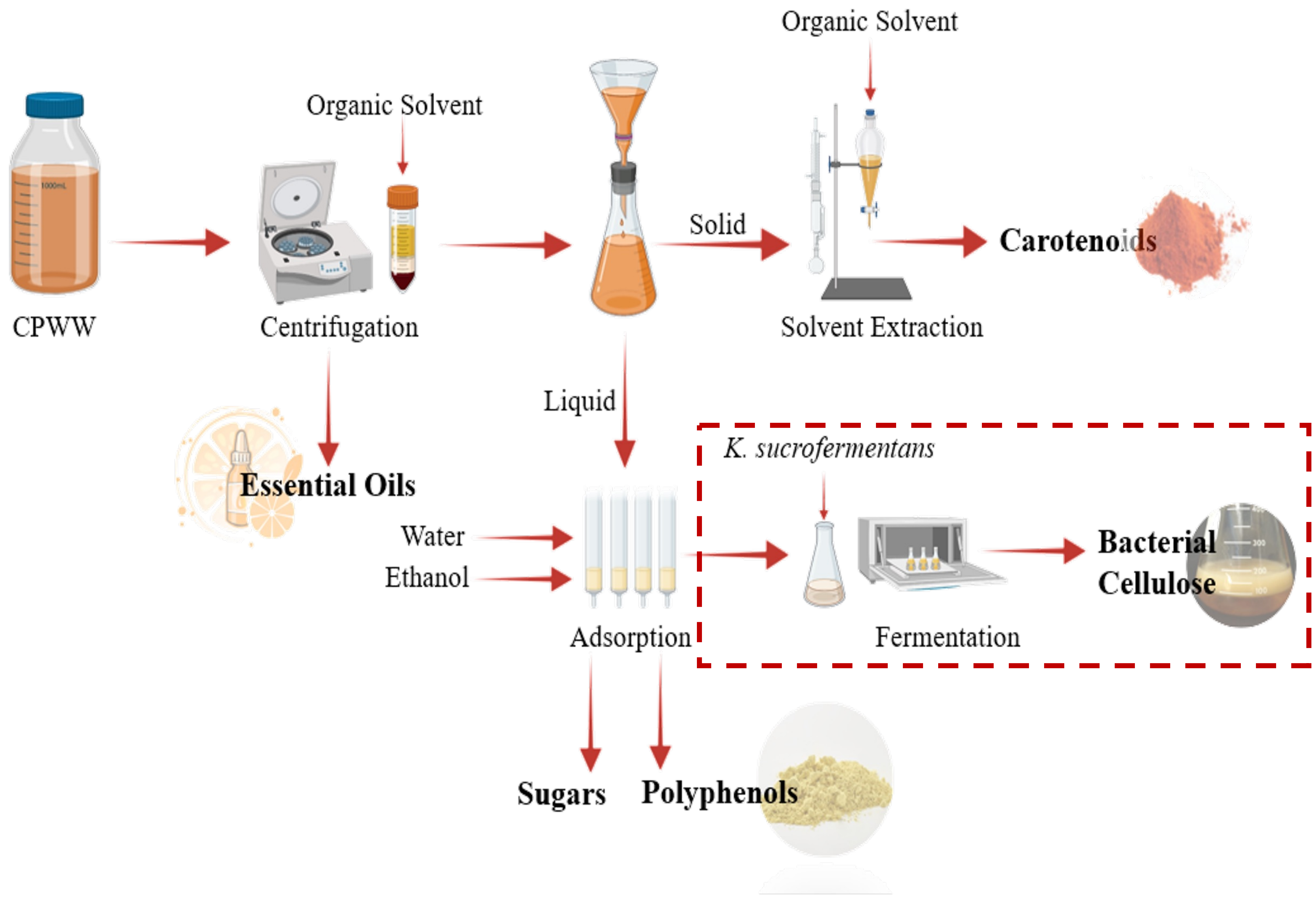


1000mL
1 m³
Organic Solvent
Centrifugation
Solid
Organic Solvent
Solvent Extraction
Carotenoids
10.3 g
Liquid
Essential Oils
0.45 kg
Water
Ethanol
Adsorption
K. sucrofermentans
Sugars
42 kg
Fermentation
Bacterial Cellulose
9.5 kg
Sugars
Polyphenols
0.5 kg

Conclusions

- ✓ **First study in our knowledge** used CPWW for **polyphenols** recovery through adsorption yielding **up to 65%**.
- ✓**0.45 kg m^{-3}** of **essential oils** can be recovered from CPWW using ***n*-heptane**.
- ✓ **1.91 mg g$_{db}^{-1}$** of **carotenoids** can be recovered employing **ethanol**.
- ✓Production of **3.9 g L^{-1}** of **bacterial cellulose** employing *K. sucrofermentans* DSM 15793.

Future Work



- Study the effect of air-supplement
- Study fed-batch and continuous fermentation modes
- Upscale in an airlift bioreactor

4Wealth

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Environmental Bioprocessing Laboratory

Department of Chemical Engineering

Cyprus University of Technology

Co-funded by the European Union

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Thank you for your attention!