School of Food Science and Nutrition UNIVERSITY OF LEEDS

Effect of rheological and structural properties of bacterial cellulose fibrils and whey protein on electro sprayed food-grade particles

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Electrospraying/electrospinning

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Method to produce particles/fibres:

Absence of heat

Absence of organic solvents

Controlled particle size

Low cost

Low productivity yield

Electrospraying/electrospinning

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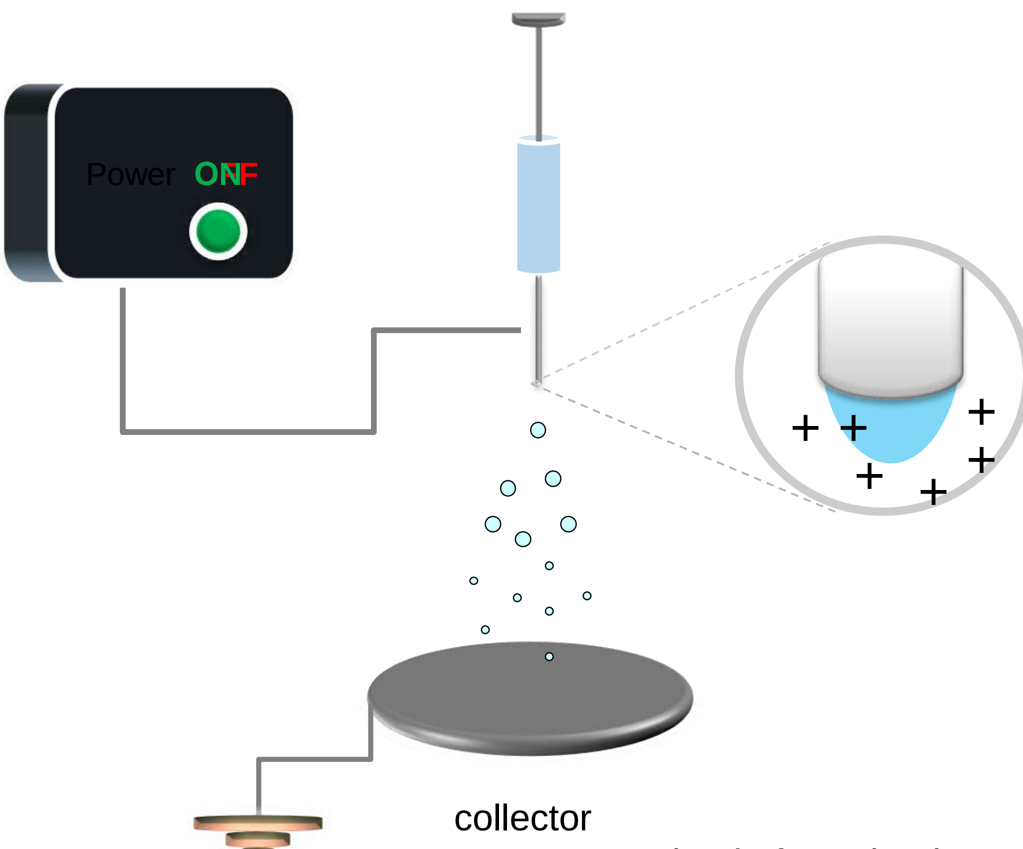

- Droplet in the edge of the needle due to surface tension
- Application of high voltage
- Creation of Taylor cone
- Overcome the surface tension → ejection of liquid jet
- Solvent evaporation
- Collection of dried particles

Bacterial Cellulose

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- *Komagataeibacter sucrofermentans* (DSM 15973)

High crystallinity

High water holding capacity

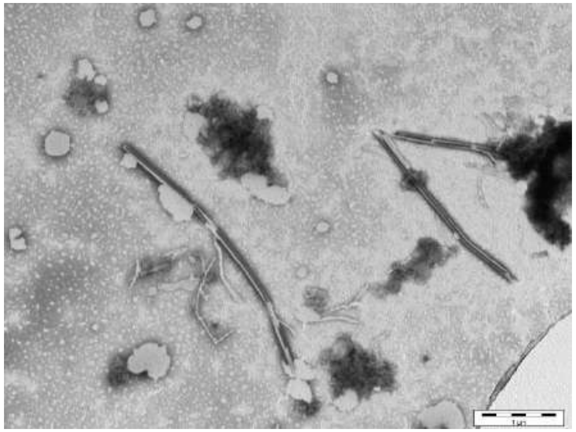
mechanical strength

High purity (does not associate with lignin or hemicelluloses)

- Bacterial cellulose (BC)

Fibrils with width: 0.1 - 9 µm

Micro-fibrils with width: 6 -15 nm



Use of BC

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Food

- Dessert Nata de coco
- **Thickening agent**
- Packaging materials

Electronics

- Substrate in electronic devices
- Organic LEDs

Bacterial Cellulose

Cosmetics

Paper substitute

Medical

- Tissue engineering
- Wound dressing
- Bone graft

Epigallocatechin gallate (EGCG)

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- Hydrophilic antioxidant
- Low cellular adsorption *in vivo*
- Chemically unstable
- Affected by light and oxygen

Research Gap

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Epigallocatechin gallate

- Few studies dealing with the encapsulation of lipophilic EGCG in food-grade materials

Encapsulation techniques

- Very few studies dealing with the possibility to produce nano-particles with non-catastrophic results for the bioactive

Bacterial Cellulose

•Less researched than the commonly used polysaccharides (cellulose, xanthan gum, locust bean gum) that industry could benefit from cheaper alternatives

Experimental Details

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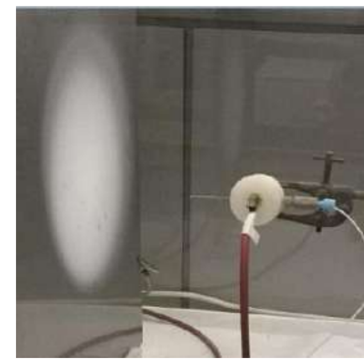
Aqueous solutions:

1-16% wt BC

10-30% wt WPI

5% wt Tween 20



* In the solution with the optimal properties:

0.1-0.2 mg/mL EGCG

Experimental Details

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Solution properties:

Surface tension

Conductivity

ζ-potential

Viscosity

- Bulk viscosity
- Interfacial viscosity

Properties of the particles:

- Structure (SEM)
- Particles size (DLS)
- Encapsulation efficiency EGCG (EE%, UV-Vis)
- Stability test for 30 days (UV-Vis)

pH = 3-9

RH = 20-80%

T = 30-90 ^{o}C

Solution properties

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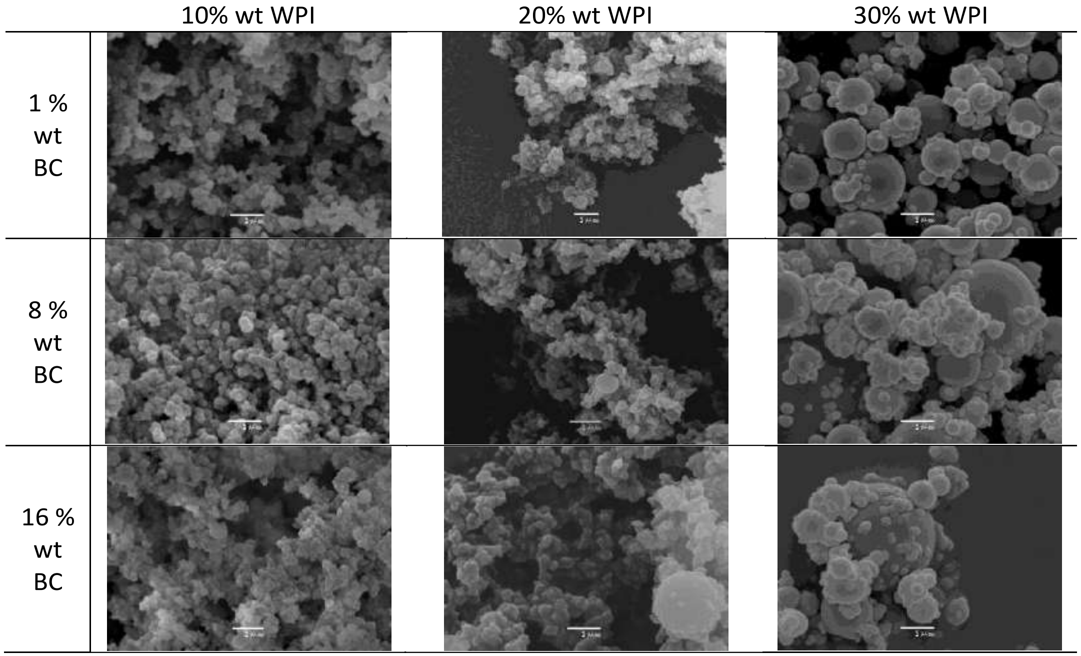
Physical Properties	%wt WPI	%wt BC				
		1	2	4	8	16
Surface tension (mN/m)	0	20.1^{a} (2.9)	27.3^{b} (2.4)	30.0^{c} (2.3)	32.1^{d} (4.7)	36.8^{e} (1.7)
	10	20.6^{a} (0.7)	26.7^{b} (0.6)	30.1^{c} (0.5)	32.7^{d} (0.7)	38.6^{f} (1.2)
	20	20.2^{a} (1.7)	27.4^{b} (1.5)	30.1^{c} (1.5)	32.8^{d} (0.5)	40.5^{f} (1.2)
	30	20.2^{a} (1.1)	27.9^{b} (1.5)	31.1^{c}(1.7)	32.9^{d} (4.3)	40. 7^{f} (0.8)
Conductivity (mS/m)	0	1.0^{a} (0.1)	2.2^{c} (0.1)	2.5^{d} (0.0)	3.1^{e} (0.0)	3.3^{f} (0.1)
	10	1.3^{b} (0.0)	2.4^{d} (0.0)	2.5^{d} (0.0)	3.1^{e} (0.2)	3.3^{f} (0.1)
	20	1.4^{b} (0.1)	2.5^{d} (0.2)	2.9^{e} (0.1)	3.1^{e} (0.1)	3.3^{f} (0.0)
	30	1.4^{b} (0.0)	2.6^{d} (0.0)	2.9^{e}(0.2)	3.1^{e} (0.0)	4.0^{g} (0.2)

Electrosprayed particles



10, 20% wt WPI → monodispersed particles

30% wt WPI → polydispersed particles, drainage of Taylor cone → unacceptable process

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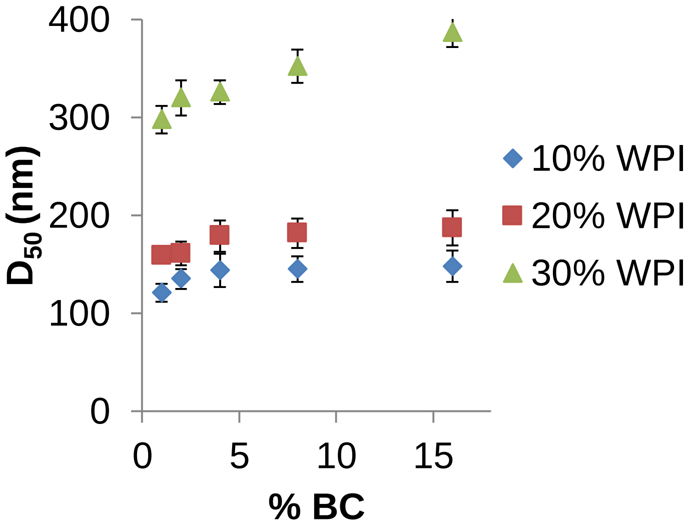
Electrosprayed particles

120-180 nm for 10-20% wt WPI

280-400 nm for 30% wt WPI

Solution with the optimal properties:

20% wt. WPI, 8% wt. BC



Encapsulation of EGCG

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	EE (%)	D_{50} (nm)
Blank	-	182[a] (5)
0.1 mg/mL EGCG	51[b] (8)	202[b] (3)
0.2 mg/mL EGCG	30[a] (5)	237[c] (6)

Stability tests

pH = 3, 6, 9

RH = 20, 40, 60, 80%

T = 37, 60 °C

Stability of EGCG

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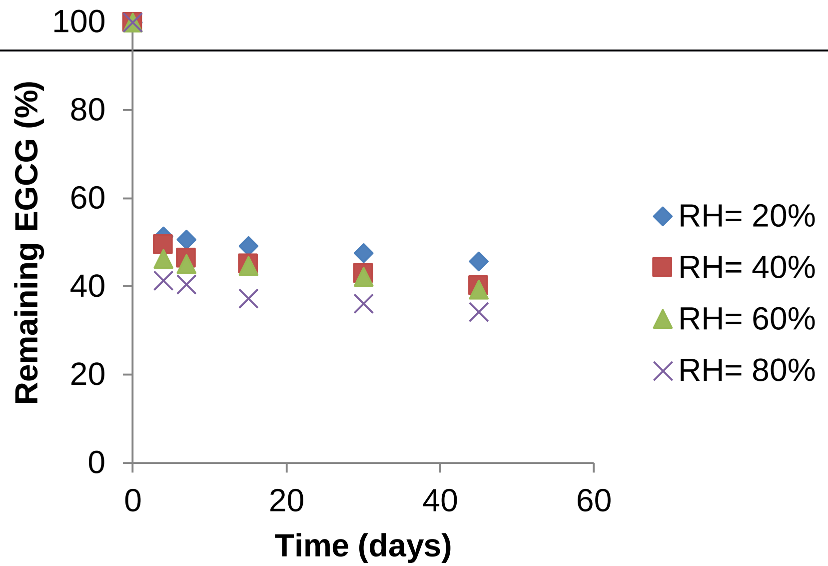
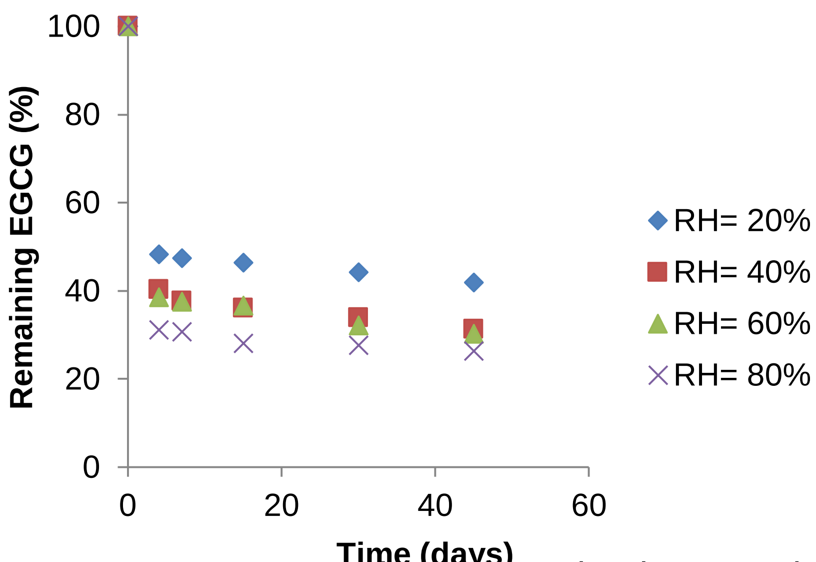
0.1 EGCG



raw EGCG



- Improved stability in all storage conditions compared to the raw EGCG
- ↑RH: ↓remaining EGCG
- ↑ pH: ↑ remaining EGCG
- Optimal conditions: low RH (up to 40%) or neutral/basic pH

Any questions?

Protein and polysaccharide co-assemblies for applications in food and biomedical sciences

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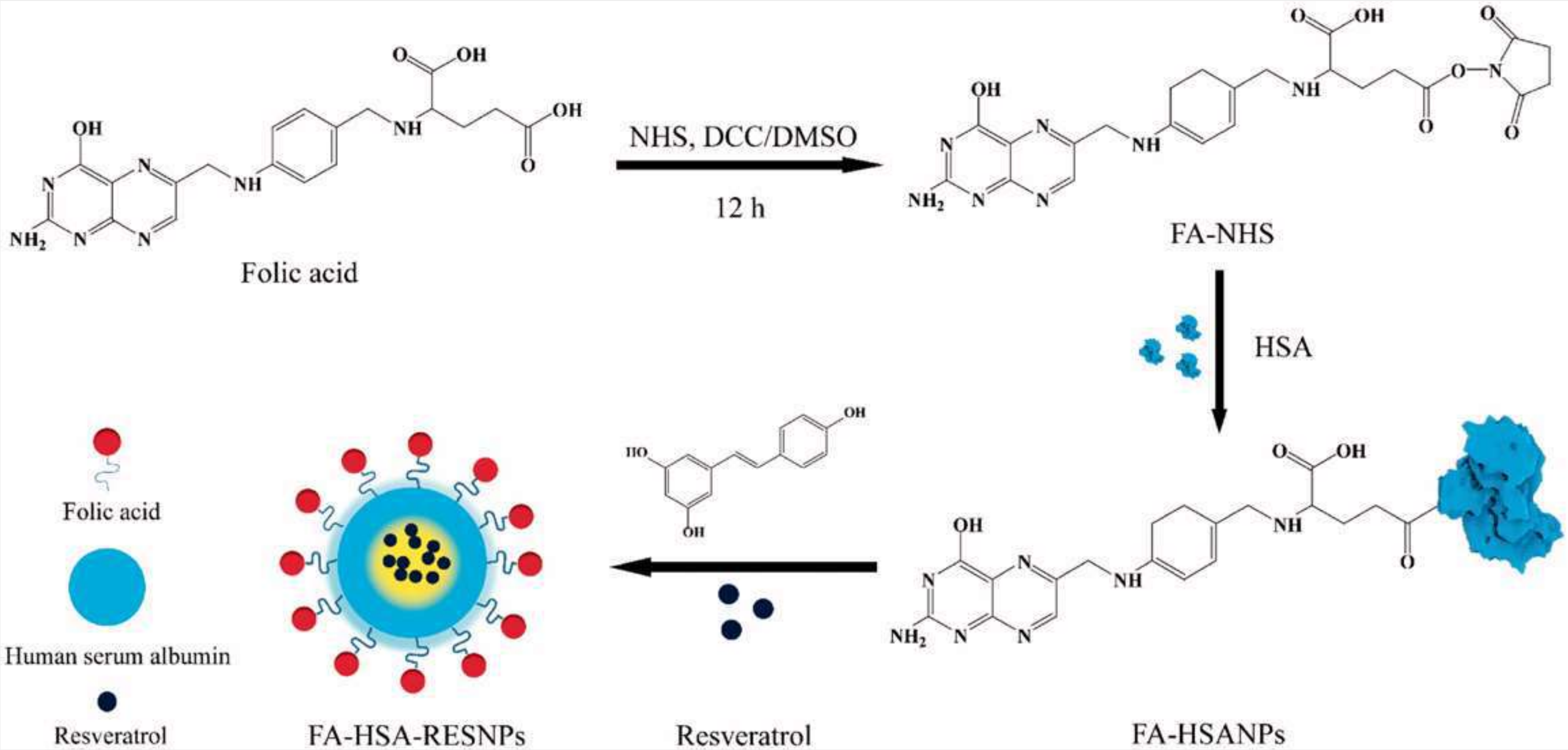
Introduction-Protein nanoparticles

- Biocompatible, biodegradable, metabolizable and nontoxic
- Attractive for the delivery of drugs, nucleic acids, growth factors and nutrients
- Bio-imaging applications
- Virus-like particle vaccine platforms

Protein NPs preparation methods

- Desolvation
- Electrospraying
- Emulsion-solvent evaporation
- Salt precipitation
- Complexation with polysaccharides

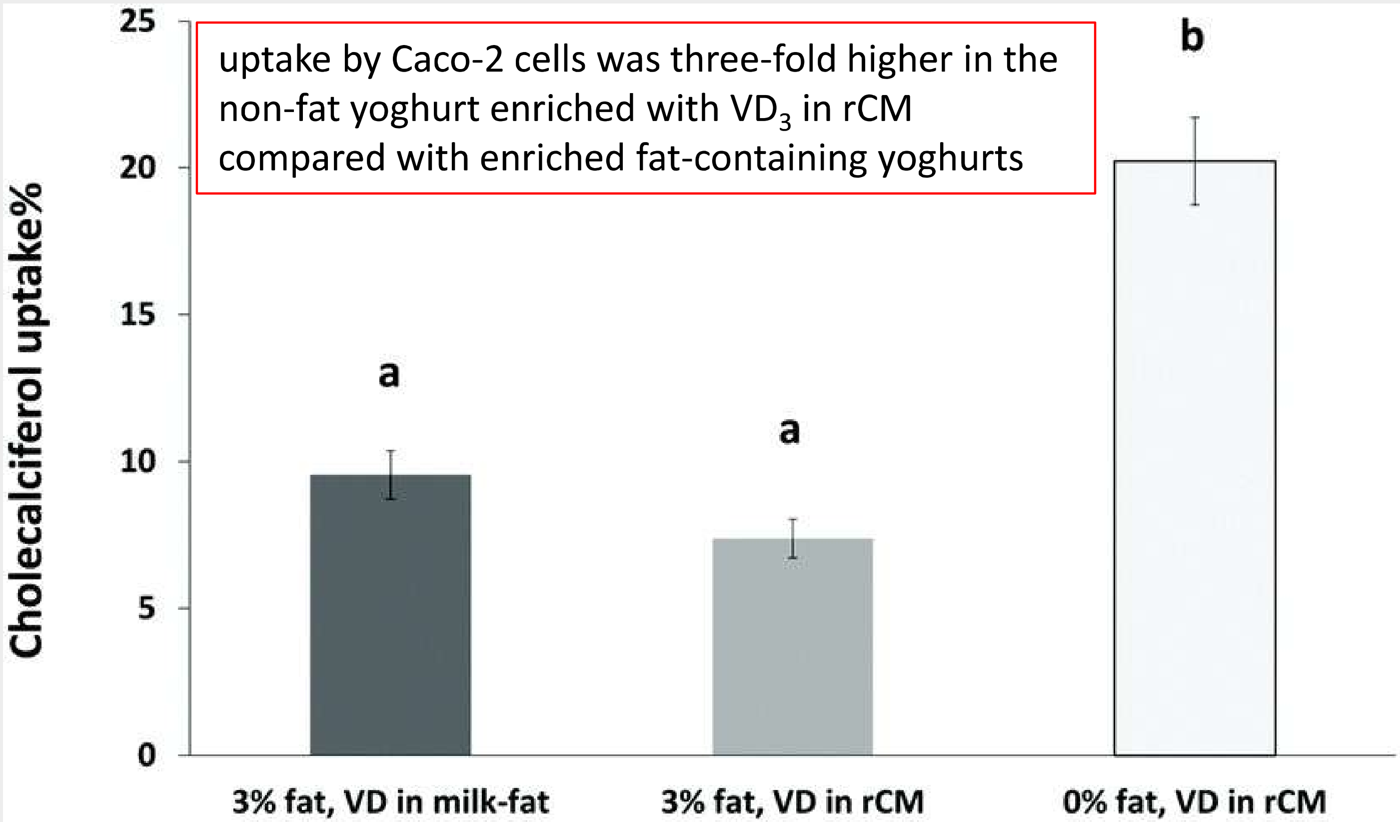
Protein NPs for drug delivery



- albumin has important functions i.e. maintenance of the pH, colloidal osmotic pressure of plasma, trapping of free radicals (antioxidant effect)
- demonstrated the ability of FA-HSA-RESNP to target liver tumor cells

Bolin Lian, Mingfang Wu, Ziqi Feng, Yiping Deng, Chen Zhong & Xiuhua Zhao (**2019**), Artificial Cells, Nanomedicine, and Biotechnology, 47:1, 154-165.

Protein NPs for nutraceutical delivery

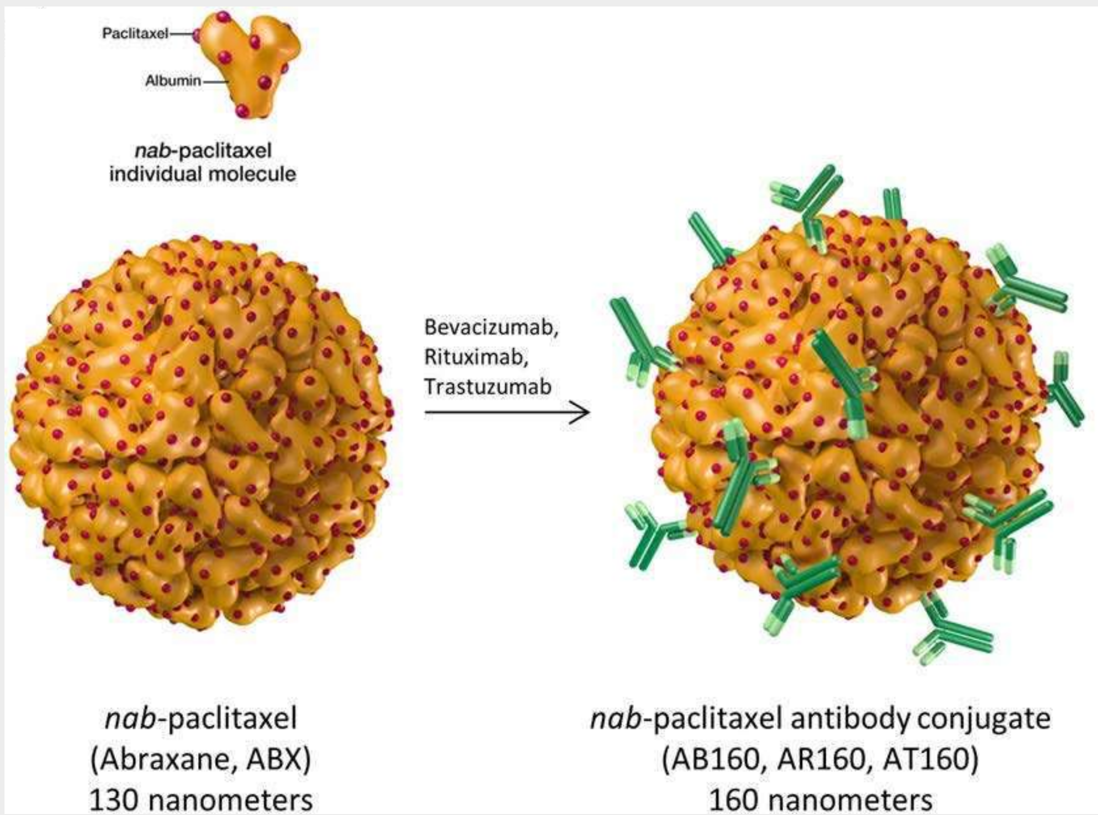


- vitamin D is a model lipophilic-nutraceutical
- reassembled casein-micelle NPs
- need for low-fat foods and beverages due to pandemic of vitamin D deficiency and global rise in obesity
- highly protective effect against vitamin D gastric degradation

Yifat Cohen, Marielle Margier, Uri Lesmes, Emmanuelle Reboul and Yoav D. Livney, Food Funct., **2021**, 12, 4935-4946
Yifat Cohen, Moran Levi, Uri Lesmes, Marielle Margier, Emmanuelle Reboulcde and Yoav D. Livney Food Funct., **2017**, 8, 2133-2141

Commercially available protein NPs

Antibody directed chemotherapy



Registered HSA-based particle formulations

- Albunex
- Abraxane

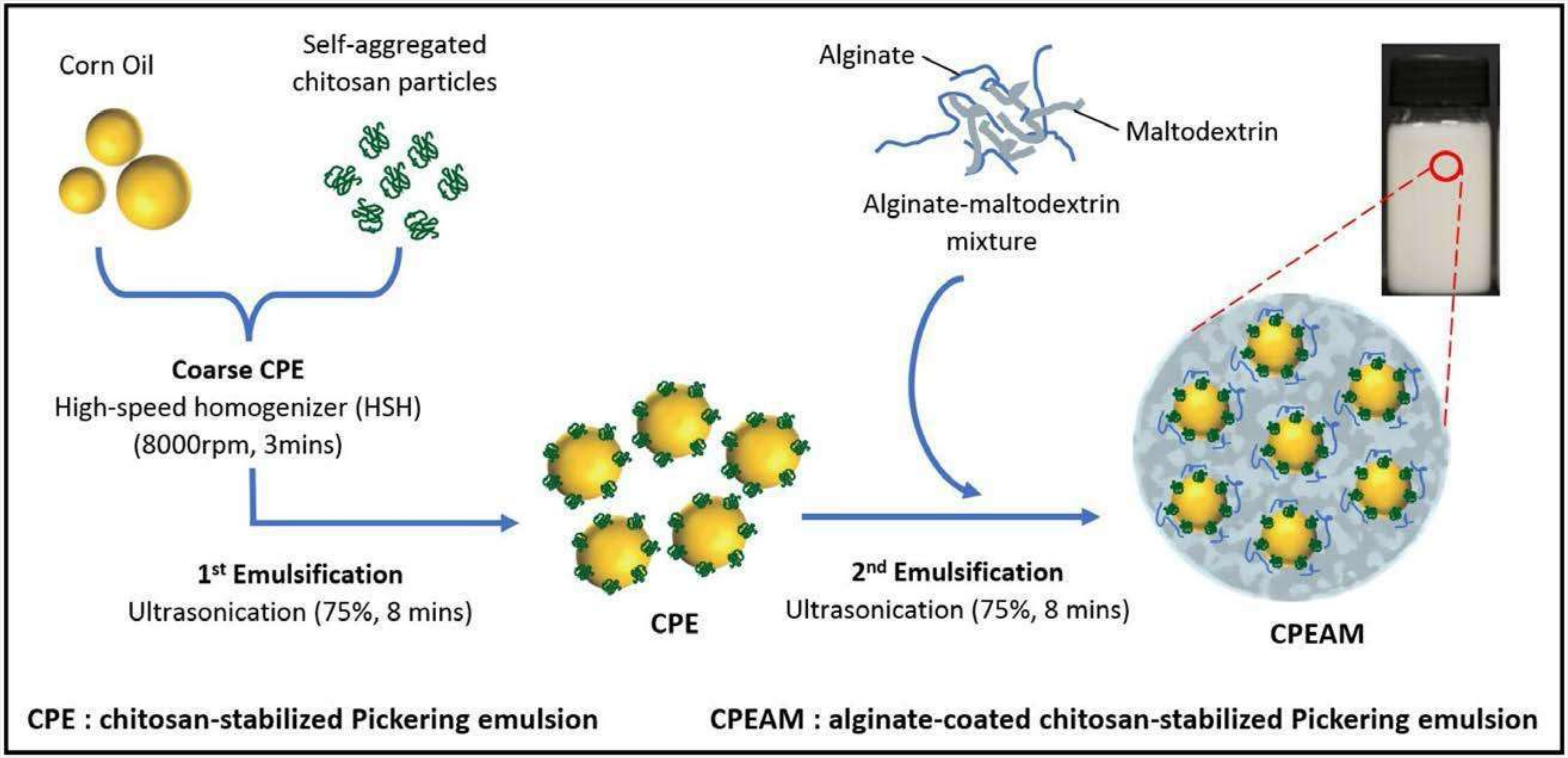
Butterfield, J.T., Kim, H., Knauer, D.J. et al., Sci Rep 7, 14476 (**2017**).

Introduction-Polysaccharides

- Polysaccharides are biocompatible, nontoxic, biodegradable, safe
- Food Science (thickeners, stabilizers)
- Medicine
- Tissue engineering, wound healing

Introduction-Polysaccharides

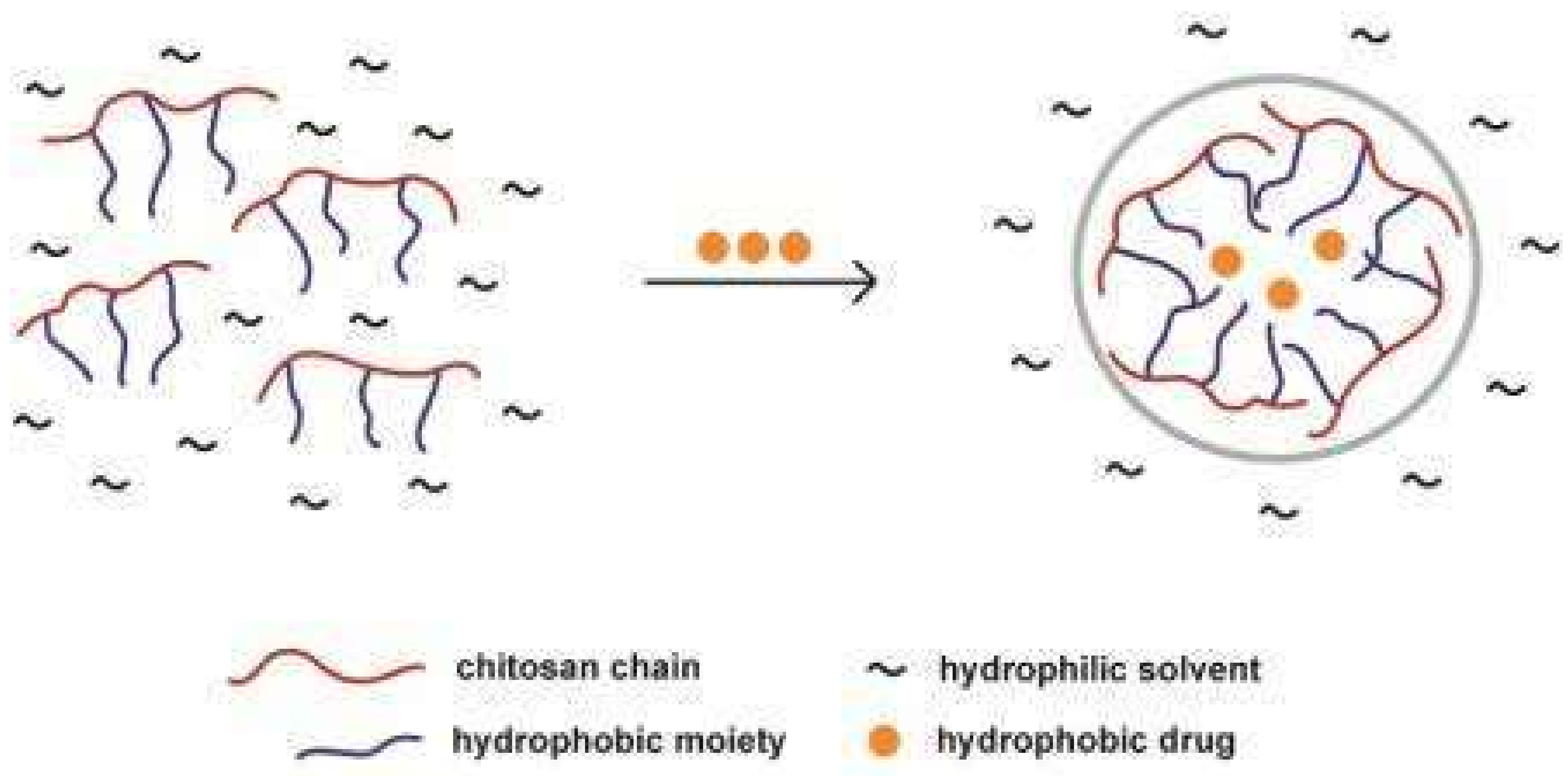
- Food Science



Charanjit Kaur Surjit Singh, Hui-Peng Lim, Beng-Ti Tey, Eng-Seng Chan, Carbohydrate Polymers, 251, 117110, **2021.**

Introduction-Polysaccharides

- Drug encapsulation

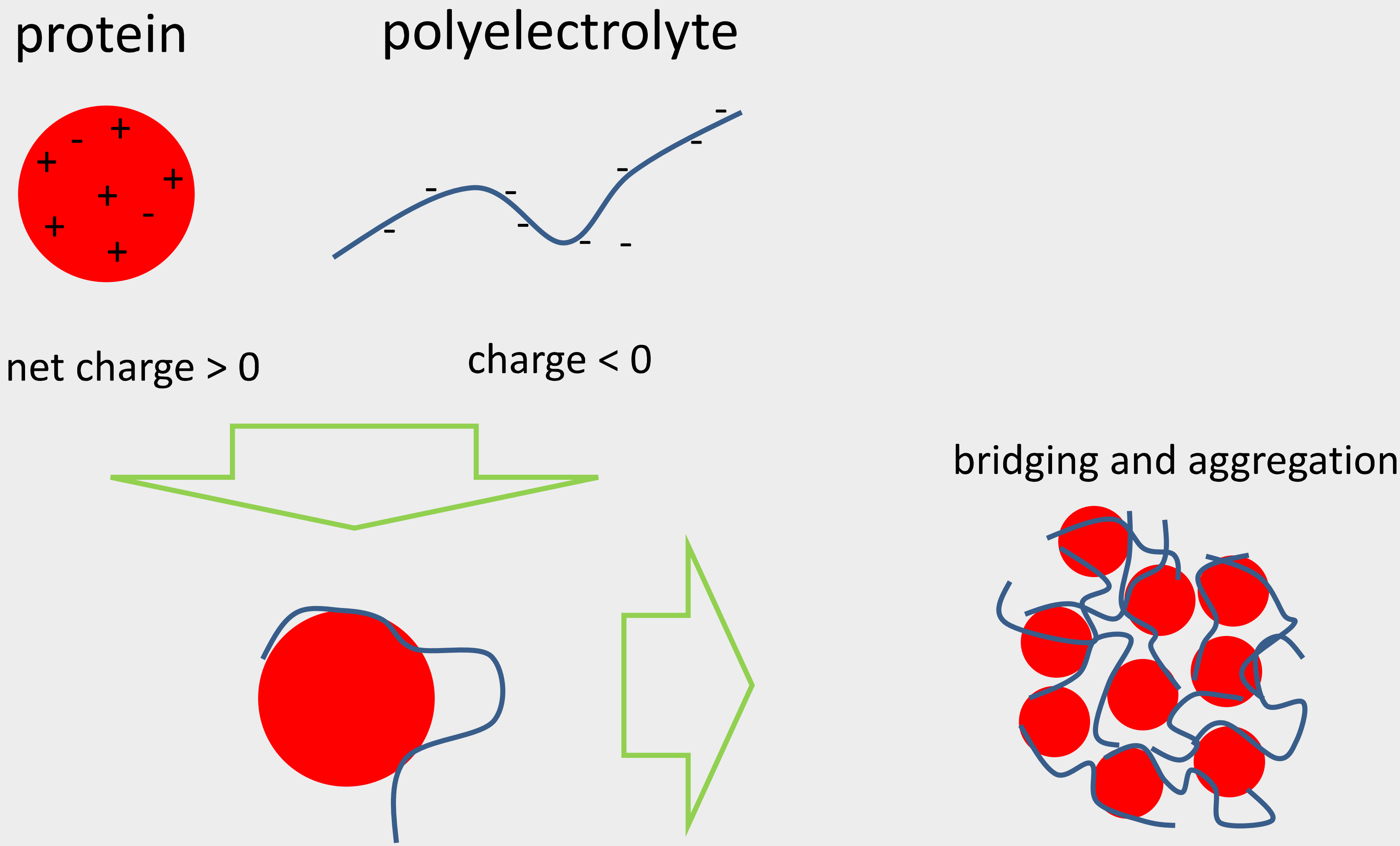


Quiñones et al. *Polymers* **2018,** 10 (3), 235

Polysaccharide-protein electrostatic complexes

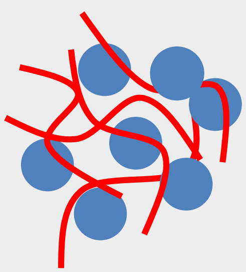
- Charged polysaccharides are polyelectrolytes of natural origin
- Proteins are complex polyelectrolytes with pH-dependent surface charge distribution

Polyelectrolyte-protein electrostatic interaction

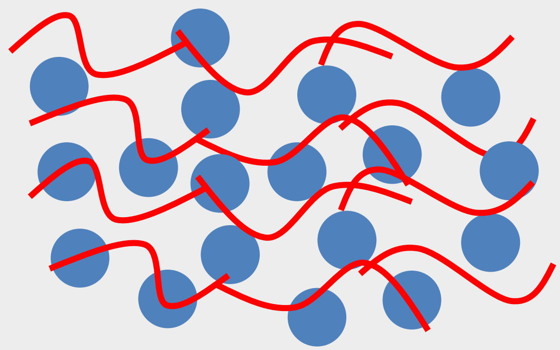


Polyelectrolyte-protein nanostructures

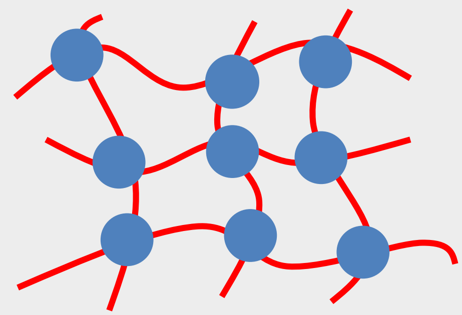
Self-organized biomaterials



Nanoparticles



Multilayers



Hydrogels/
nanogels

Macromol **2021**, *1*, 155-172 (review)

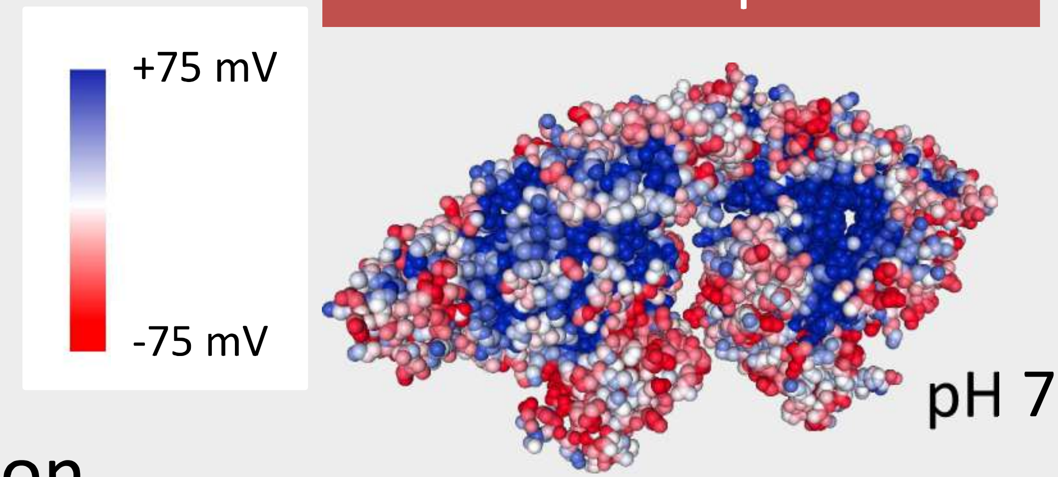
Protein surface properties: multifunctionality

Charge Patch

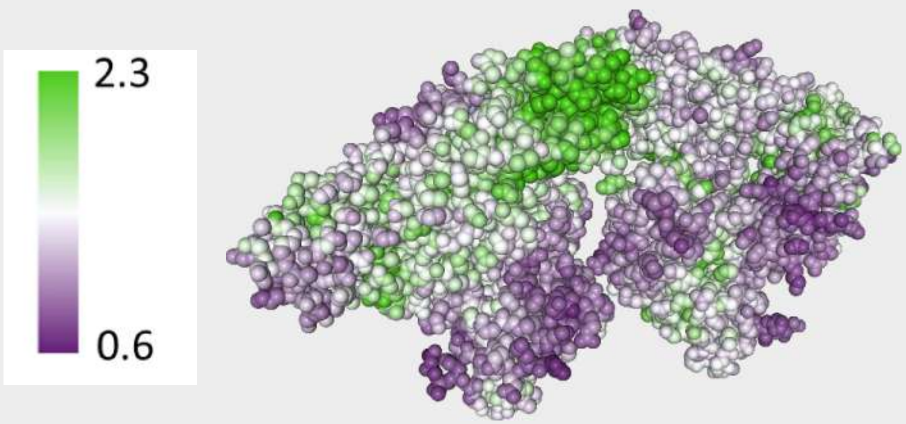
Interactions for organization and binding

Hydrophobicity patch

electrostatic potential



non-polar/polar ratio



Development of protein/polysaccharide NPs

Opportunities for

- Biocompatible and ecofriendly methods
- Use of proteins as building blocks for multifunctionality
- Stimuli responsiveness
- Loading of bioactive substances

Physicochemical Characterization Methods

Static and Dynamic Light Scattering

- Molar mass and size of nanoparticles
- Assembly/disassembly kinetics
- Size distribution of nanoparticles
- Protein aggregation
- Nanoparticle stability/stimuli responsiveness

(temperature, pH, salt)

Physicochemical Characterization Methods

Light Spectroscopy Methods

- UV-vis absorbance (drug/nutrient loading and release)
- Fluorescence spectroscopy (drug/nutrient binding, hydropathy, protein conformation)
- ATR-FTIR and circular dichroism (protein secondary structure, denaturation)

Advanced Experimental Methods at Large Scale Facilities

Small Angle Neutron Scattering

- morphology at length scales 1-100 nm
- internal structure of NPs
- protein size and shape
- swelling transitions within NPs
- intra- and inter- NP interactions
- superior contrast
- non-invasive measurement

Small angle neutron scattering diffractometer

Advanced Experimental Methods at Large Scale Facilities

Small Angle X-ray Scattering

- same principles as SANS
- short acquisition times
- superior resolution
- resolve of fast kinetics (10 ms)

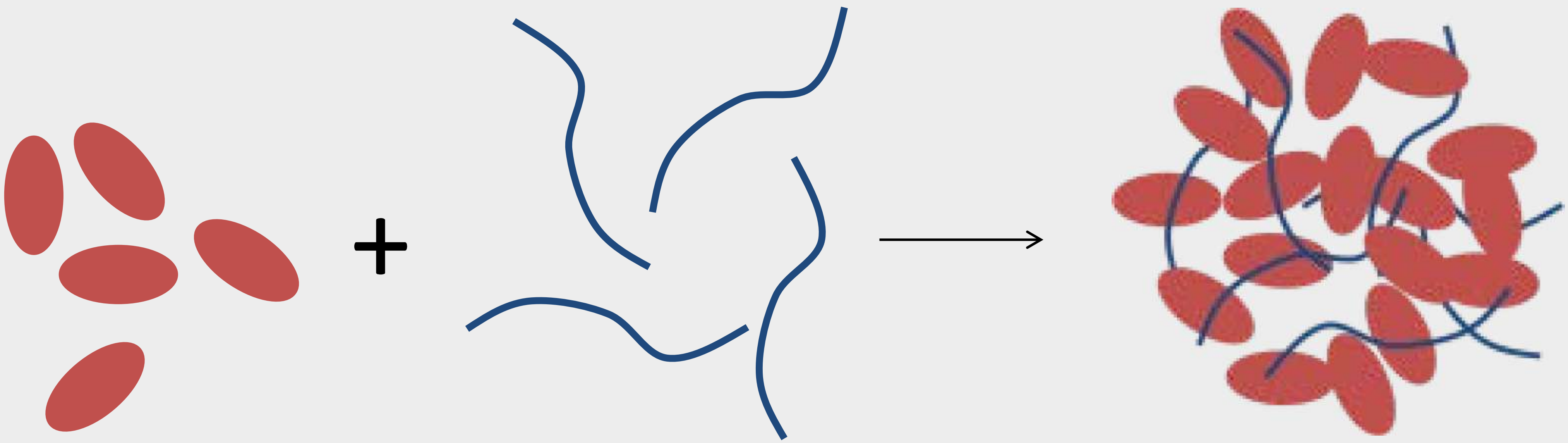
Studied systems

Protein/polysaccharide NPs

1. BSA/chondroitin sulfate
2. BSA/xanthan gum
3. Fibrinogen/hyaluronic acid
4. Trypsin/chondroitin sulfate

Anionic polysaccharide/protein at pH<pI

Complexation at acidic pH 4.2: First step of the ecofriendly protocol

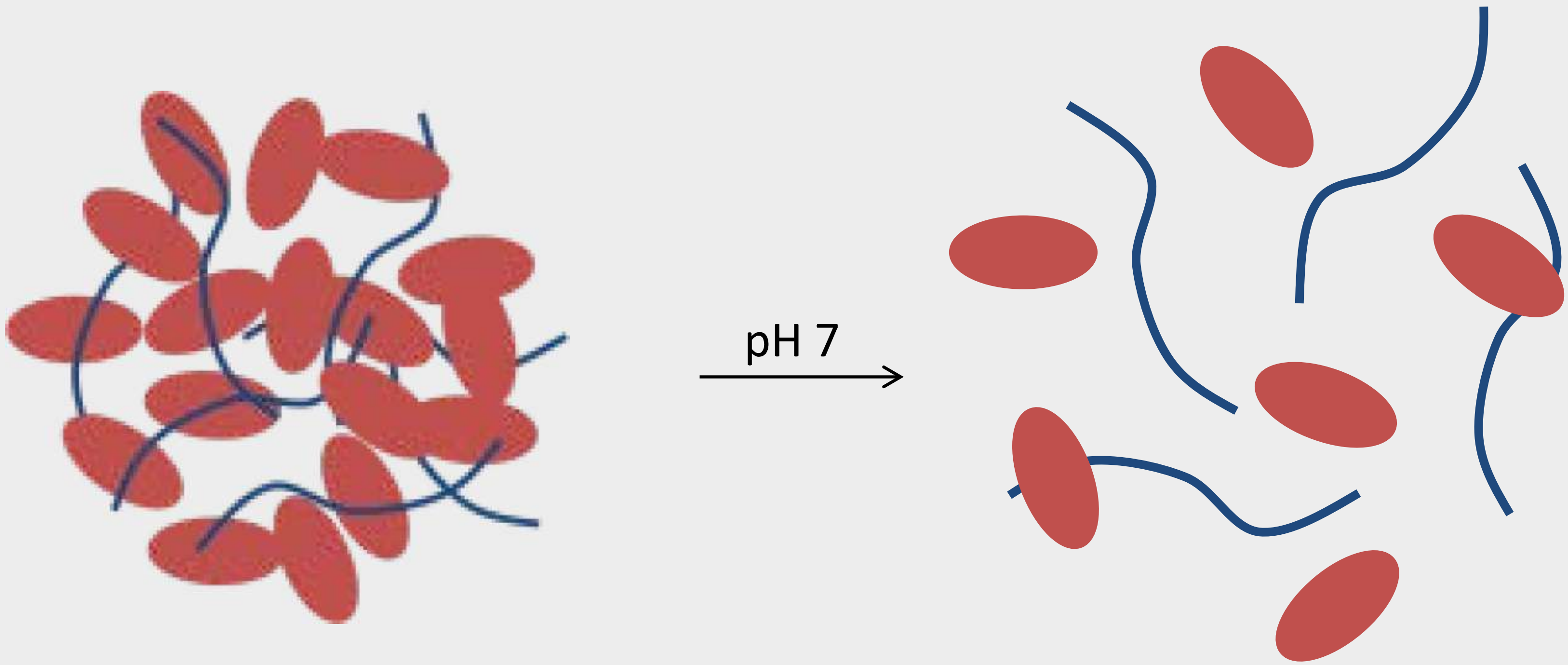


At pH 4.2, ζ_{BSA} =+25

Isoelectric point ~5.5

Anionic polysaccharide/protein at pH>pI

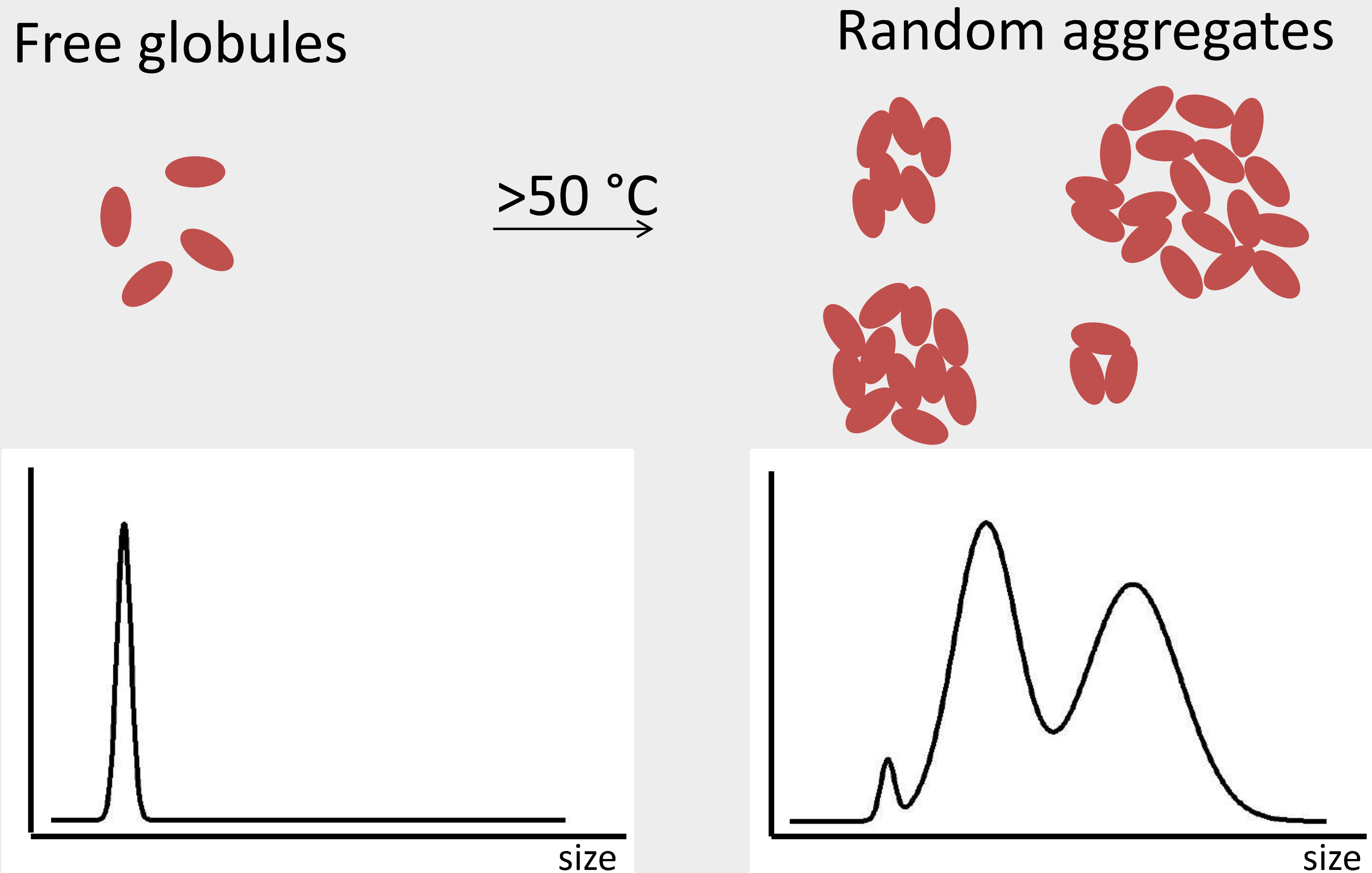
Disintegration



At pH 7, ζ_{BSA} =-9

Isoelectric point ~5.5

Aggregation induced by thermal treatment



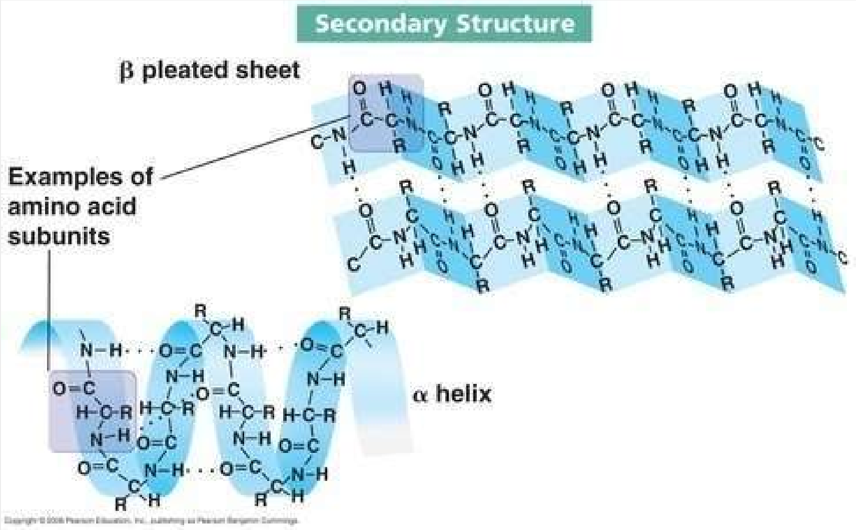
Aggregates of high polydispersity and multiple populations are usually observed.

Protein denaturation: thermal treatment

Second step of the ecofriendly protocol

BOVINE SERUM ALBUMIN

$$\alpha\text{-helix} \xrightarrow{T>50\ °C} \beta\text{-sheet}$$



Unfolding of the cysteine-containing pocket enables disulfide bridges.
Irreversible intermolecular β-sheets are formed at 50–52 °C and are enhanced up to 80–85 °C.

Fibrinogen – thermal aggregation

Blood protein with critical role

- blood clotting
- aggregation of platelets
- interaction of blood with biomaterials

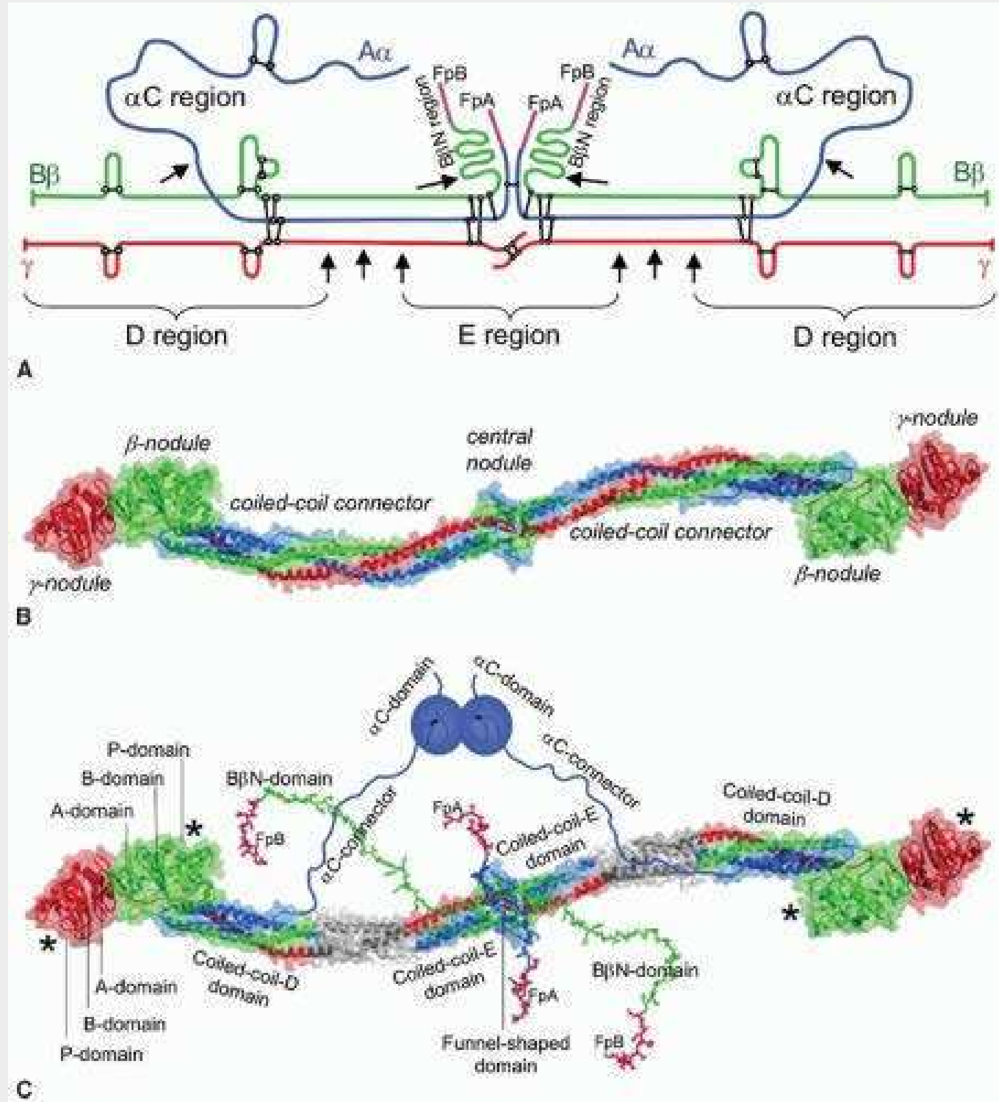
Effective agent in wound healing and tissue engineering

- active sites that able to bind fibroblasts, endothelial cells and their growth factors

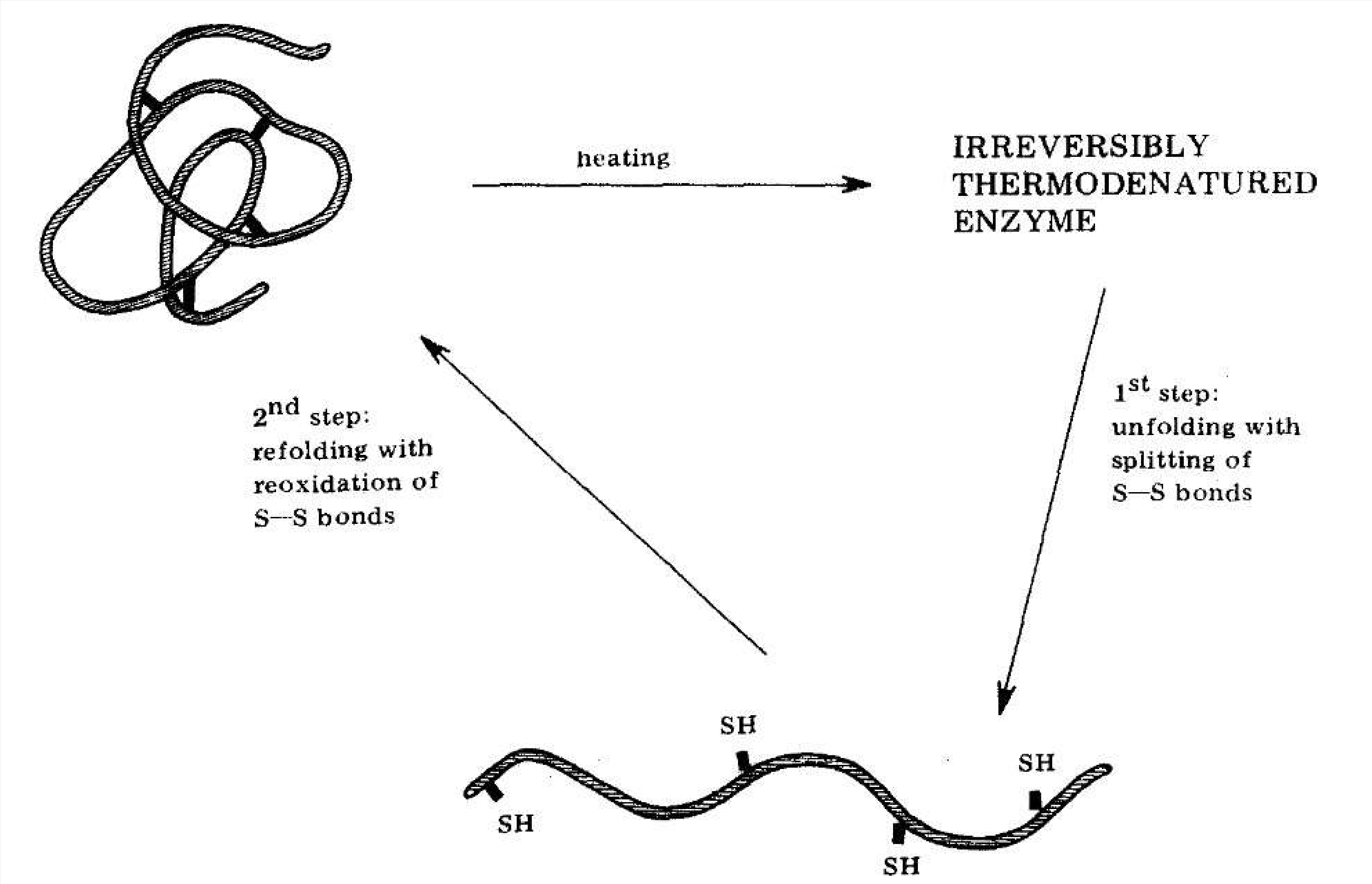
Fibrinogen molecules

Trinodular structure

At 90 °C the central globular region is still intact while the lateral globules are melted (Tm ≈ 77 °C) **causing irreversible intermolecular bridges**



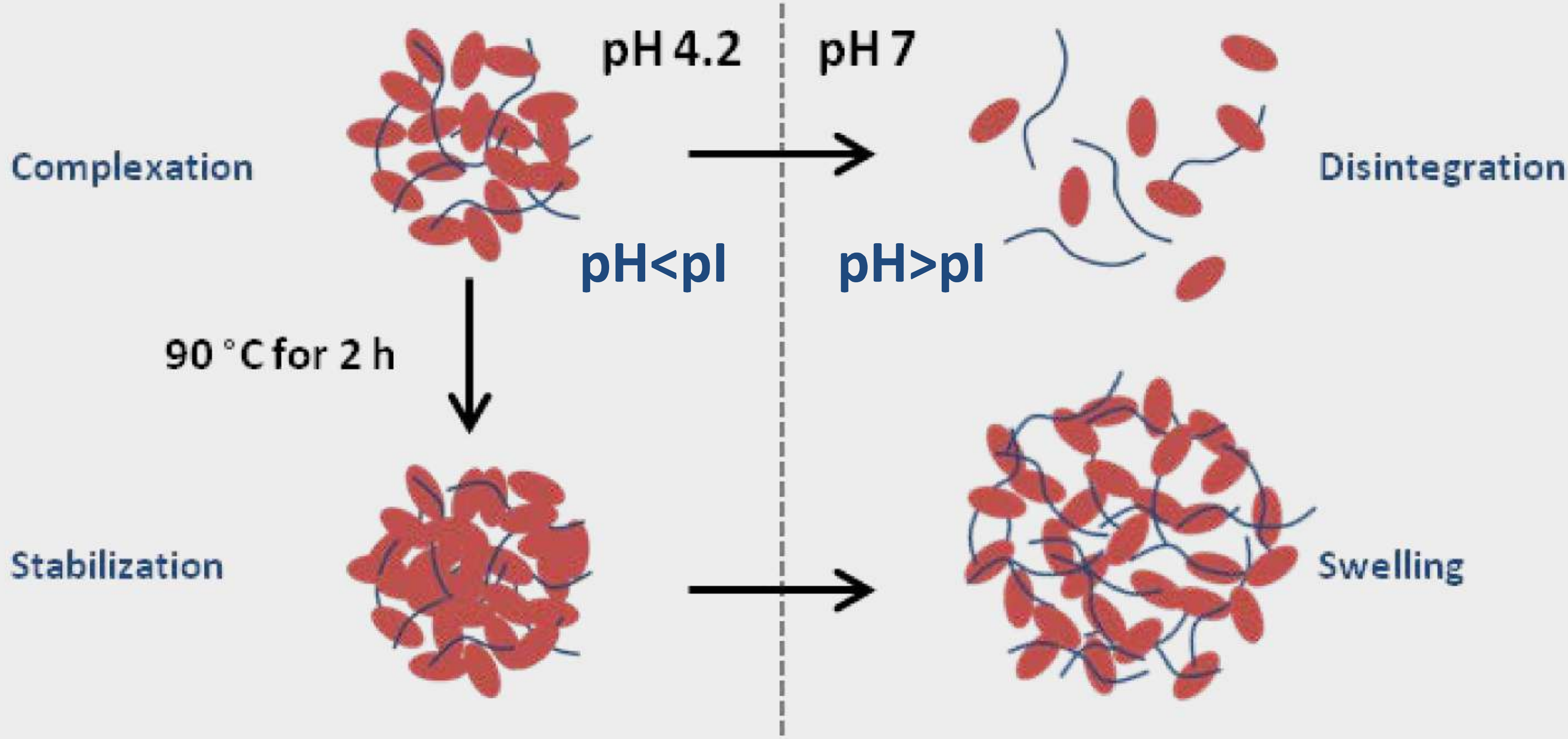
Trypsin: heat-induced denaturation



- secreted by the pancreas
- acts as a proteolytic enzyme in the small intestine

Biochimica et Biophysica Acta (BBA) – Enzymology Volume 615, Issue 2, 7 October 1980, Pages 426-435

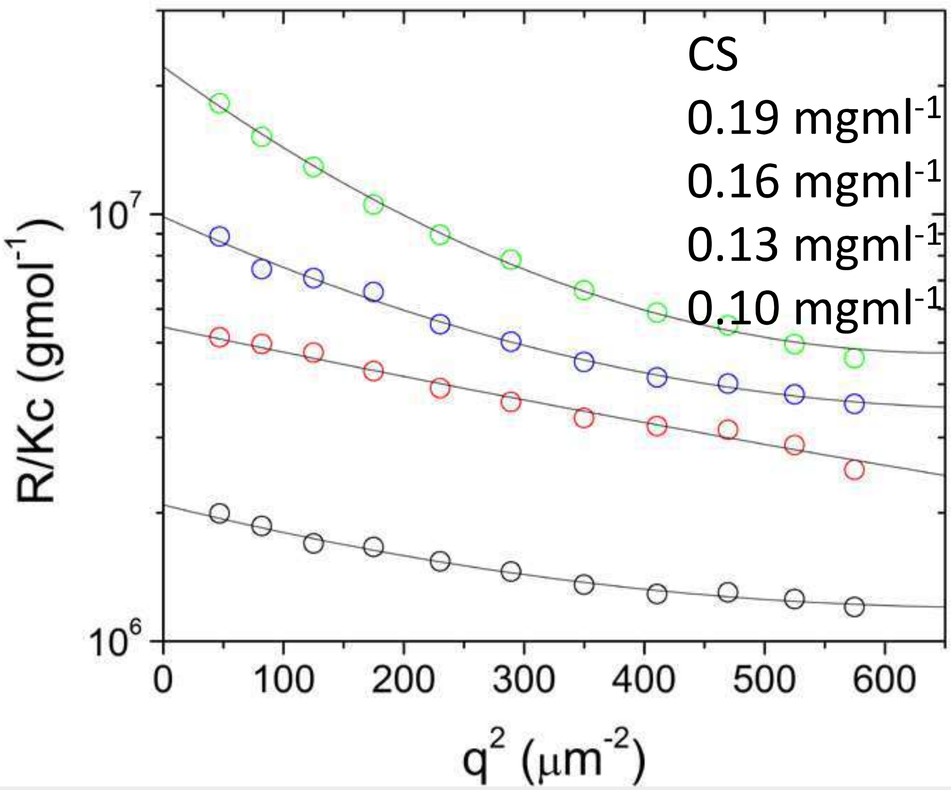
The main concept: biocompatible method



electrostatic complexation = control size distribution
thermal treatment = stability against pH changes

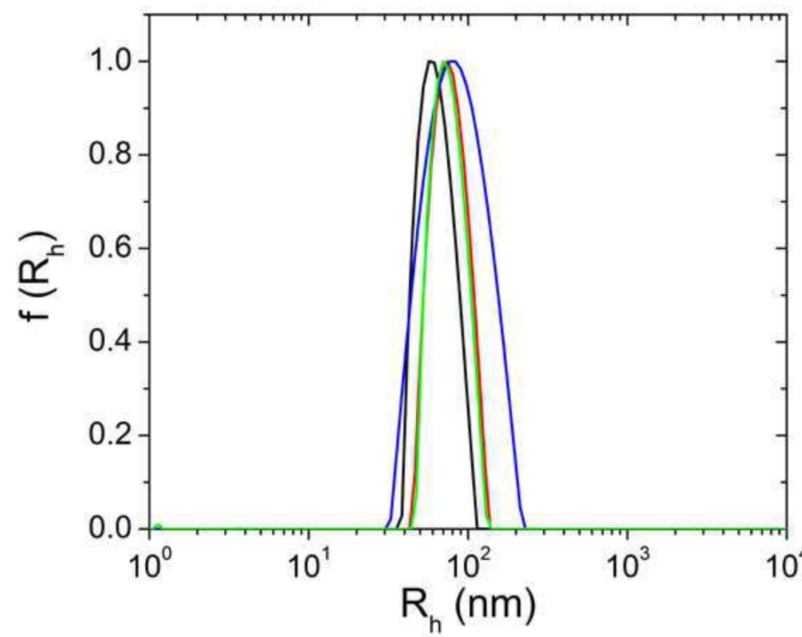
Food Hydrocolloids 87 (**2019**) 602–610

CS/BSA complexation-tuning molar mass



STATIC LIGHT SCATTERING:
Scanning concentration range for detection of complexation

DYNAMIC LIGHT SCATTERING:
Size distribution/populations



BSA at 1.0 $mgml^{-1}$

Stoichiometric Neutrality at C_{CS}/C_{BSA}~0.14 or 1 CS chain per 2.5 BSA globules

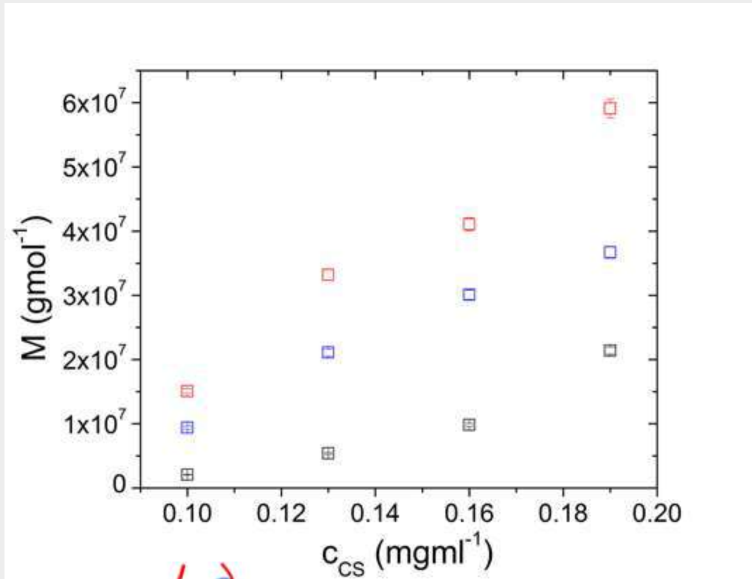
Tunning molar mass / temperature treatment

Second step of the ecofriendly protocol

Acidic pH

Thermal treatment (acidic pH)

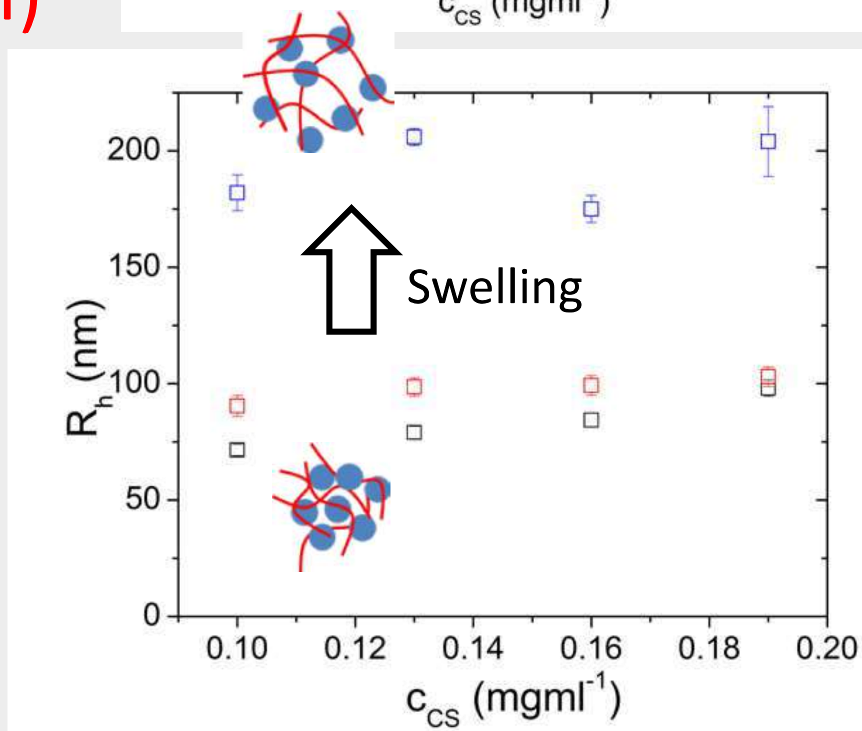
Neutral pH

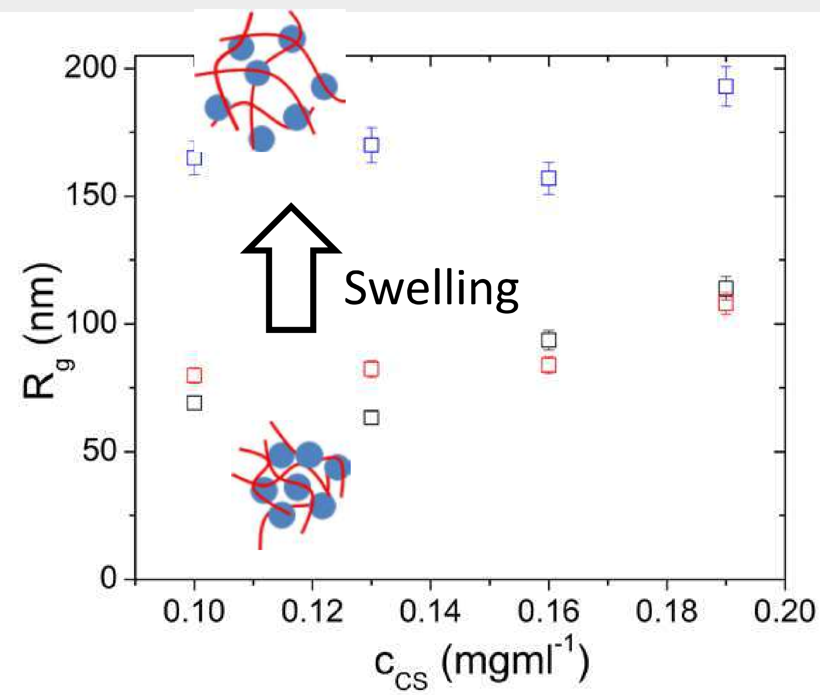


Themal protocol:

90 °C for 2 hours

stabilization

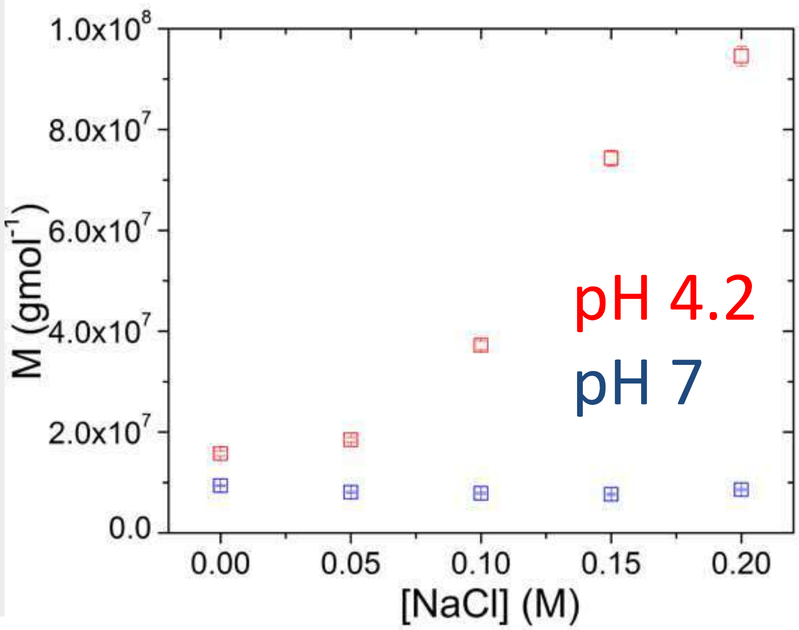


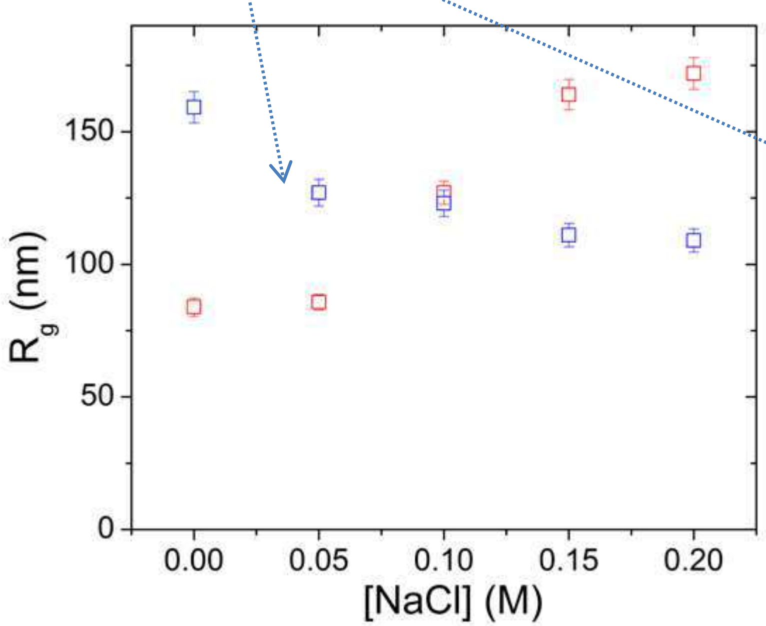


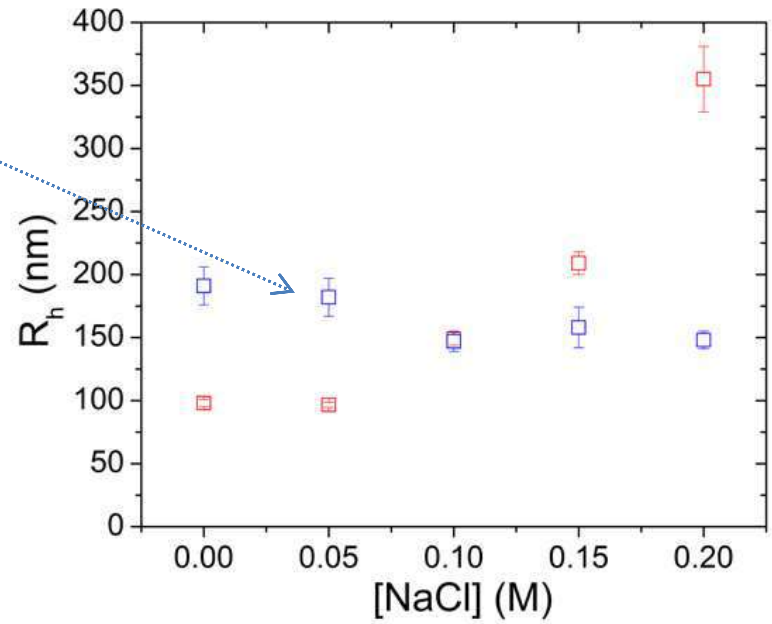
Thermally stabilized NPs at “physiological” conditions

T=37 °C

shrinkage:
responsive nanogel (salt)

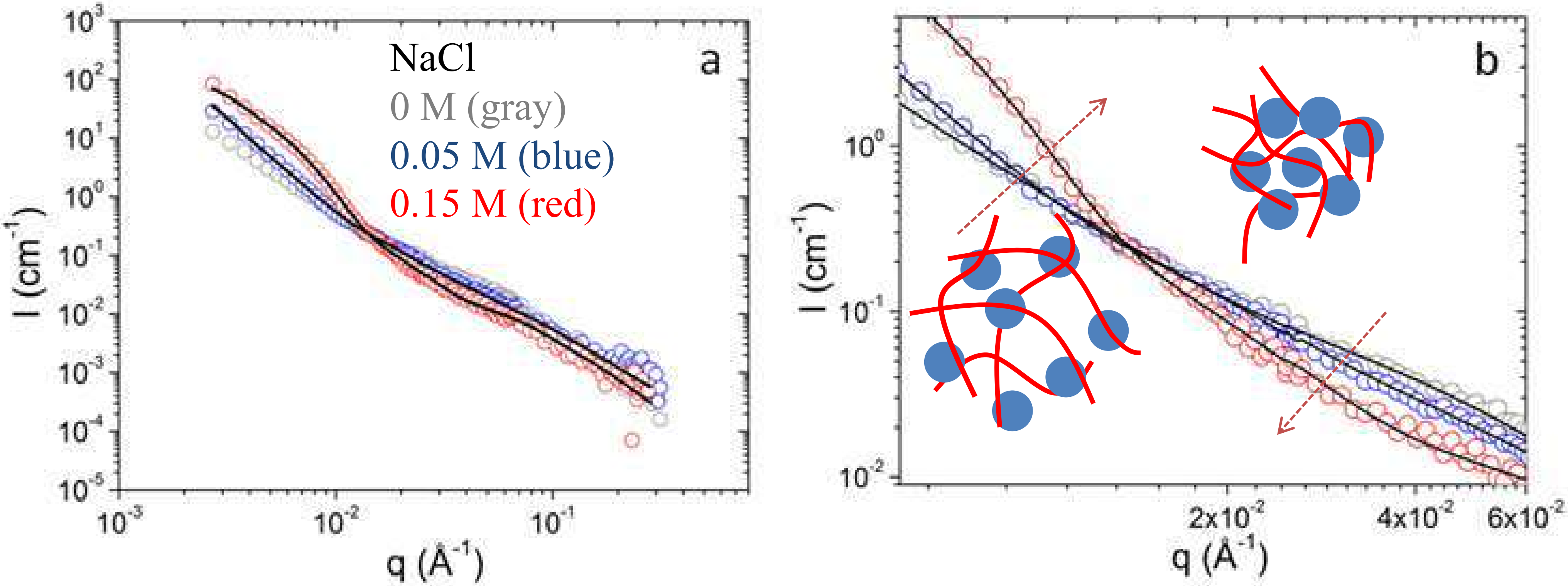






Polysaccharide/protein nanoparticles: SANS

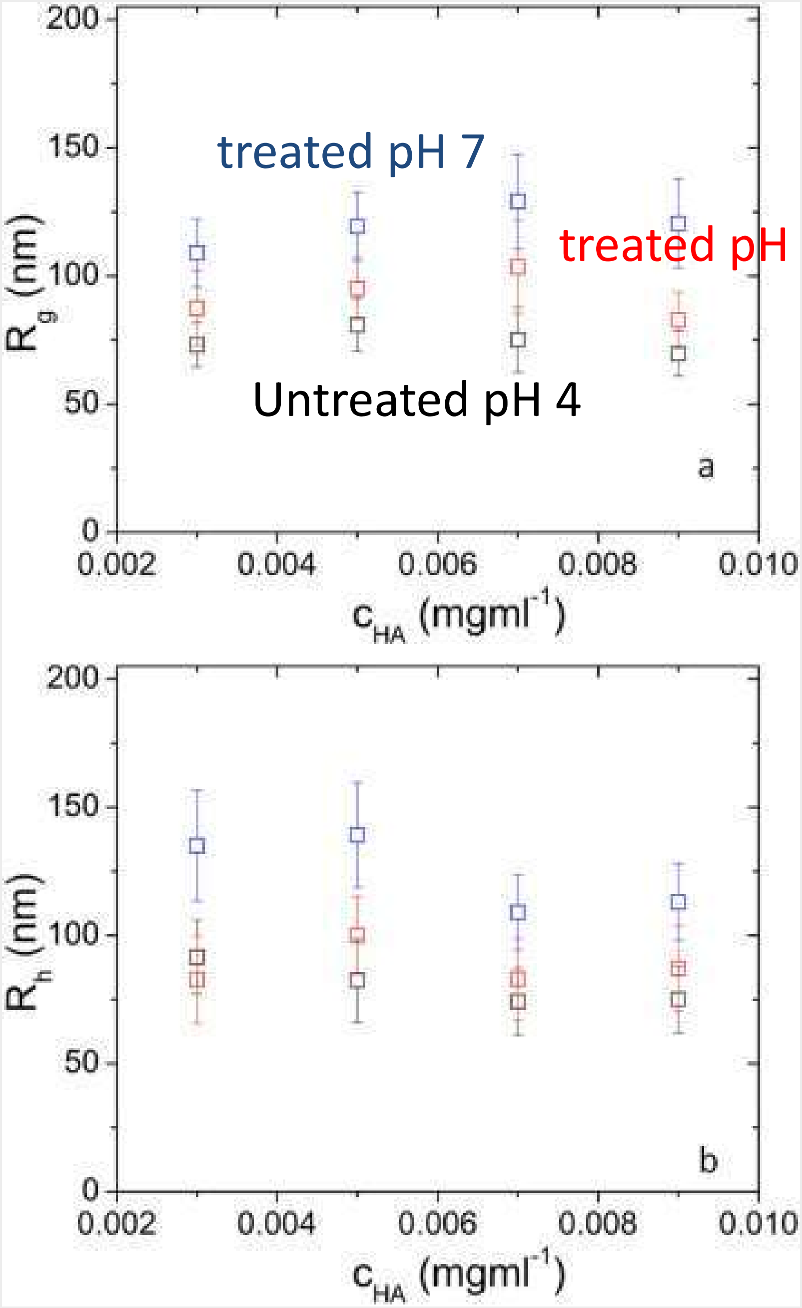
Ionic strength dependence: nanogel



Diswelling: Two-level morphology (nanogel) – to – three-level hierarchical morphology

Carbohydrate Polymers, 218, 2019, 218-225

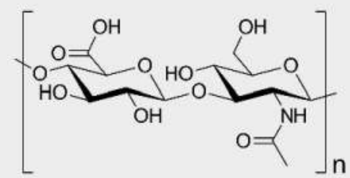
Nanoformulation by hyaluronic acid



Thermal Protocol

85 °C for 20 min

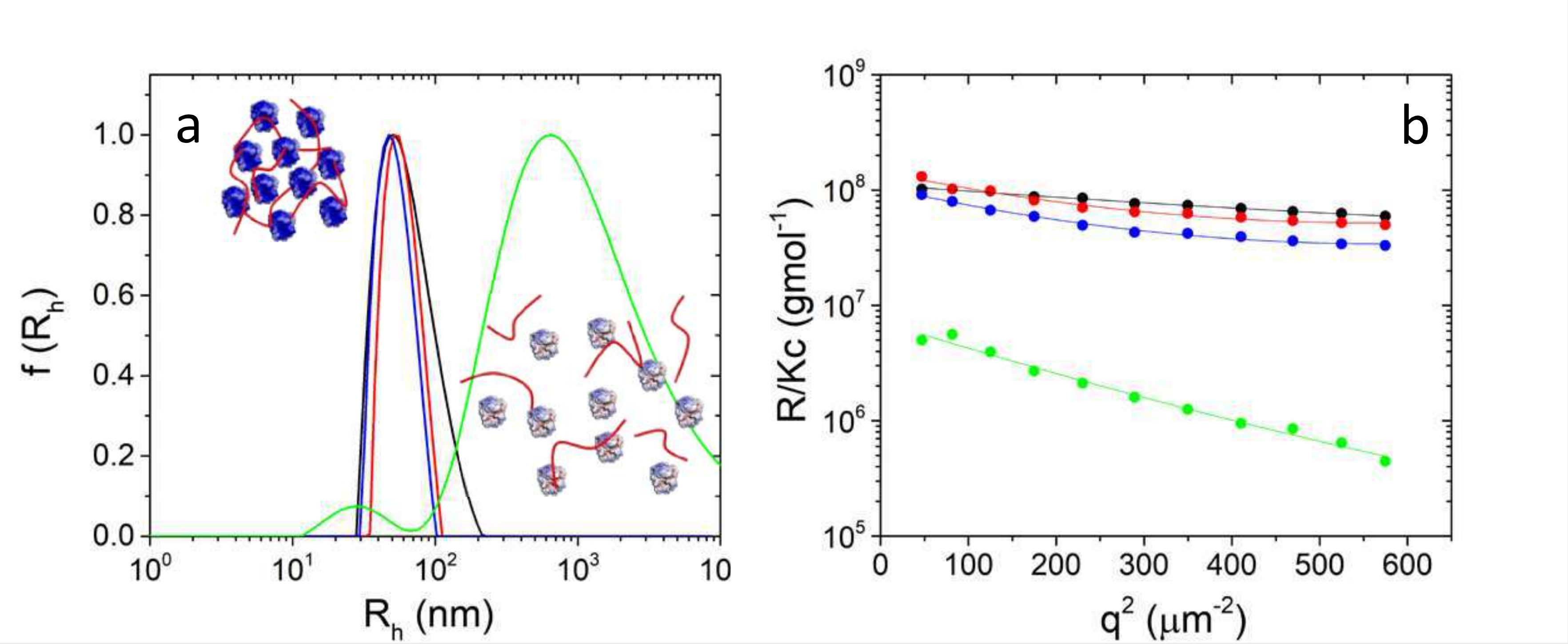
at pH 4



Sodium Hyaluronate

International Journal of Biological Macromolecules,
158, 2020, 251-257

Trypsin/chondroitin sulfate NPs



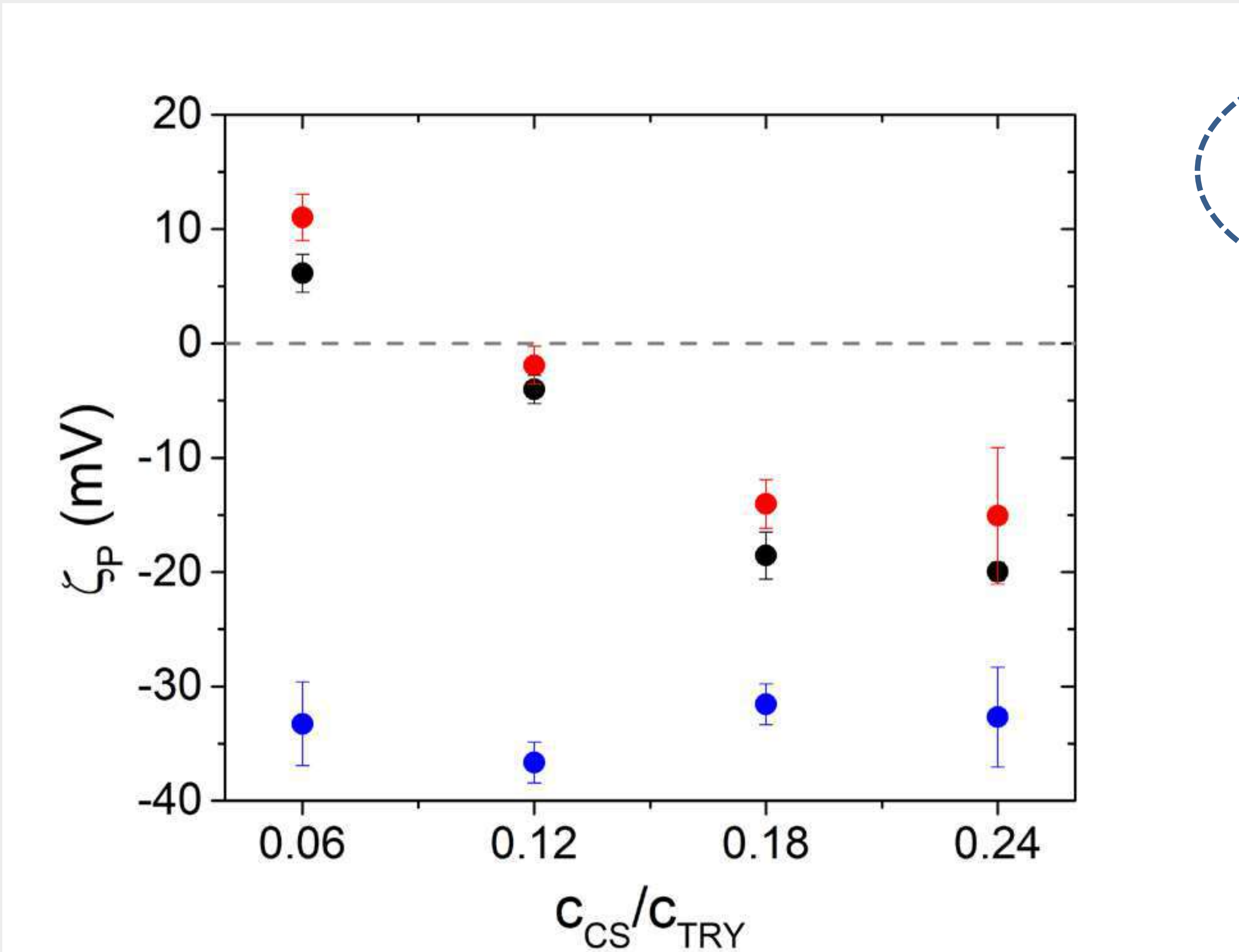
Complexes at pH 4 (black), at pH 4 after thermal treatment (red), at pH 7 after thermal treatment (blue) and at pH 7 without thermal treatment (green).

- Use thermal treatment to stabilize TRY/CS NPs (T=60 °C for 20 min)
- Develop drug/nutrient loaded TRY/CS NPs

Trypsin/chondroitin sulfate NPs: surface charge

Tunable Surface charge

Dependence on CS amount



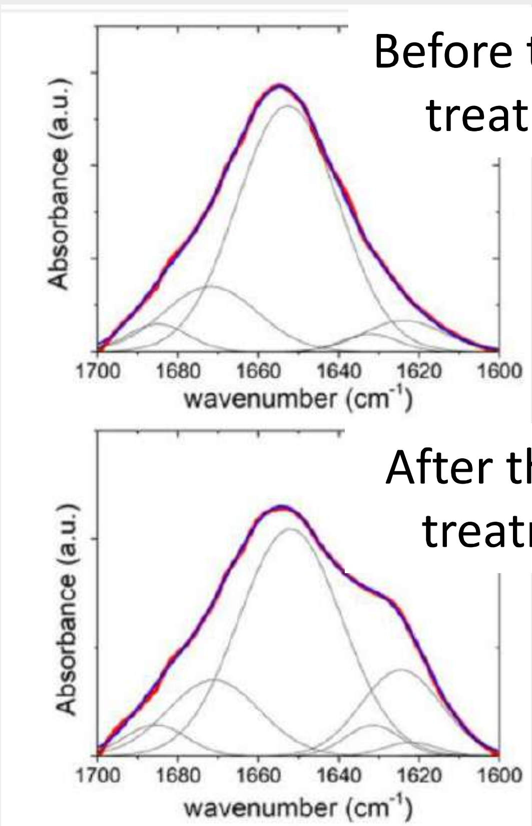
Untreated pH 4

Treated pH 4

Treated pH 4

Protein conformation in NPs

ATR-FTIR amide I region (1700-1600 cm^{-1})



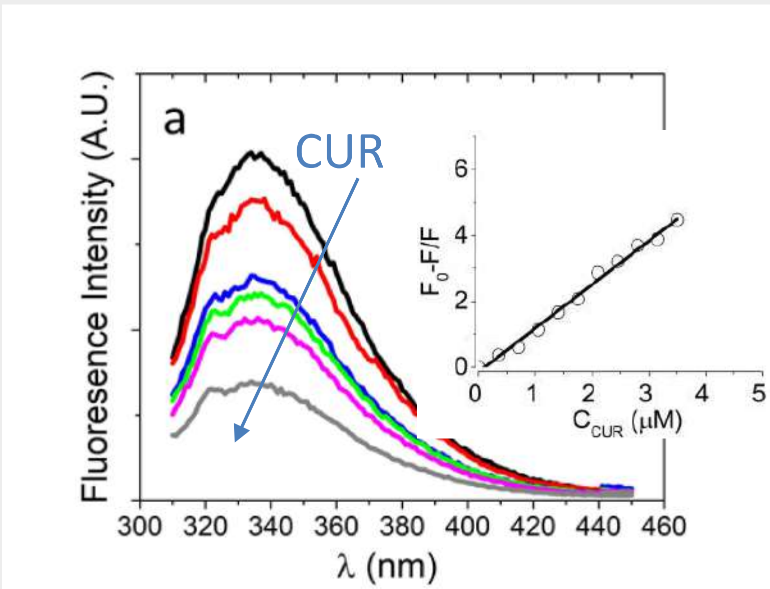
Assignment	β-sheets and β-turns	α-helix	Short-segment chains connecting α-helical segments	Intermolecular β-sheet
Wavenumbers (cm^{-1})	1678–1688	1654–1658	1635–1639	1615–1630
BSA pH 5	19.6	71.8	1.94	6.66
BSA treated pH5	21.0	69.2	2.71	7.04
BSA treated pH 7	20.2	68.3	2.95	8.55
XG-BSA pH5	21.1	68.8	2.34	7.73
XG-BSA treated pH 5	20.2	59.1	3.23	17.5
XG-BSA treated pH 7	23.1	56.3	4.45	16.0
approx. uncertainty in estimation	1.5	3	0.5	1.6

significant increase in intermolecular β-sheet conformation from about 8 to about 17 % upon thermal treatment in complexes

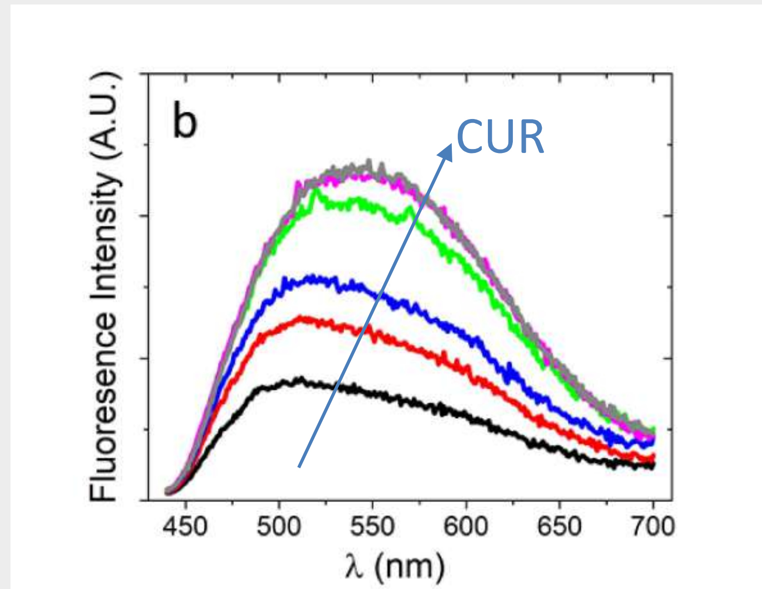
Carbohydrate Polymer Technologies and Applications, 2, **2021**, 100075.

BSA/Xanthan Gum NPs: binding of CUR

Tryptophan fluorescence from thermally treated XG-BSA NPS



CUR fluorescence from thermally treated XG-BSA NPS



Curcumin binds to the hydrophobic domains of the NPs

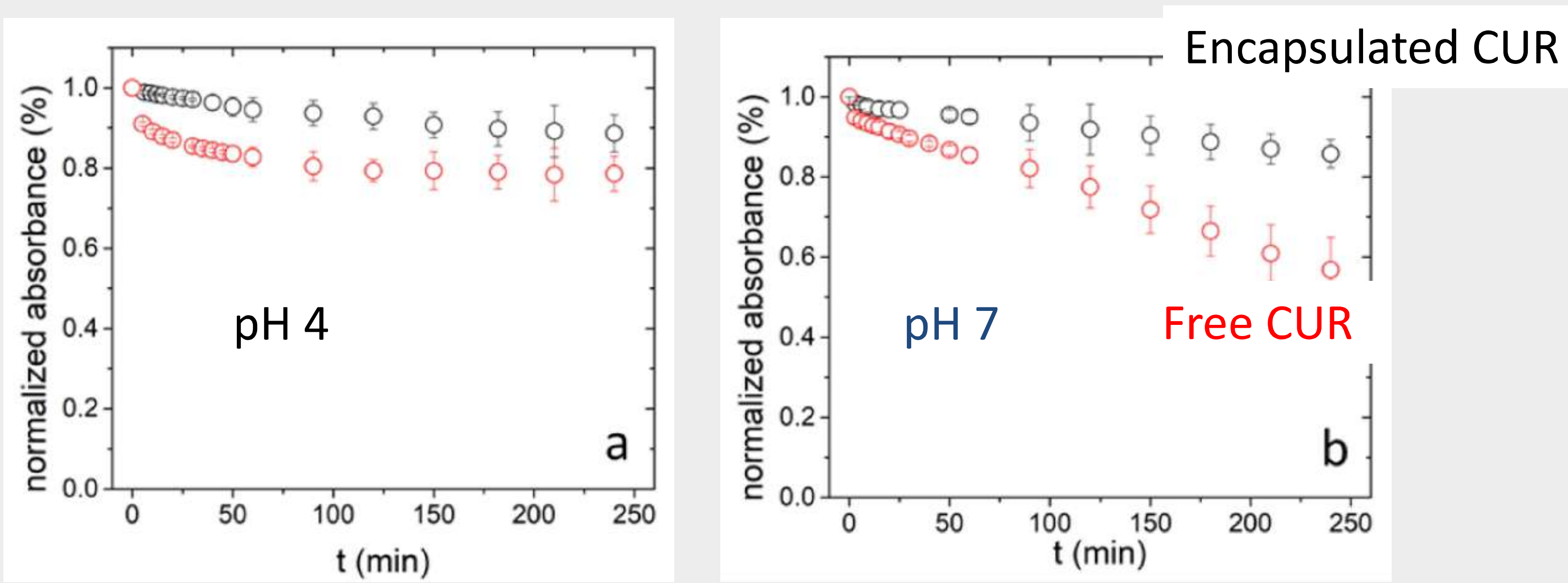
Stern-Volmer constant K_{SV}, binding constant K_A and number of binding sites n from tryptophan fluorescence and binding constant K_B from CUR fluorescence of XG-BSA NPs (thermally treated) at pH 7.

Parameter/sample	$r_m = 6•10^{-2}$	$r_m = 8•10^{-2}$
K_{SV} ($10^6 M^{-1}$)	1.32 ± 0.05	1.29 ± 0.07
K_A ($10^6 M^{-1}$)	0.96 ± 0.12	0.76 ± 0.10
n	1.18 ± 0.13	1.43 ± 0.09
K_B ($10^6 M^{-1}$)	0.10 ± 0.24	0.078 ± 0.013

Carbohydrate Polymer Technologies and Applications, 2, **2021**, 100075

Kinetics of curcumin's degradation

XG-BSA NPs protect curcumin's structure against degradation at neutral pH



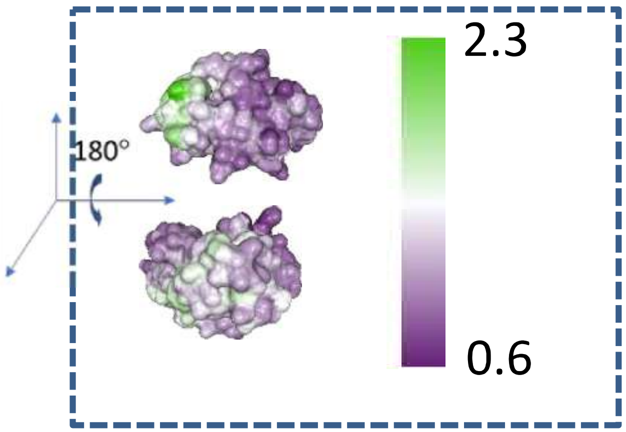
Curcumin's absorbance at 425 nm in free solution (red) and loaded on xanthan -BSA NPs (back) at pH 5 (**a**) and pH 7 (**b**).

Carbohydrate Polymer Technologies and Applications, 2, **2021**, 100075

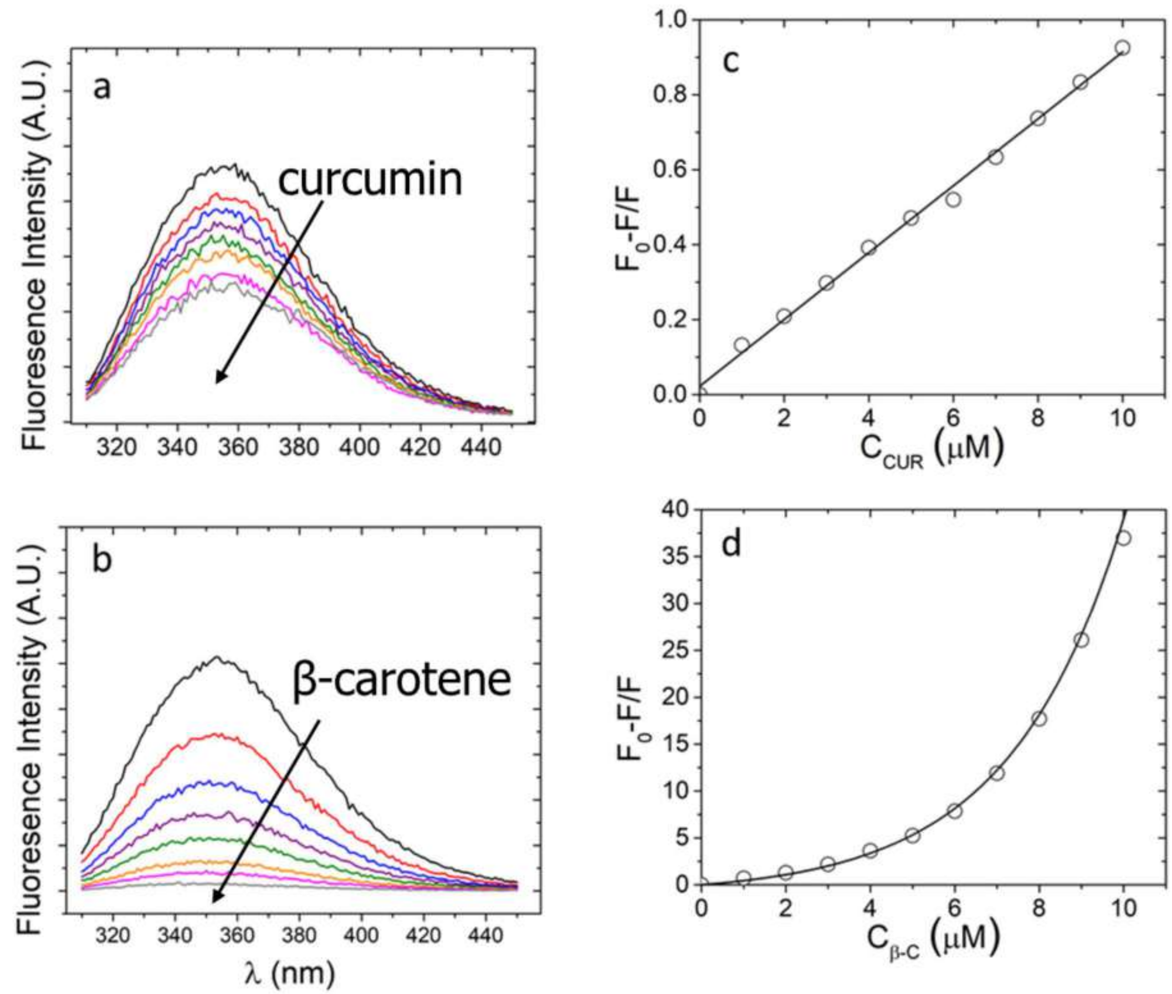
Trypsin/chondroitin sulfate NPs

Binding of nutraceuticals

With protein-sol



Hydrophobicity patch
(non-polar/polar ratio)



Concluding remarks

• Ecofriendly methods with no chemical reactions or toxic solvents/components were used.

• Stable multifunctional NPs by electrostatic polysaccharide/protein complexation and subsequent thermal treatment.

• Tunable size and molar mass.

• Responsive to pH and ionic strength.

• Potential for nanocarriers of nutraceutical compounds.

• Promising for applications in food technology and drug delivery.

• The developed protocols and experimental methods can be applied on other protein-based biomaterials.

Acknowledgements

Prof. Christine M. Papadakis (TUM)
Johannes Allwang (TUM)
Yanan Li (TUM)
Dimitris Selianitis (NHRF)
Angeliki Chroni (NHRF)
Dr. Aurel Radulescu (JCNS)
Dr. Eleni Vlassi (NHRF)
Aggeliki Sklapani (NTUA)

Acknowledgements

Thank you for your attention!

Upcycling of by-products from the agri-food industry into sustainable packaging applications

Rosa González · Packaging Group / Technology Area

rgonzalez@aimplas.es · **10th November 2021**

Index

- AIMPLAS
- Contex
- R&D&i lines
- Upcycling of by-products for packaging applications.

What is AIMPLAS?

A **technology centre** with more than 30 years' experience in the plastic sector.

Our Mission

Add value to companies to generate **wealth** and create **employment**.

Add value to society to improve quality of life and ensure environmental sustainability.

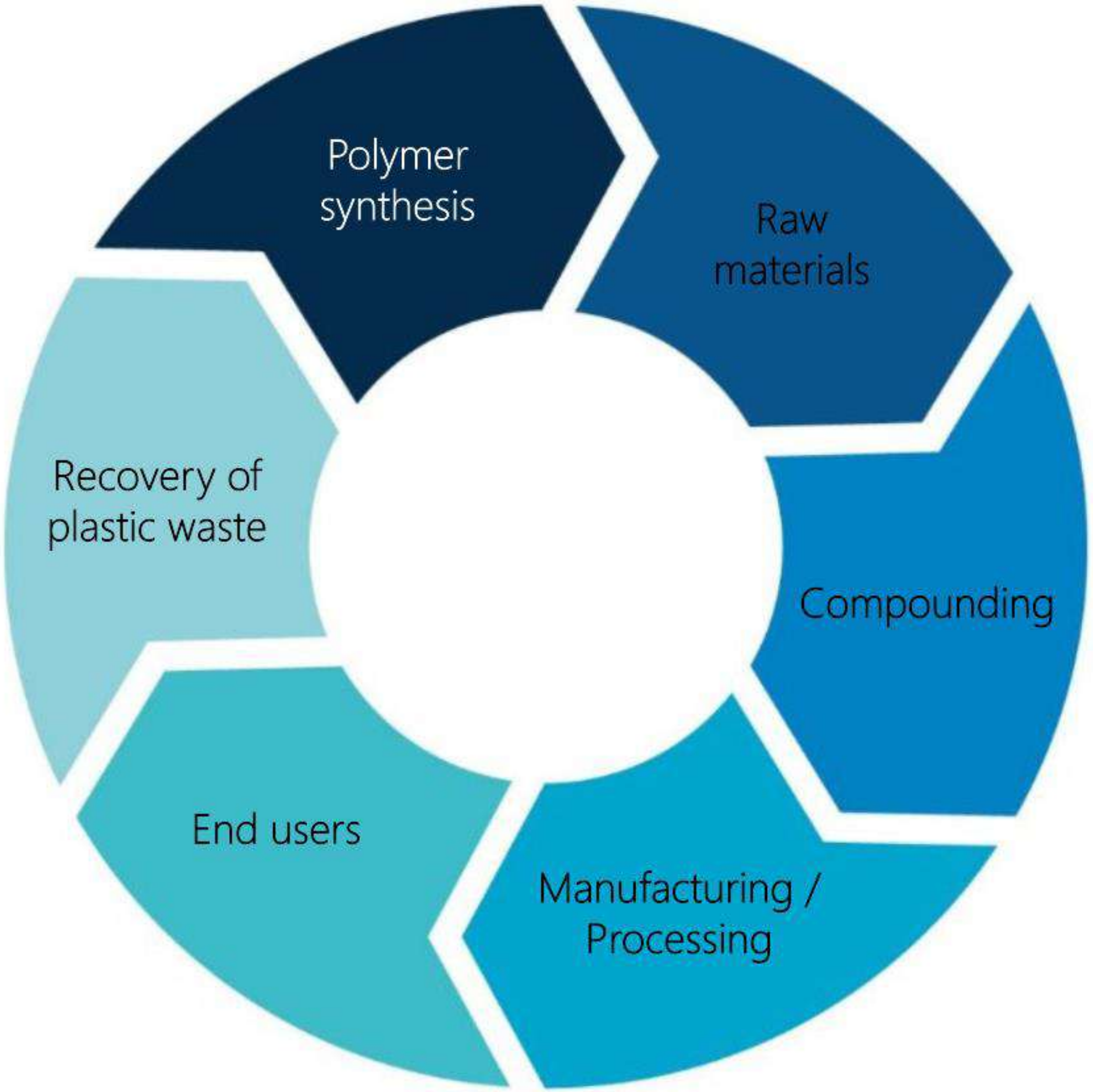
Market Oriented

More than **10,500 m^2** of cutting-edge facilities

Pilot plants (6,000 m^2)

Laboratories (4,500 m^2)

Expertise across the entire plastics value chain



Figures

14.6M€
ingresos

+2.600
clientes

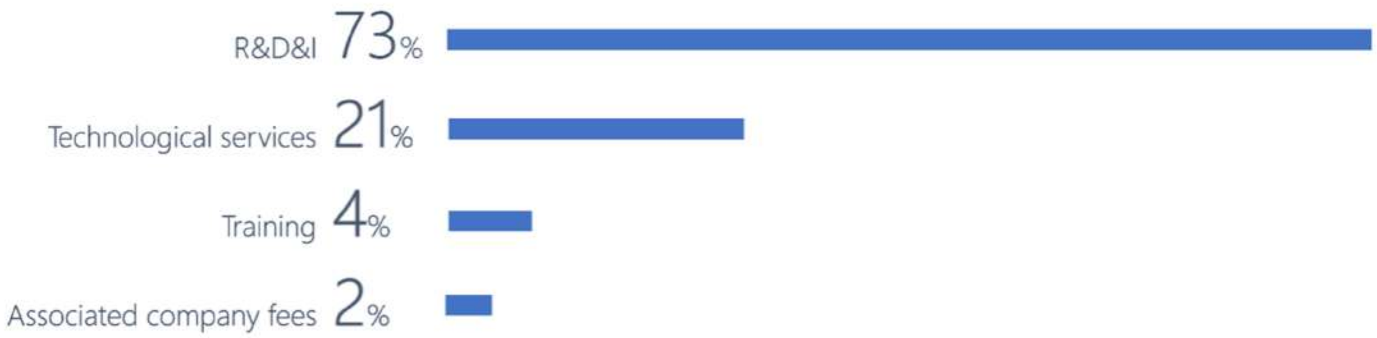
+700
asociados

218
R&D&i projects

5,129
Services

158
Training actions

Revenue by Activity



DATA 2020

SUSTAINABLE DEVELOPMENT GOALS

Decoupling economy growth from environmental degradation, increasing resource efficiency and promoting sustainable lifestyles.

Global efforts focused on the valorization of by-products and wate from different sources.

R&D&i LINES

CIRCULAR eCOnomy by AIMPLAS

SUSTAINABLE AND FUNCTIONAL PACKAGING

- Biopolymers with improved performances (barrier, thermal, mechanical, ...)
- Functional coatings and adhesives

  Bio-coatings and bio-adhesives
- Bipolymers for single use applications
- Design for reusable packaging and assessment
- Recycled materials and packaging recyclability
- By-products and waste valorization in polymers, additives, ...

Biodegradability/ Compostability testing

Ecolabels certification

Safety according to current legislation

UPCYCLING OF BY-PRODUCTS FOR PACKAGING PRODUCTION

Different sources




Different components to valorize

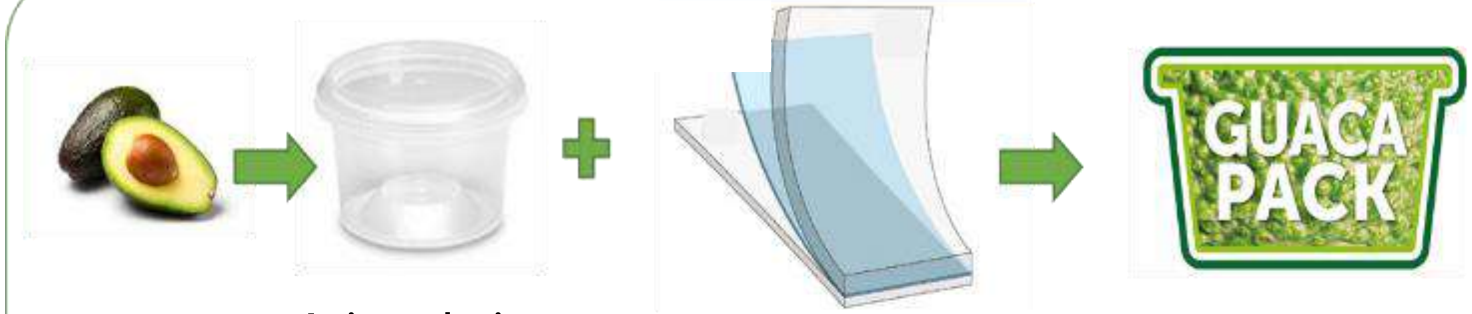
UPCYCLING OF BY-PRODUCTS FOR PACKAGING PRODUCTION

AVOCADO

Skins and seeds from guacamole production

Polyphenols (antioxidants)

Starch (oxygen barrier)



Active packaging (Antioxidant)

Barrier label IML (In-mould labelling)

Sustainable active barrier packaging

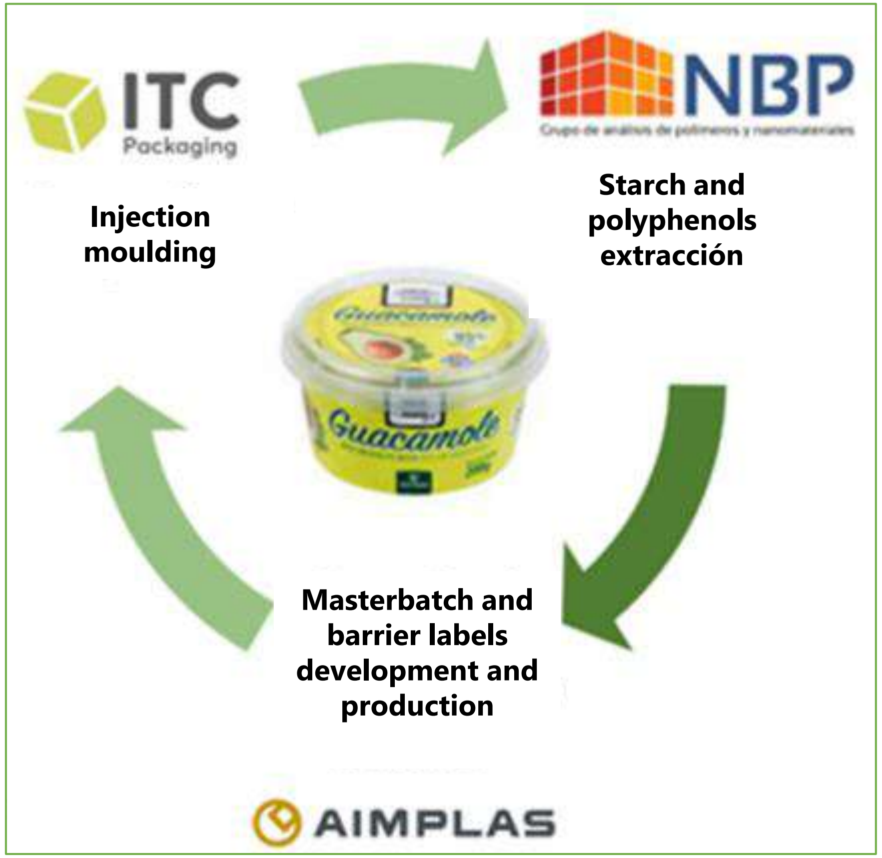
GUACA PACK

GENERALITAT VALENCIANA | TOTS A UNA VEU

AVI AGÈNCIA VALENCIANA DE LA INNOVACIÓ

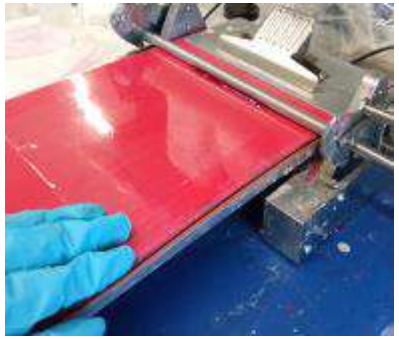
El proyecto GUACAPACK 'Desarrollo de envases compostables con propiedades barrera al oxígeno a partir de residuos de aguacate ha sido financiado por la Agencia Valenciana de la Innovación.

UPCYCLING OF BY-PRODUCTS FOR PACKAGING PRODUCTION



Duration: 2 years (2020-2021)
Strategic Project in Cooperation Comunidad Valenciana

AIMPLAS



IML Label	OTR cm3/(m2·día) (23ºC, 50% HR)
Film PLA commercial (30 microns)	555
Film PLA commercial + coating AS (30 microns)	**<10**

BARRIER ACTIVE PACKAGING, COMPOSTABLE WITH REDUCED ENVIRONMENTAL IMPACT

GUACA PACK
GENERALITAT VALENCIANA | TOTS A UNA VEU
AVI AGÈNCIA VALENCIANA DE LA INNOVACIÓ

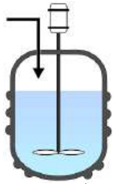
El proyecto GUACAPACK 'Desarrollo de envases compostables con propiedades barrera al oxígeno a partir de residuos de aguacate ha sido financiado por la Agencia Valenciana de la Innovación.

UPCYCLING OF BY-PRODYCTS FOR PACKAGING PRODUCTION

Barley, malt

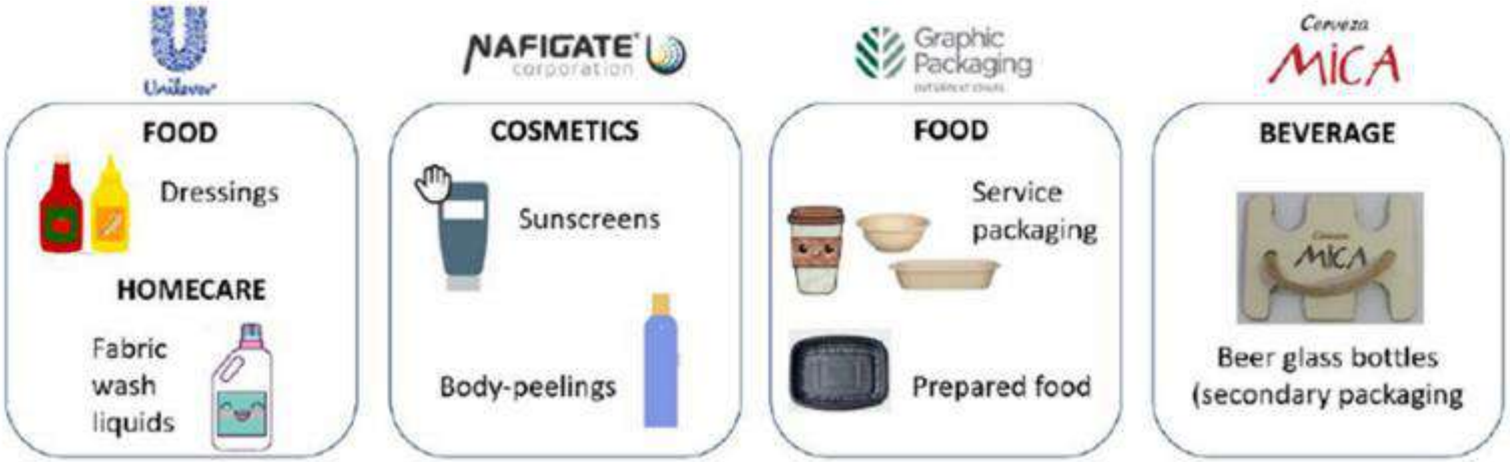
Brew Spent Grains from beer production

Sugars for enzymatic fermentation



PHAs

Packaging for food, beverage and cosmetics



MATERIALS & PACKAGING WITH LOWER ENVIRONMENTAL IMPACT

New biobased packaging solutions.

Enzymatic recycling of future packaging waste.

BIO SUP PACK

Creation of a new value chain

This project has received funding from the Bio Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme.

BBI JU
Bio-based Industries Consortium
Horizon 2020 European Union Funding for Research & Innovation

BIOSUPPACK PROJECT

Starting: 1st June 2021

17 partners

8 EU countries

Multisectorial project

42 months

TRL final: 7-8

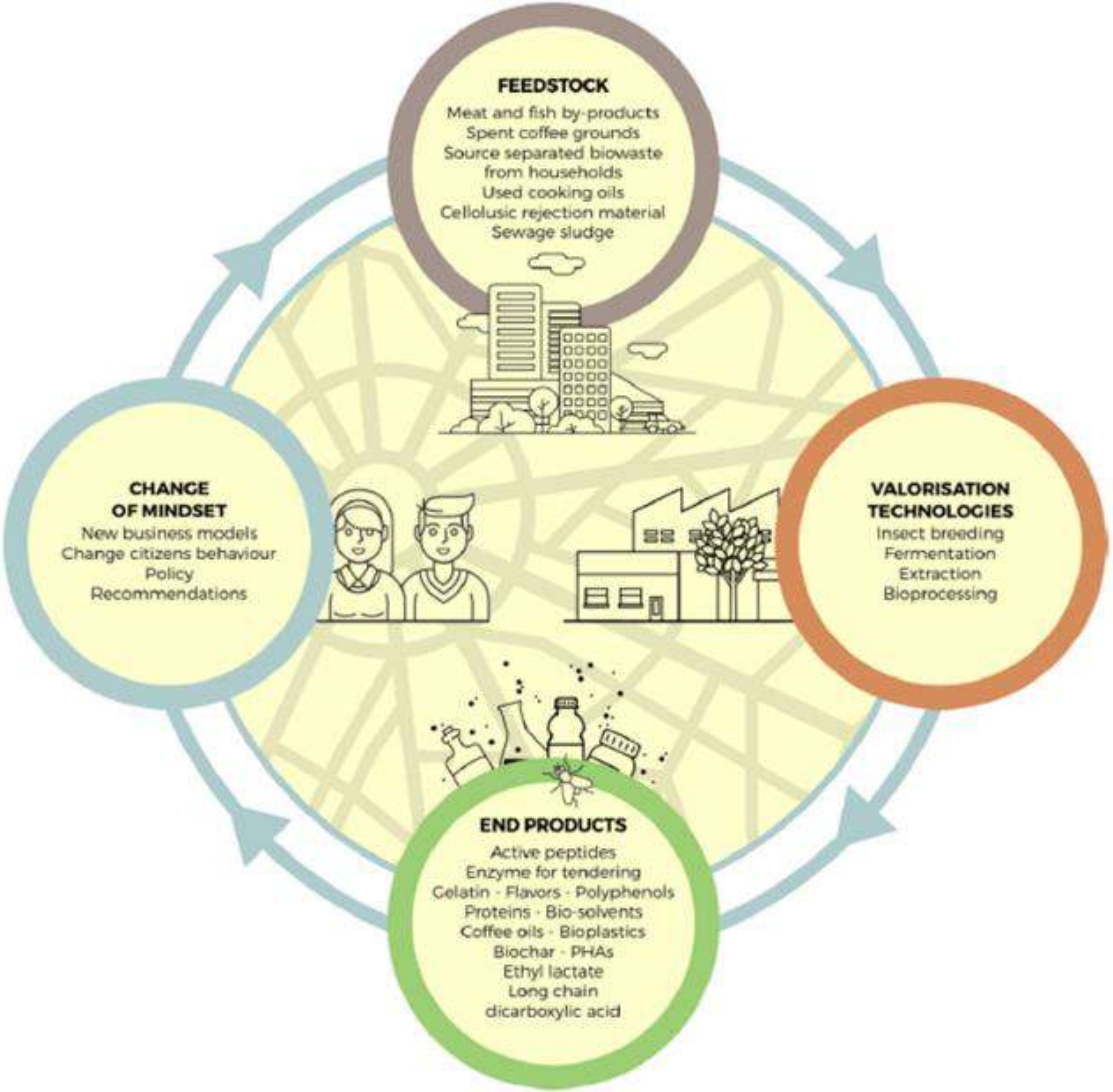
This project has received funding from the Bio Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme.

UPCYCLING OF BY-PRODUCTS FOR PACKAGING PRODUCTION

WaysTUP!

VALUE CHAINS FOR DISRUPTIVE TRANSFORMATION OF URBAN BIOWASTE INTO BIOBASED PRODUCTS IN THE CITY CONTEXT

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 818308.



UPCYCLING OF BY-PRODUCTS FOR PACKAGING PRODUCTION

COFFEE

HORECA waste

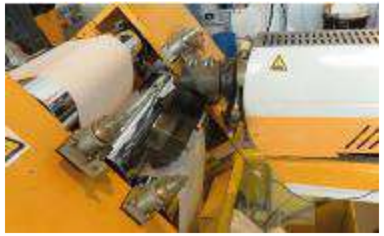
Flavours, polyphenols, oils, carotenoids
Active Ingredients food/pharma

Oils for enzymatic fermentation

Biopolyesters

AIMPLAS

Formualtion development for film extrusion



Hygiene and personal care packaging

WaysTUP!

VALUE CHAINS FOR DISRUPTIVE TRANSFORMATION OF URBAN BIOWASTE INTO BIOBASED PRODUCTS IN THE CITY CONTEXT

COMPOUNDS, MATERIALS AND PACKAGING WITH LOWER ENVIRONMENTAL IMPACT

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 818308.

UPCYCLING OF BY-PRODUCTS FOR PACKAGING PRODUCTION

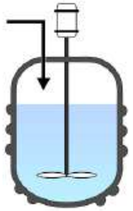
BiOrangePack

Active coatings

AIMPLAS

CITRUS FRUIT

Orange skin and pulp



Biocative serums

AIMPLAS

Edible active coatings

PRIMA

The PRIMA programme is supported under Horizon 2020, the European Union's Framework Programme for Research and Innovation

ACTIVE COATINGS AND PACKAGING WITH REDUCED ENVIRONMENTAL IMPACT

THANK YOU!

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MANCHESTER
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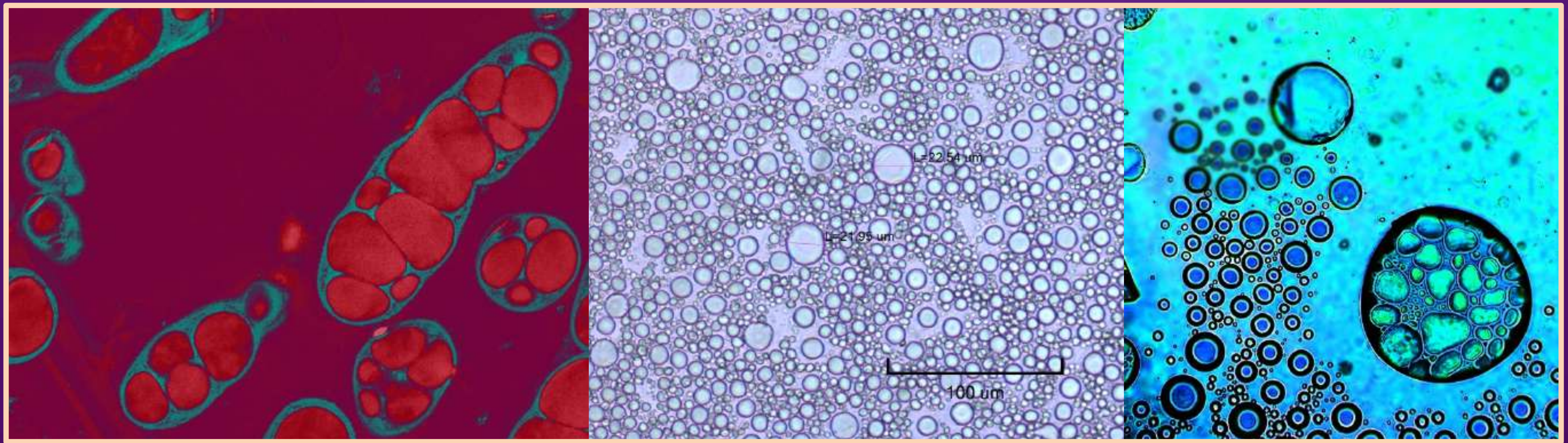
Integrated biorefining for the production of biobased chemicals from a range of feedstocks

Dr James Winterburn

Department of Chemical Engineering and Analytical Science

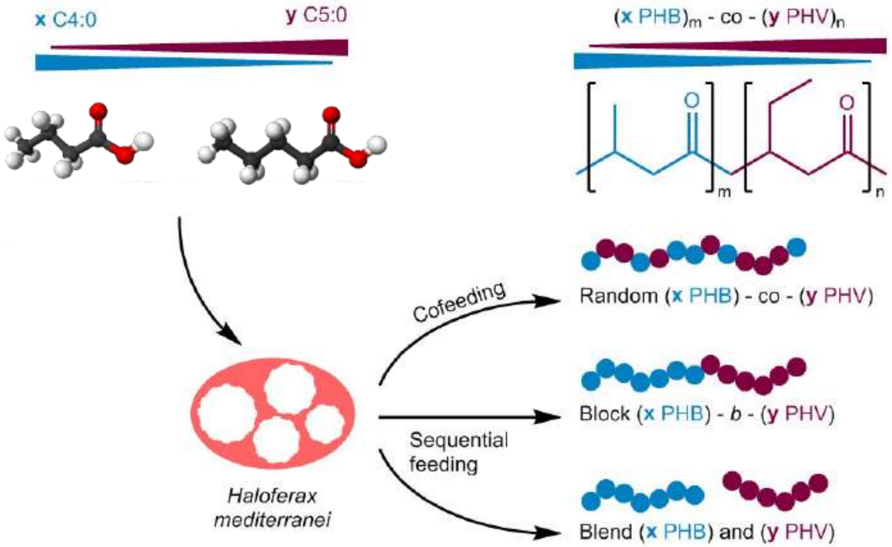
The University of Manchester, UK

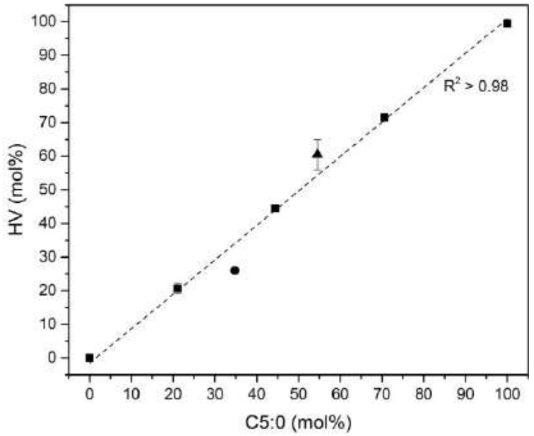


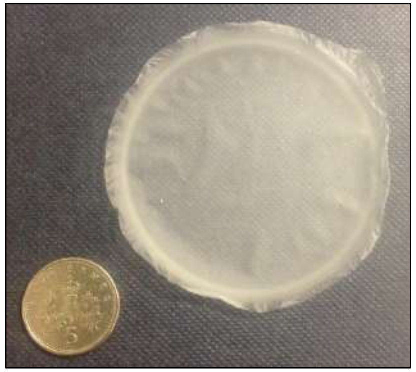
Biopolymers

Bespoke biopolymer production - control over PHBV composition

- *Haloferax mediterranei* – produces PHBV copolymer
- C4:0/C5:0 ratio in the feed is proportional to HB/HV fraction in PHBV

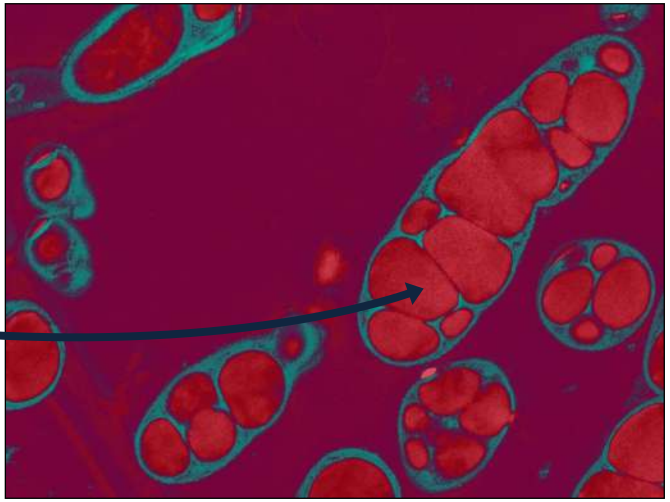




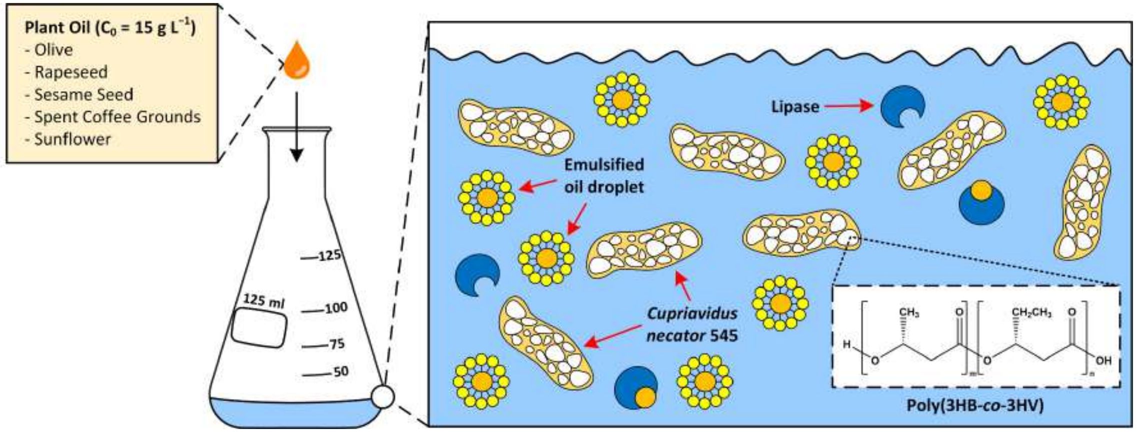


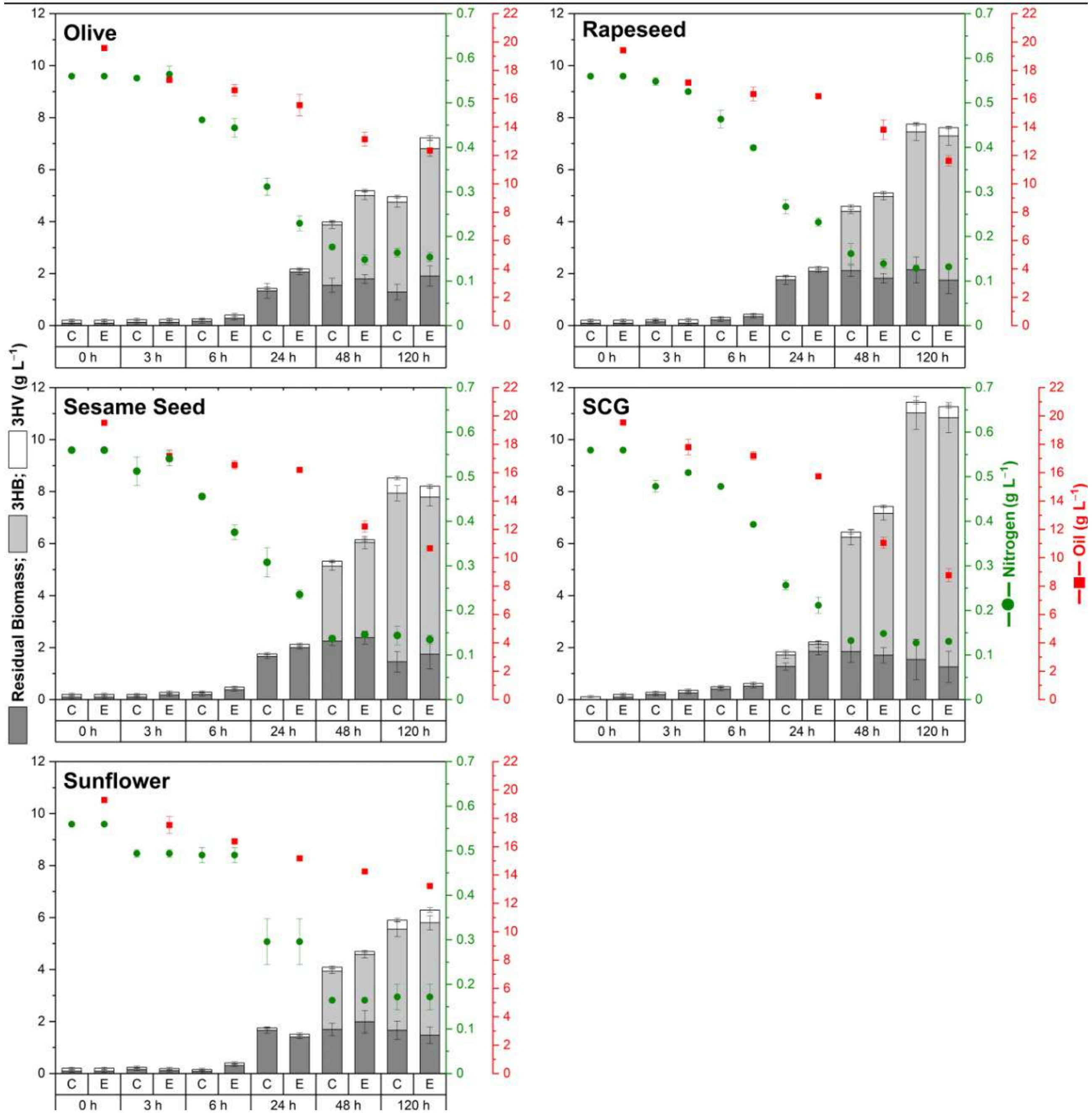
Waste biomass valorisation

- Production of PHB from waste biomass



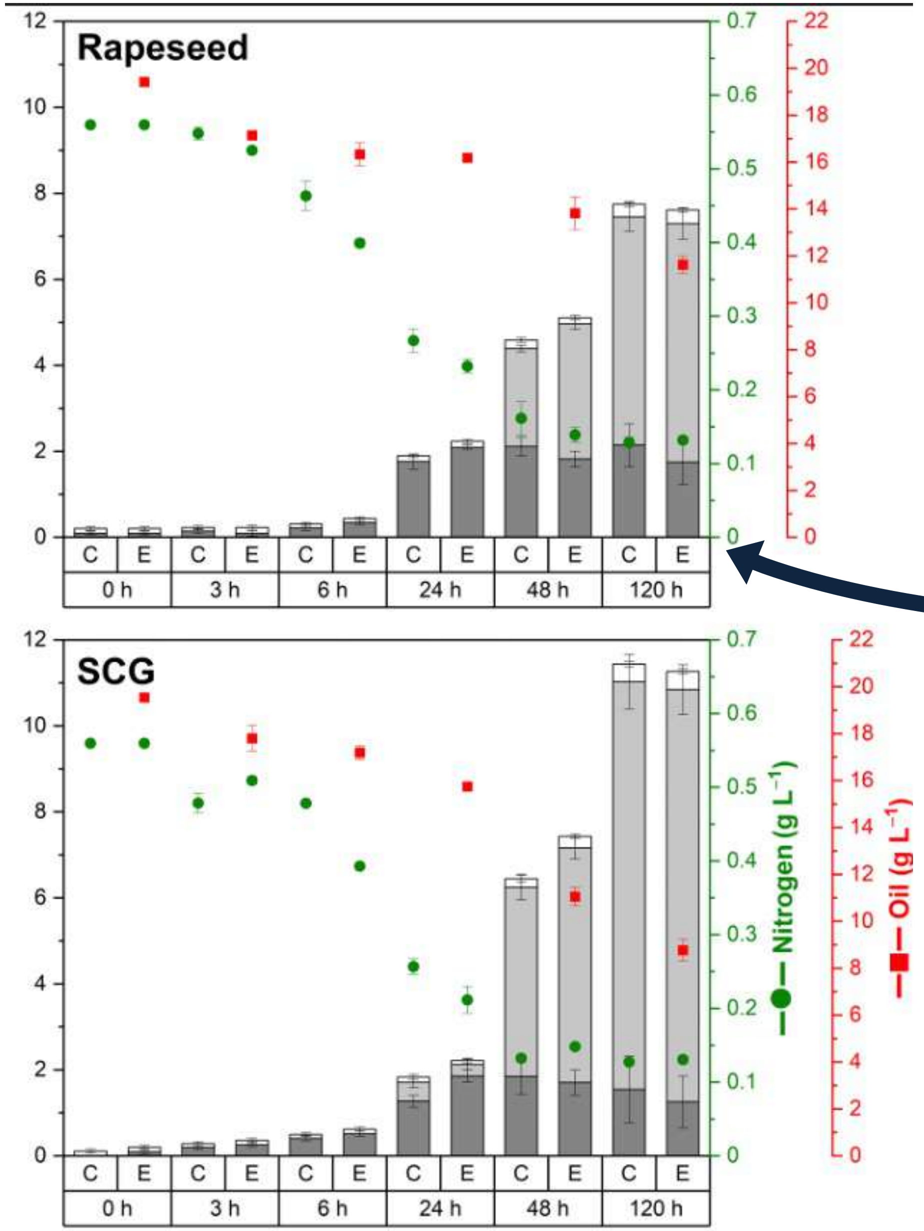
PHBV Production from SCG

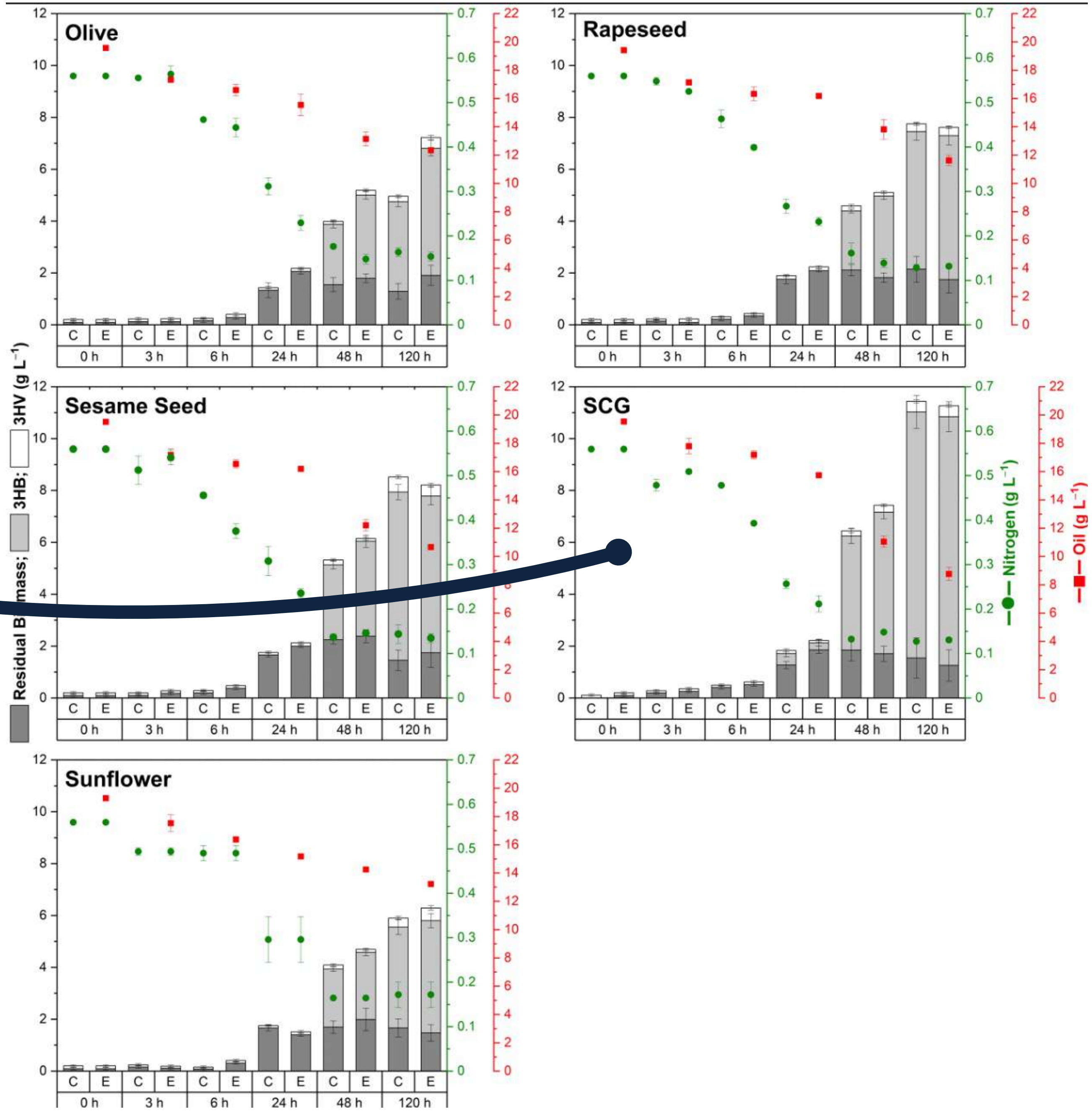




Ingram H and Winterburn JB (2020) Anabolism of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by *Cupriavidus necator* DSM 545 from Spent Coffee Grounds Oil. *New Biotechnology*, 60, 12-19

PHBV Production from SCG





Ingram H and Winterburn JB (2020) Anabolism of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by *Cupriavidus necator* DSM 545 from Spent Coffee Grounds Oil. *New Biotechnology*, 60, 12-19

Rapeseed meal (RSM)

MANCHESTER 1824
The University of Manchester

What is Rapeseed Real (RSM)?

Rapeseed Production

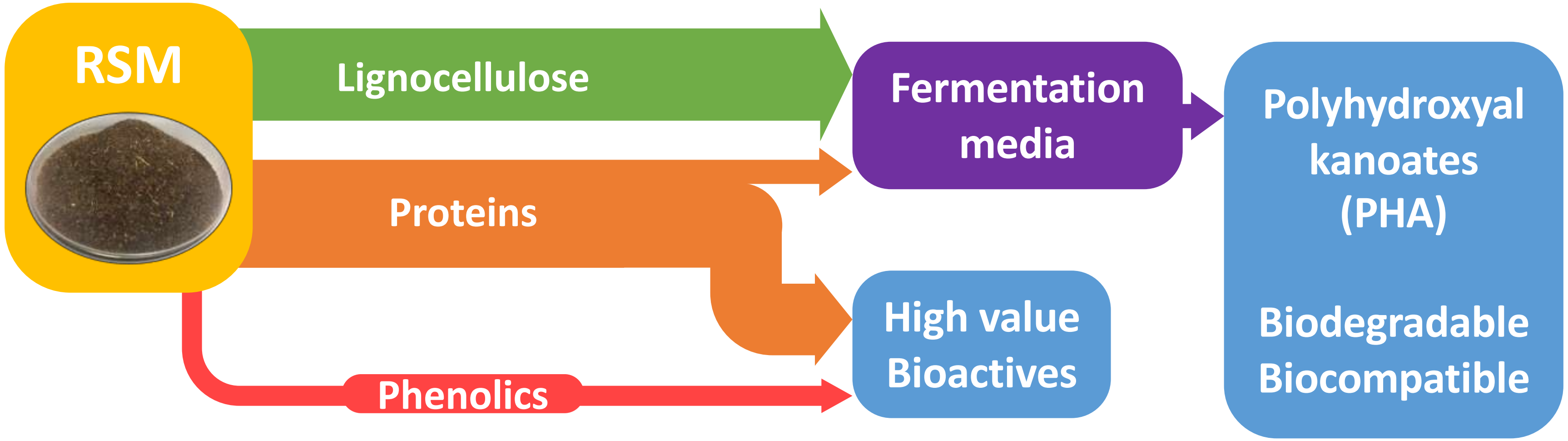
<u>Global annual production</u>[1]

- Rapeseed: ~90 million tons per annum
- 2-3 million tons per annum in the UK
- **RSM:**
    - **~250 USD per ton**

RSM Valorisation

RSM Composition

Glucose	10.9	% wt
Xylose	2.41	% wt
Galactose	3.56	% wt
Arabinose	5.22	% wt
Proteins	28.1	% wt
Phenolics:	16.1	mg/g



1. George, B., Caldwell, A. & Snyder, M. 2020. Oilseeds: World Markets and Trade. USDA.

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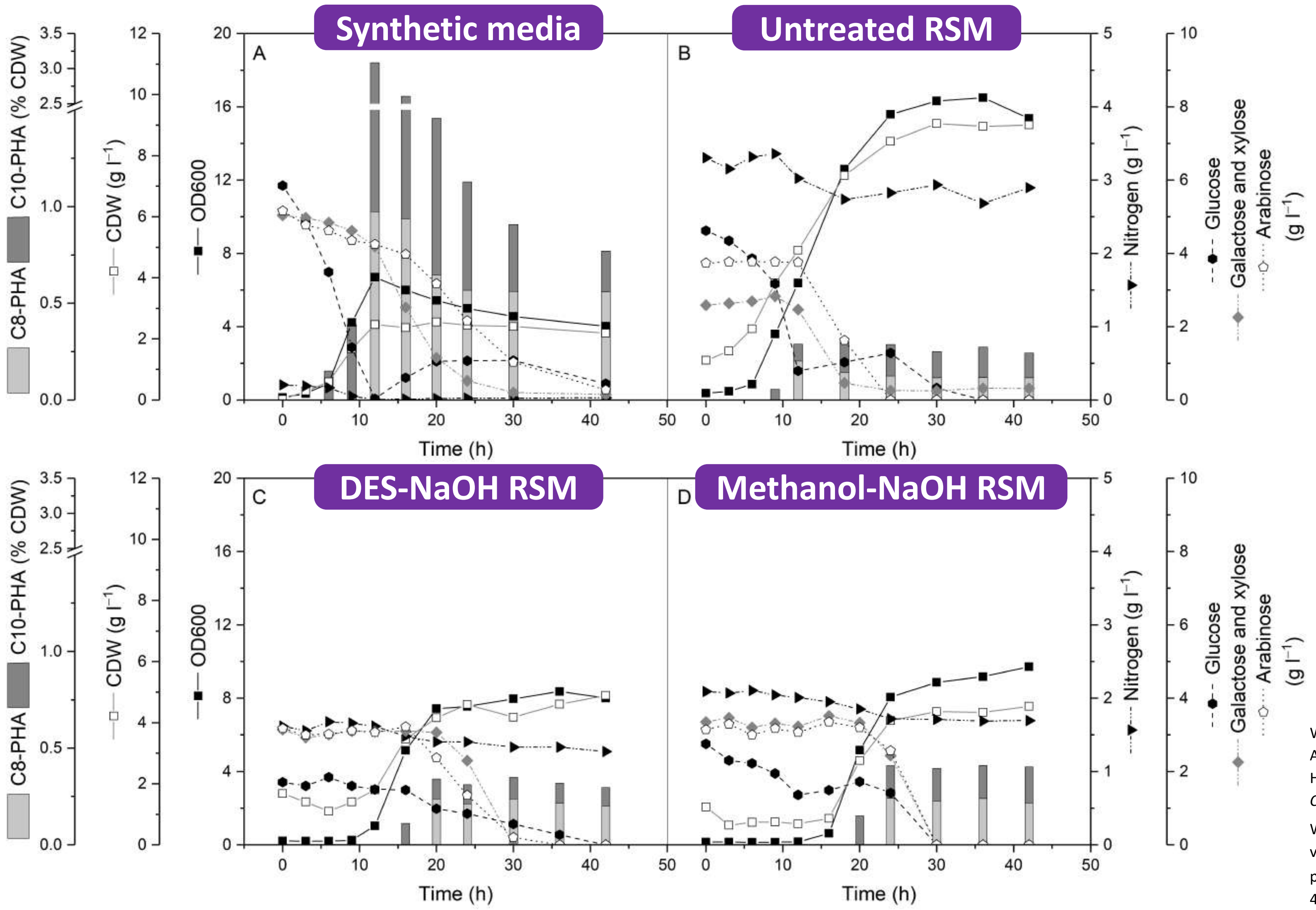
The University of Manchester

RSM fermentation viability after integration

P. putida bioreactor fermentation

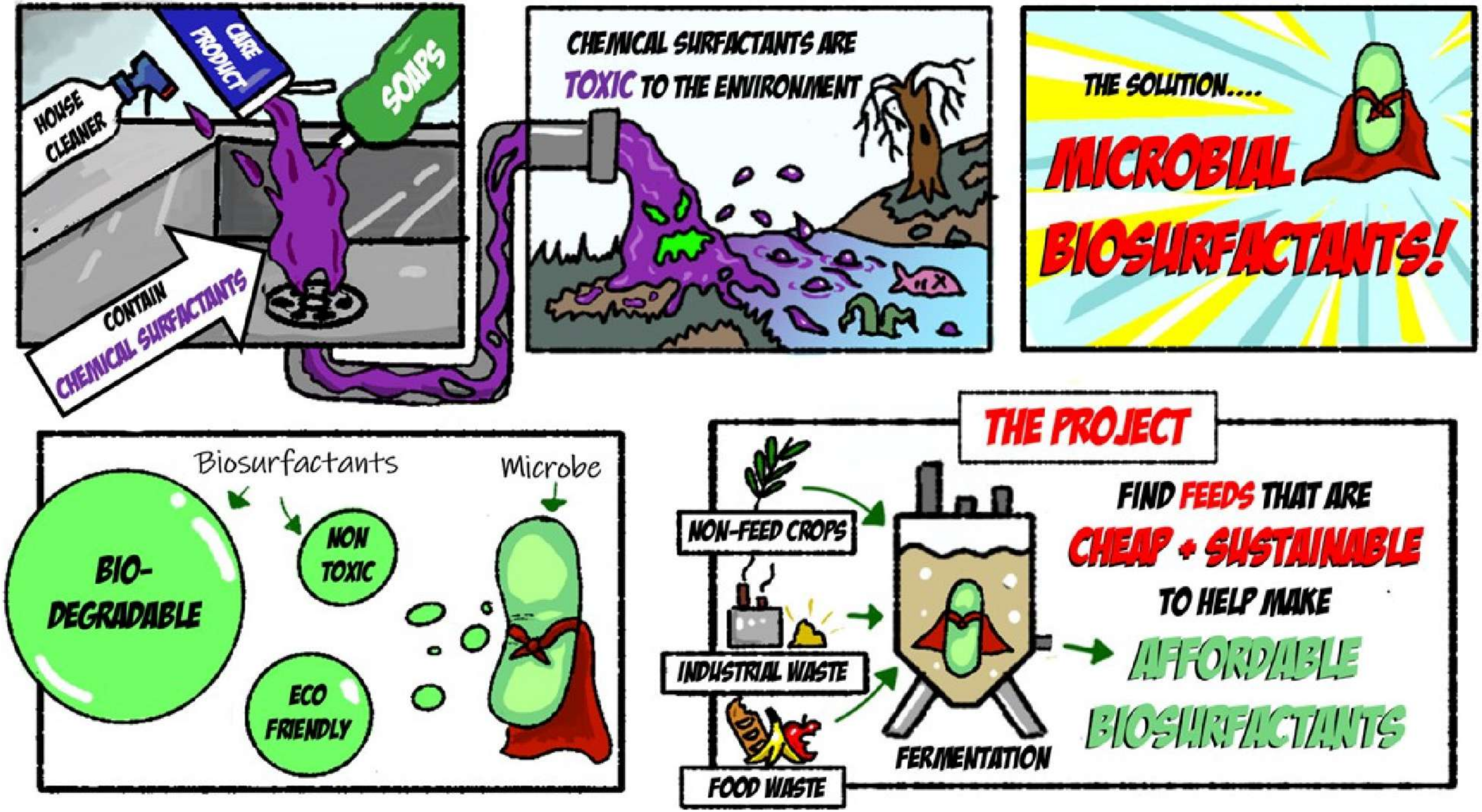


Key findings:

- RSM viable at bioreactor scale
- **Nitrogen-limited** conditions crucial for PHA accumulation

Wongsirichot P, Gonzalez-Miquel M and Winterburn JB (2020) Integrated Biorefining Approach for the Production of Polyhydroxyalkanoates from Enzymatically Hydrolyzed Rapeseed Meal under Nitrogen-Limited Conditions. *ACS Sustainable Chemistry and Engineering*, 8(22), 8362–8372.

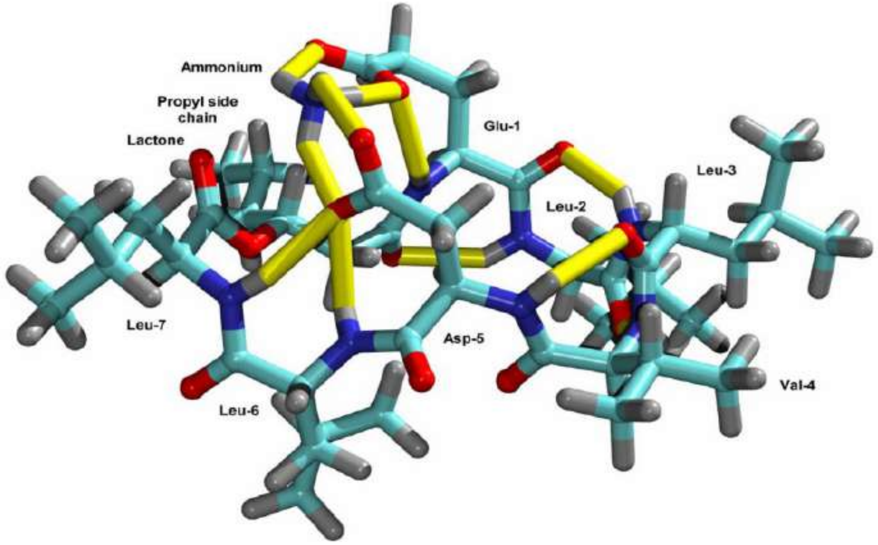
Wongsirichot P, Gonzalez-Miquel M and Winterburn JB (2020) Rapeseed meal valorization strategies via nitrogen- and oxygen-limited production of polyhydroxyalkanoates with *Pseudomonas putida. Waste Management*, 105, 482-491.

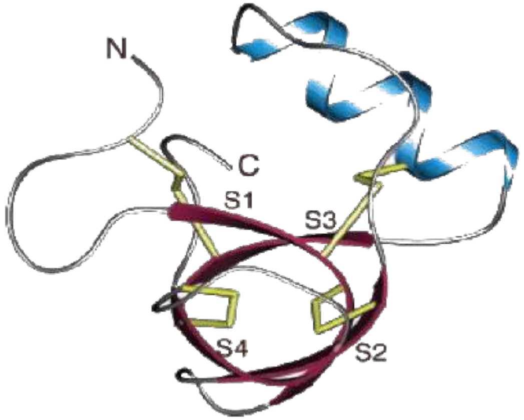


HOUSE CLEANER
CARE PRODUCT
SOAPS
CONTAIN CHEMICAL SURFACTANTS
CHEMICAL SURFACTANTS ARE TOXIC TO THE ENVIRONMENT
THE SOLUTION....
MICROBIAL BIOSURFACTANTS!
Biosurfactants
Microbe
BIO-DEGRADABLE
NON TOXIC
ECO FRIENDLY
THE PROJECT
NON-FEED CROPS
INDUSTRIAL WASTE
FOOD WASTE
FERMENTATION
FIND FEEDS THAT ARE CHEAP + SUSTAINABLE TO HELP MAKE AFFORDABLE BIOSURFACTANTS

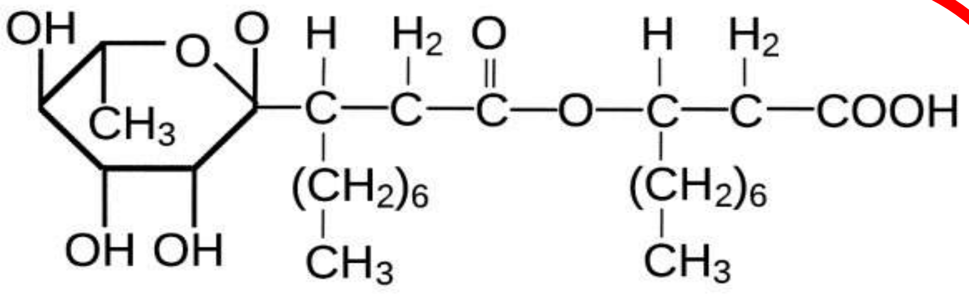
Biosurfactants

Surfactin

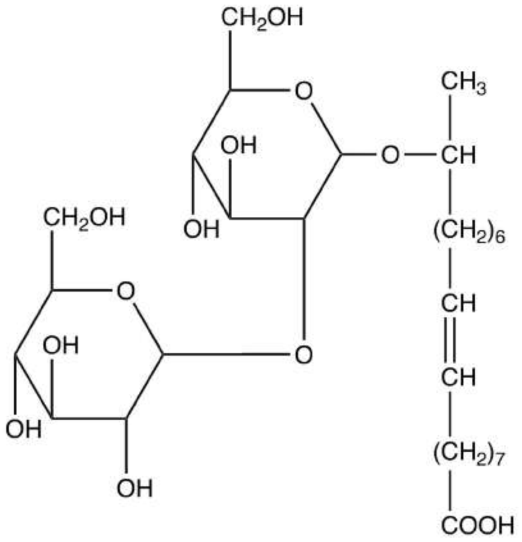


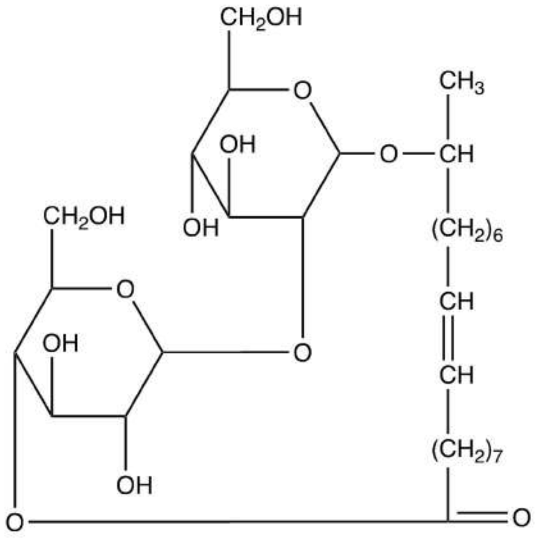


HFBII



Rhamnolipid





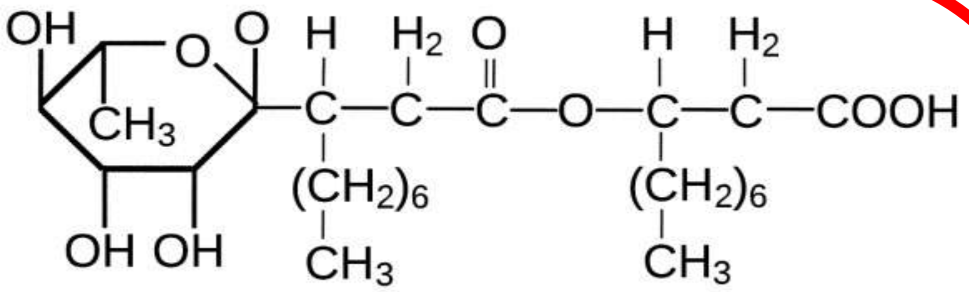
Acidic

Lactonic

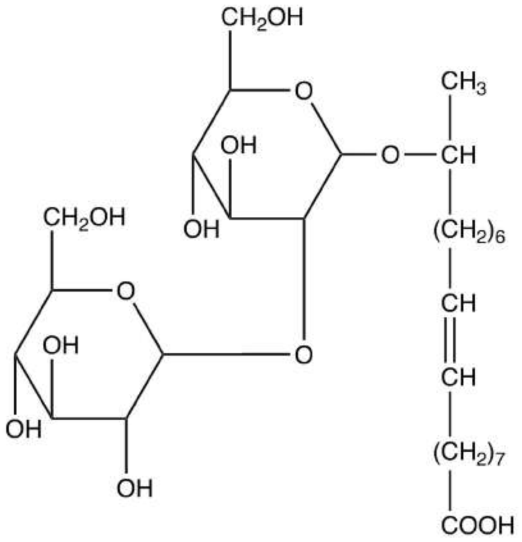
Sophorolipids

Chen C-Y et al (2006) *J. Chem. Technol. Biotechnol.* **81**. Hakanpää J et al (2004) *J. Bio. Chem.* **279.** Marchant R et al (2012) *Biotechnol. Lett.*

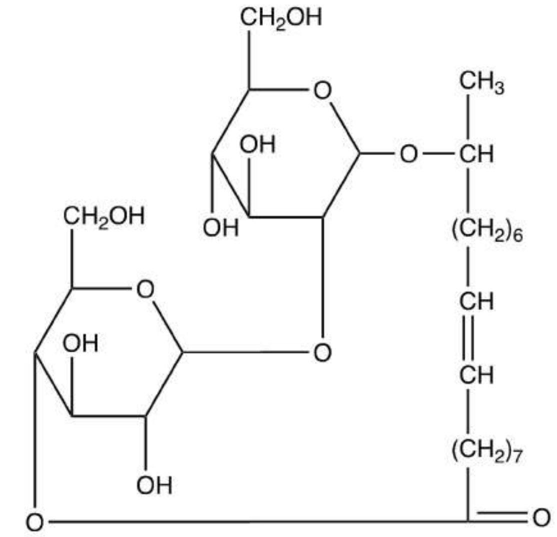
Biosurfactants



Rhamnolipid



Acidic

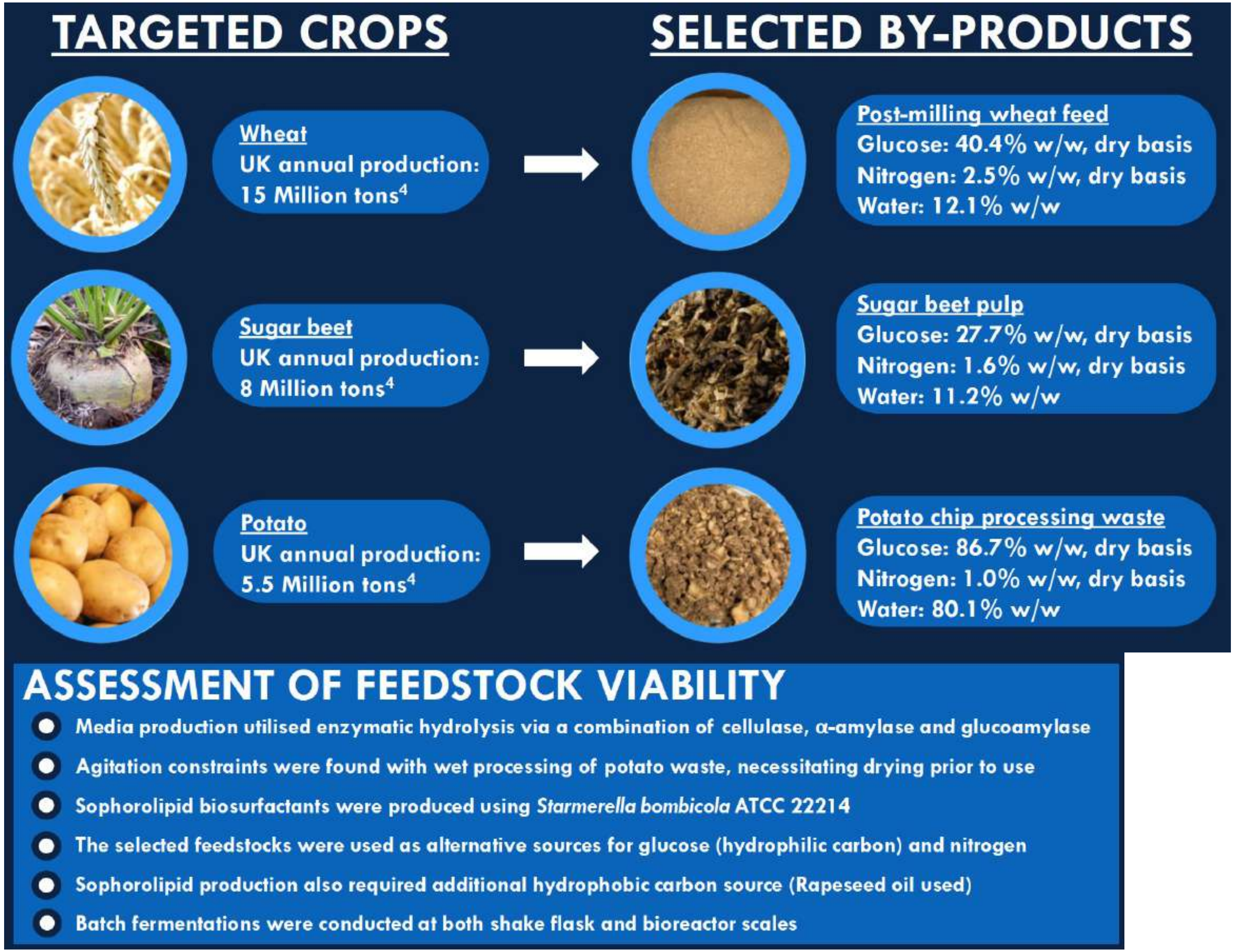


Lactonic

Sophorolipids

Chen C-Y et al (2006) *J. Chem. Technol. Biotechnol.* **81**. Hakanpää J et al (2004) *J. Bio. Chem.* **279.** Marchant R et al (2012) *Biotechnol. Lett.*

Sophorolipids from Waste

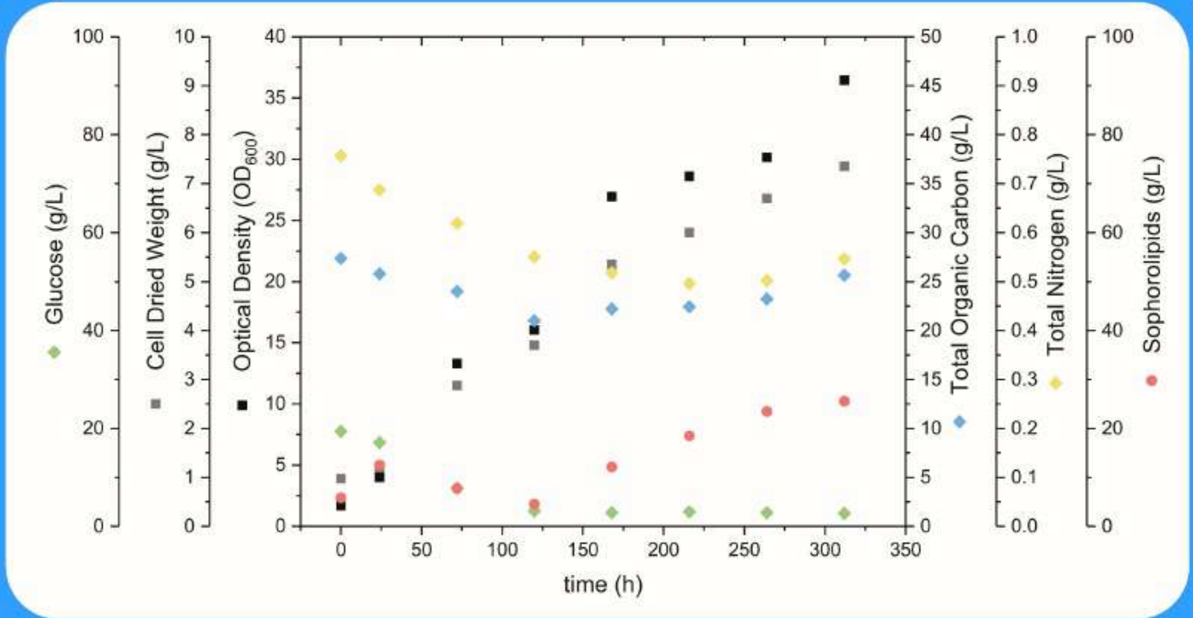


Wongsirichot, P., Ingham, B., Winterburn, J. 2021. A review of sophorolipid production from alternative feedstocks for the development of a localized selection strategy. Journal of Cleaner Production, 319, 128727.

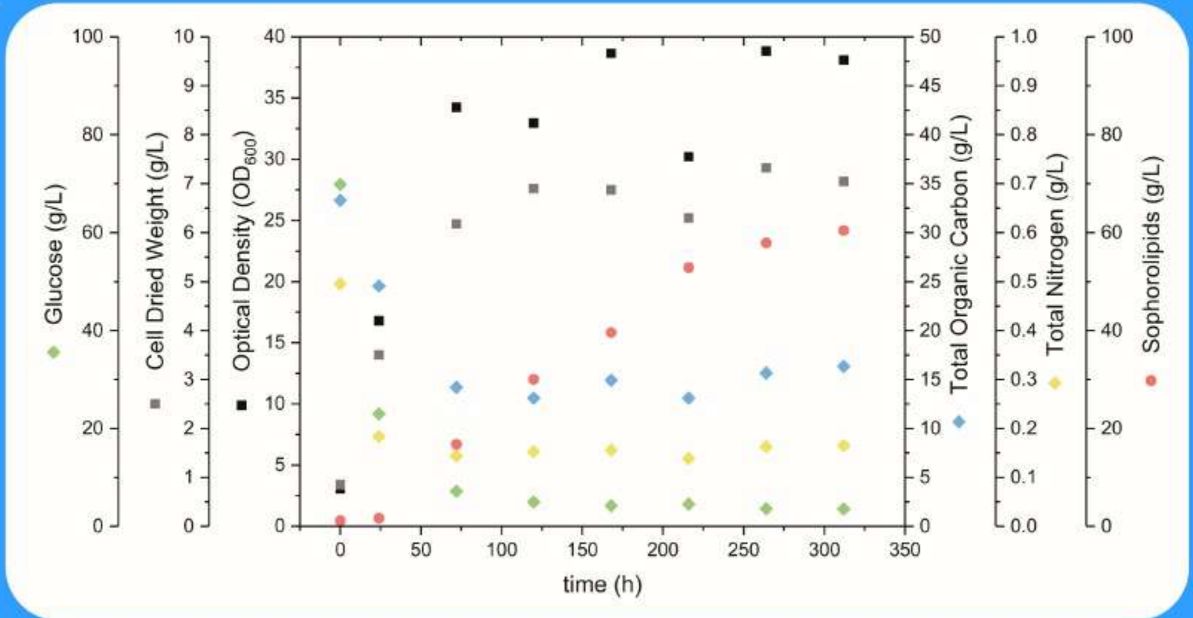
Sophorolipids from Waste

BIOREACTORS

Sugar beet



Potato



ASSESSMENT OF FEEDSTOCK VIABILITY

- Media production utilised enzymatic hydrolysis via a combination of cellulase, α-amylase and glucoamylase
- Agitation constraints were found with wet processing of potato waste, necessitating drying prior to use
- Sophorolipid biosurfactants were produced using *Starmerella bombicola* ATCC 22214
- The selected feedstocks were used as alternative sources for glucose (hydrophilic carbon) and nitrogen
- Sophorolipid production also required additional hydrophobic carbon source (Rapeseed oil used)
- Batch fermentations were conducted at both shake flask and bioreactor scales

Wongsirichot, P., Ingham, B., Winterburn, J. 2021. A review of sophorolipid production from alternative feedstocks for the development of a localized selection strategy. Journal of Cleaner Production, 319, 128727.

Acknowledgements

holiferm

FoodWasteNet

EPSRC
Pioneering research and skills

BBSRC
bioscience for the future

BioProNET

UKRI
Innovate UK

MANCHESTER 1824
The University of Manchester

james.winterburn@manchester.ac.uk

ARISTOTLE UNIVERSITY OF THESSALONIKI
DEPARTMENT OF CHEMISTRY

CENTRE FOR RESEARCH AND TECHNOLOGY-HELLAS
CHEMICAL PROCESS & ENERGY RESOURCES INSTITUTE

"Integrated biorefinery for waste lignocellulosic biomass valorization to fuels, chemicals and polymers"

Konstantinos S. Triantafyllidis

(1) Department of Chemistry and Center for Interdisciplinary Research and Innovation (CIRI), Aristotle University of Thessaloniki (AUTH), Greece

(2) Chemical Process & Energy Resources Institute (CPERI/CERTH), Thessaloniki, Greece

* email: ktrianta@chem.auth.gr

Online Workshop

"Sustainable Production of Biobased Products in the Bioeconomy Era"

10 November 2021, Agricultural University of Athens

FOOD INNOVATION Research Infrastructure

European Union
European Regional Development Fund

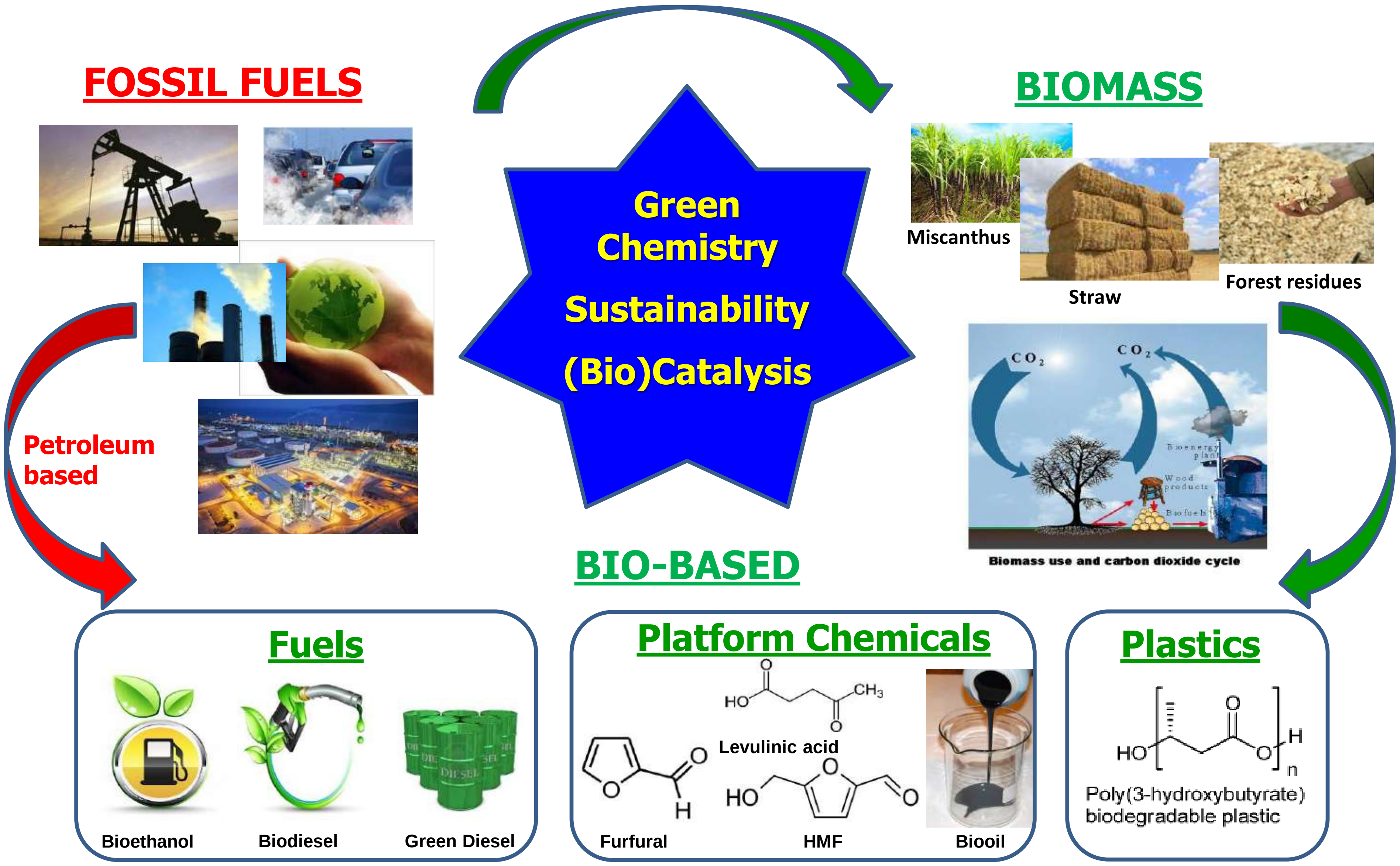
HELLENIC REPUBLIC
MINISTRY OF DEVELOPMENT AND INVESTMENTS
SPECIAL SECRETARIAT FOR ERDF & CF PROGRAMMES
MANAGING AUTHORITY OF EPAnEK

EPAnEK 2014-2020
OPERATIONAL PROGRAMME
COMPETITIVENESS
ENTREPRENEURSHIP
INNOVATION

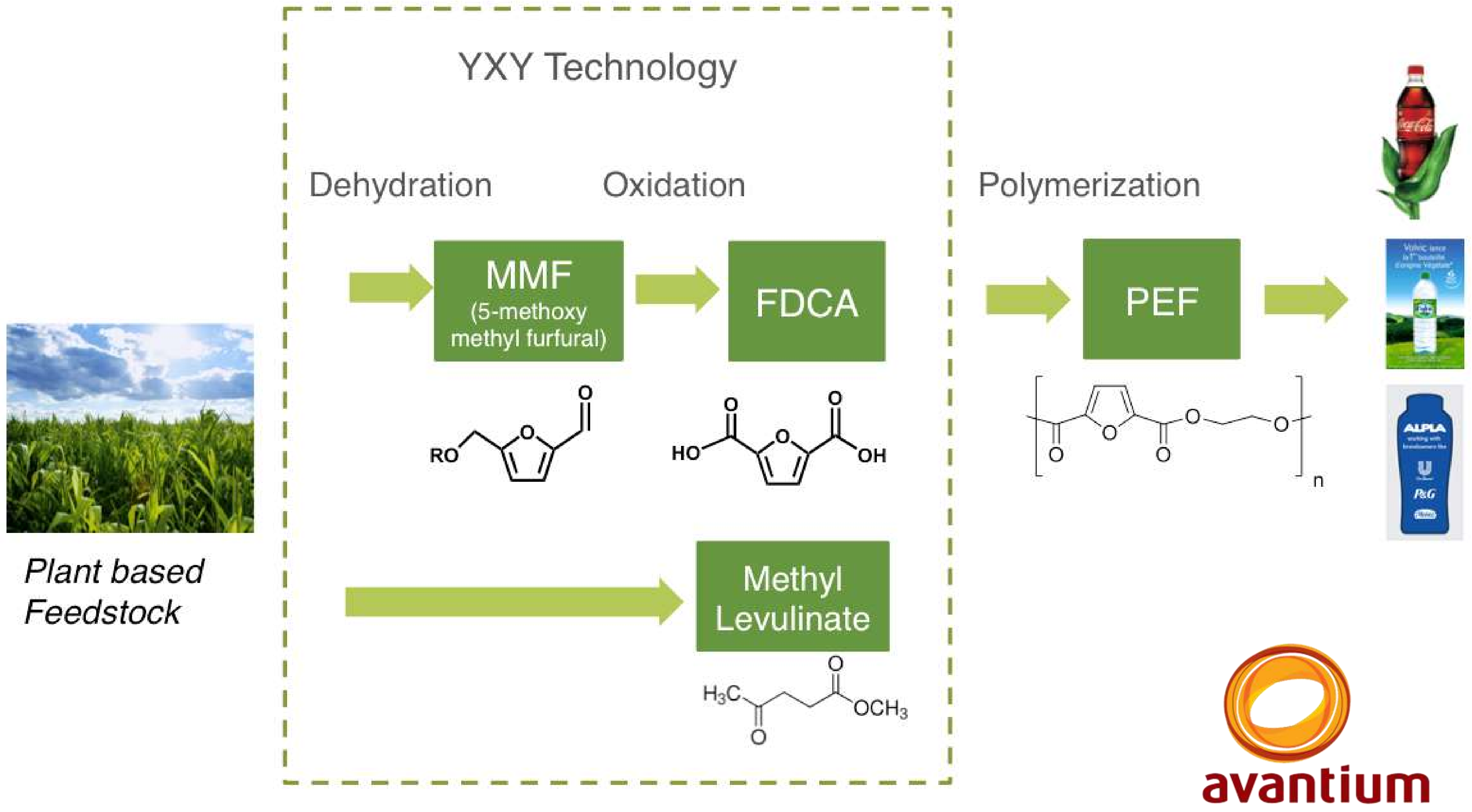
ΕΣΠΑ 2014-2020
ανάπτυξη - εργασία - αλληλεγγύη
Partnership Agreement
2014 - 2020

Co-financed by Greece and the European Union

Utilization of Biomass



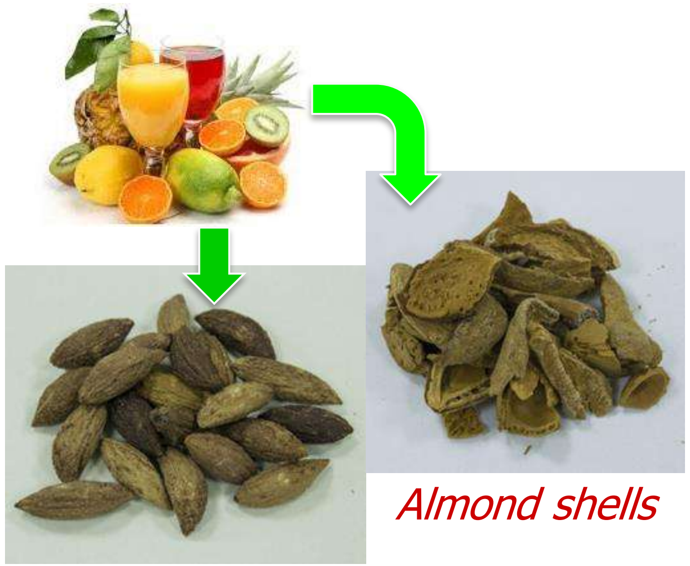
A successful commercial example



https://www.avantium.com/yxy/yxy-technology/

Lignocellulosic biomass (*residues, wastes or dedicated crops*)

- Agricultural and forestry residues/waste (wheat straw, trimmings, tree branches)
- Industrial wood processing residues (e.g. sawdust)
- Food industry waste (e.g. kernels, shells)
- Municipal organic solid waste (e.g. waste paper)
- Perennial or annual dedicated crops with high yield 1-4 ton/1000m^2 year (e.g. eucalyptus, pseudoacacia, willow, miscanthus, switch grass, sweet sorghum baggase,..)



Almond shells

Olive kernels

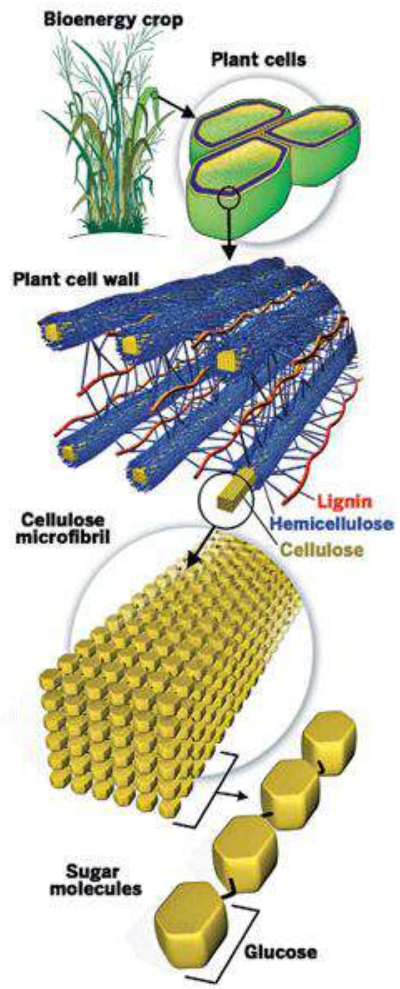
Agricultural & forestry Residues/wastes

Miscanthus

Robinia pseudoacacia

Lignocellulosic Biomass

Structure



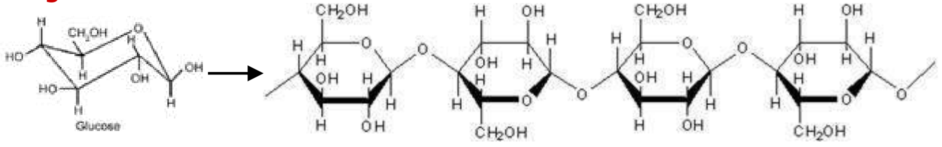
Source: Ritter S.K., Lignocellulose: A Complex Biomaterial, Plant Biochemistry, 86(49) (2008) 15

Composition

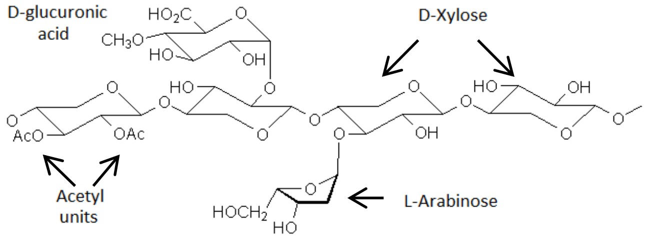
- Cellulose:

general formula $(C_6H_{10}O_5)_n$,
MW: 300.000-500.000

glucose

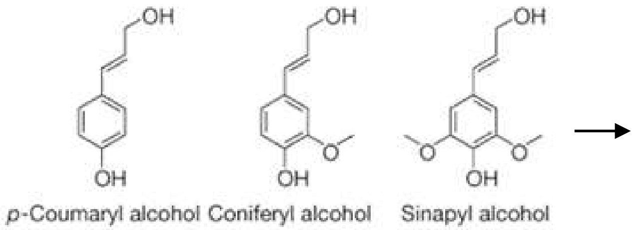


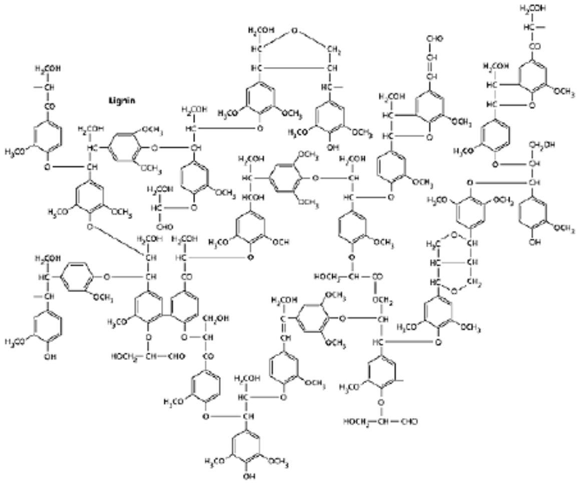
- Hemicellulose: general formula$(C_5H_8O_4)_n$



C_5 & C_6 sugars, uronic acids, acetyl units

- Lignin:

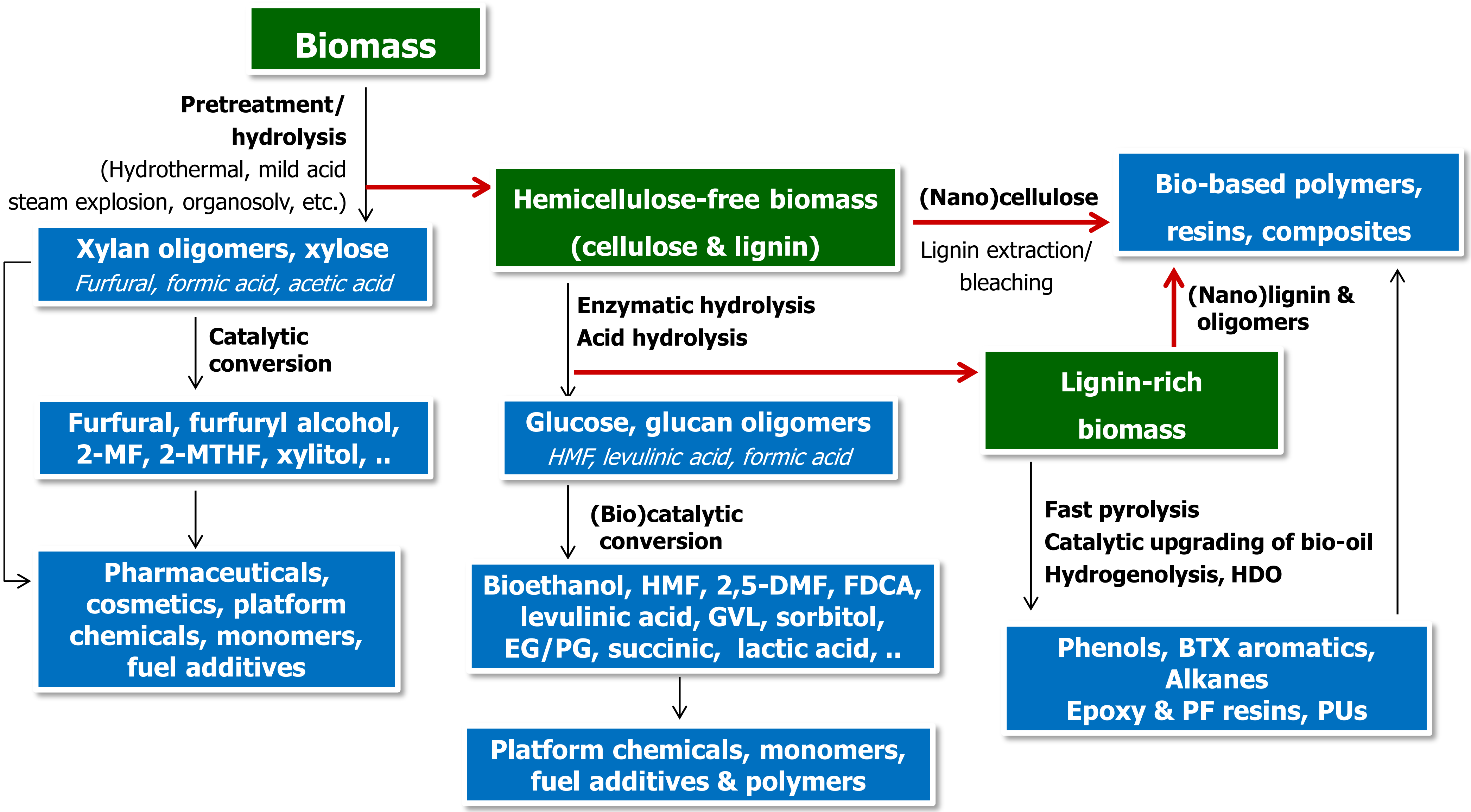




Monolignol building blocks of lignin

Cellulose: 30-50%, Hemicellulose: 20-40%, Lignin: 15-25%
Others 5-35%: Ash 3-10% (Si,Al,Ca,Mg,K,Na), Extractives: resins, fatty acids, waxes, phenolics, sterols, etc

Example of an integrated process valorization scheme - "Whole biomass refinery"



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Pre-treatment & Fractionation of biomass

- **Selection depends on targeted products and conversion process:**

- Paper & pulp industry – Kraft pulping uses $NaOH+Na_2S$ → **produces cellulose pulp and black liquor (lignin, hemicellulose, extractives)**

- 2nd Generation Bio-ethanol/butanol – Hydrothermal, steam explosion, dilute acid → **removes hemicellulose and increases enzymatic digestibility of cellulose**

- Integrated Biorefinery for chemicals, plastics, fuels – Acid hydrolysis, alkaline and alkaline/H_2O_2, Organosolv, etc. → **isolates hemicellulose and/or lignin**

- **Aim: Selective recovery of "clean" fractions (hemicellulose, cellulose, lignin) for more efficient down-stream upgrading**

Hydrothermal Pre-treatment (in pure water)

Biomass

Beech wood
Lignocel 150-500 μm

Autoclave reactor

Solid product

- SEM
- FTIR
- XRD
- BET
- TGA

Enzymatic Hydrolysis

Glucose

Liquid product

- Xylose
- Furfural
- Acetic & formic

Severity factor (logRo)

Combined effect of time (t: min) and Temperature (T: °C)

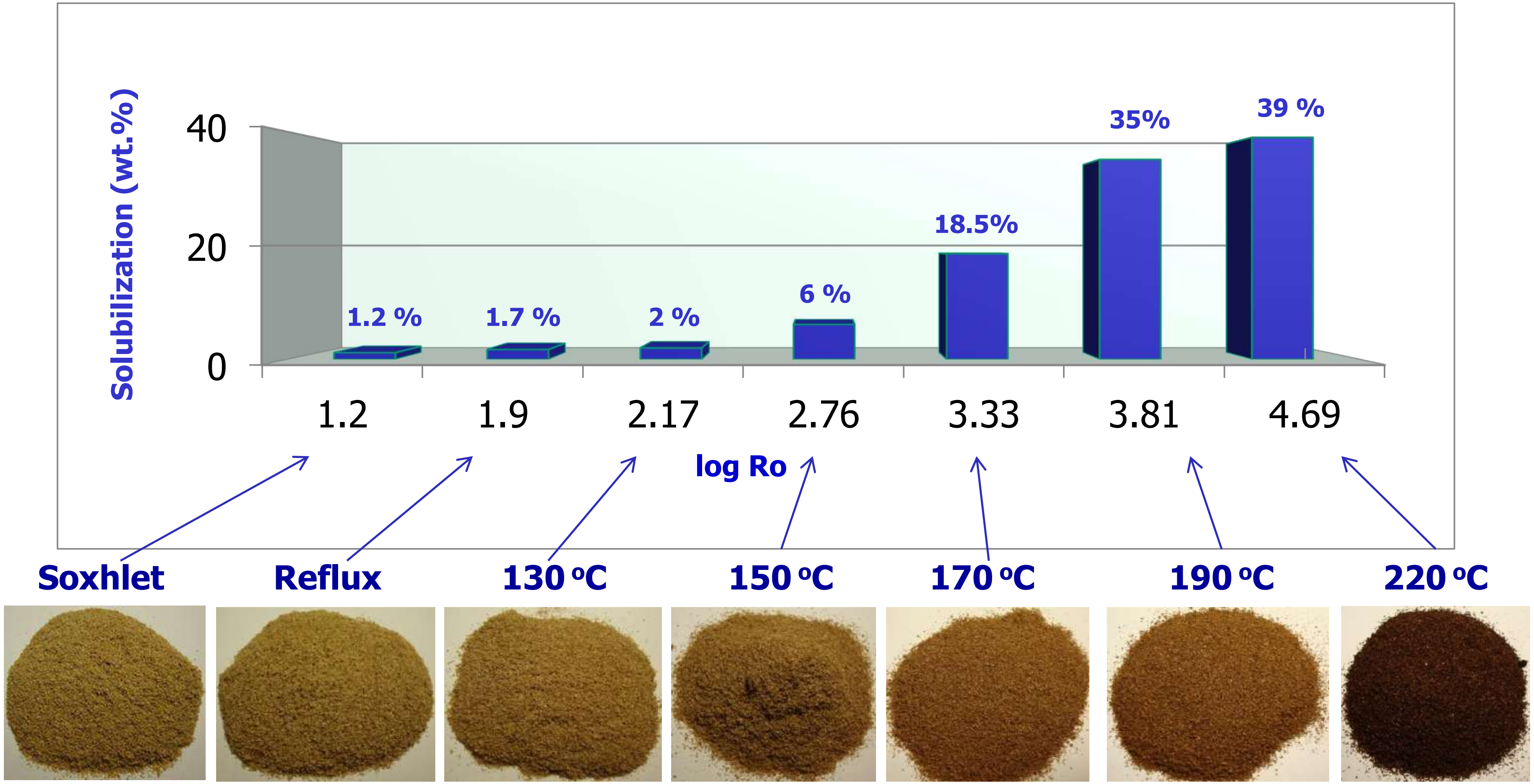
$$R_0 = t \cdot \exp\left[\frac{T - 100}{14.75}\right]$$

Experimental conditions

- Temperature : 130-220°C
- Time : 15-180 min
- LSR: 15
- Stirring: 150 rpm
- Heating Rate: 7 °C min^{-1}

C.K. Nitsos, K.A. Matis, K.S. Triantafyllidis, ChemSusChem, 6 (2013) 110 – 122
C.K. Nitsos, T. Choli-Papadopoulou, K.A. Matis, K.S. Triantafyllidis, ACS Sust. Chem. & Engin. 4 (2016) 4529-4544
C. K. Nitsos, P. A. Lazaridis, A. Mach-Aigner, K. A. Matis, & K. S. Triantafyllidis, ChemSusChem (2019) 12 (6): 1179

Biomass solubilization at increasing HT severities

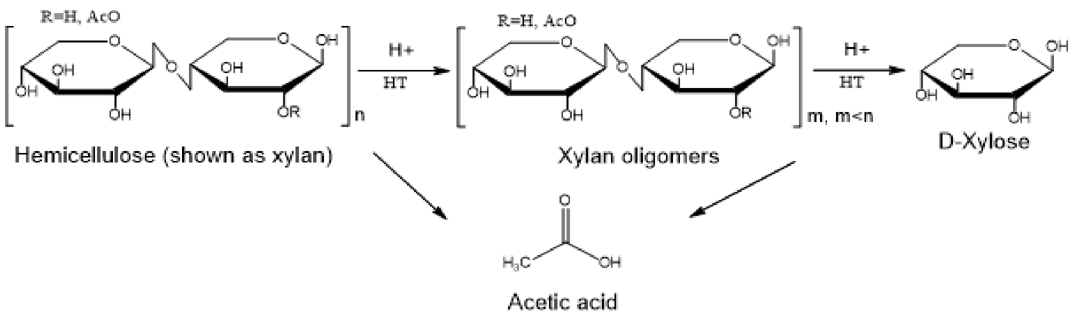


At higher severities:

- **A large portion of the biomass (35-40%) is solubilized**
- **The biomass particles color becomes dark brown due to recondensation of lignin on the surface**

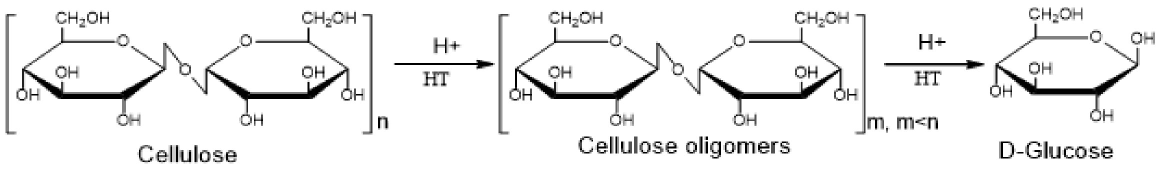
Generalized reaction scheme

Hemicellulose hydrolysis at subcritical water

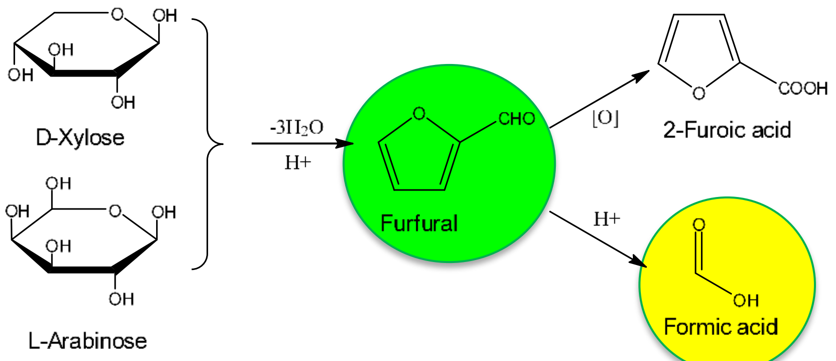


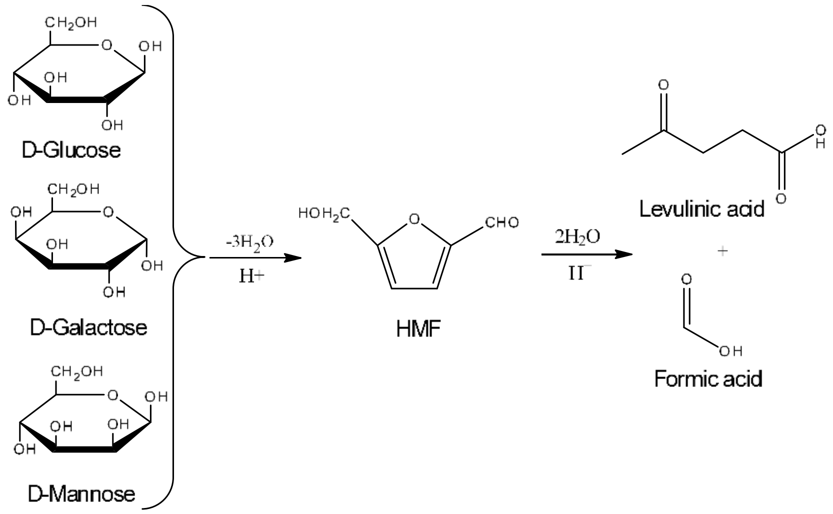
- ❑ **Self-catalyzed hydrolysis (pH 5 → 2.5)**
- ❑ **The catalyst (acetic acid) is a biomass component**

Cellulose hydrolysis at subcritical water



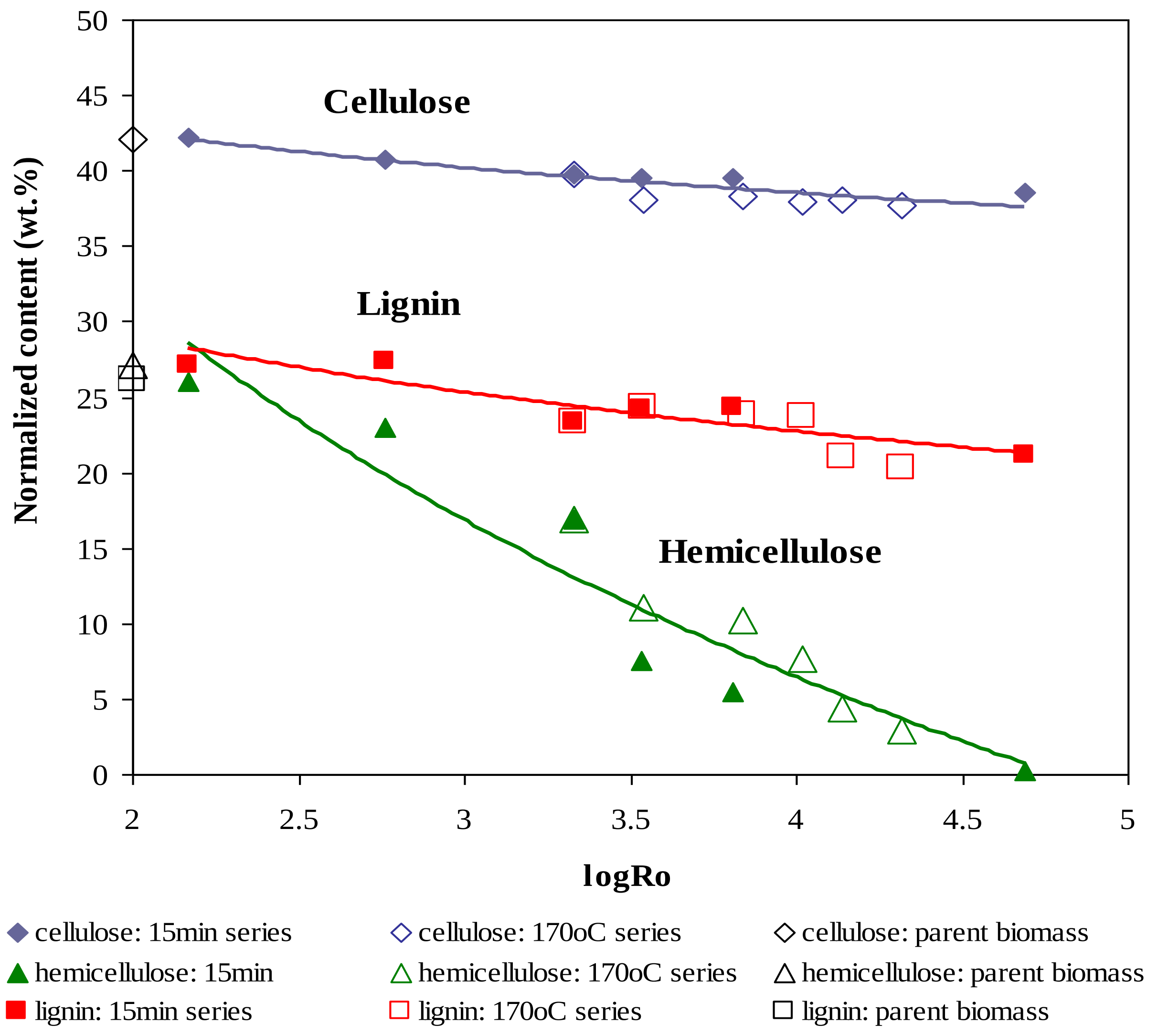
Sugars dehydration products



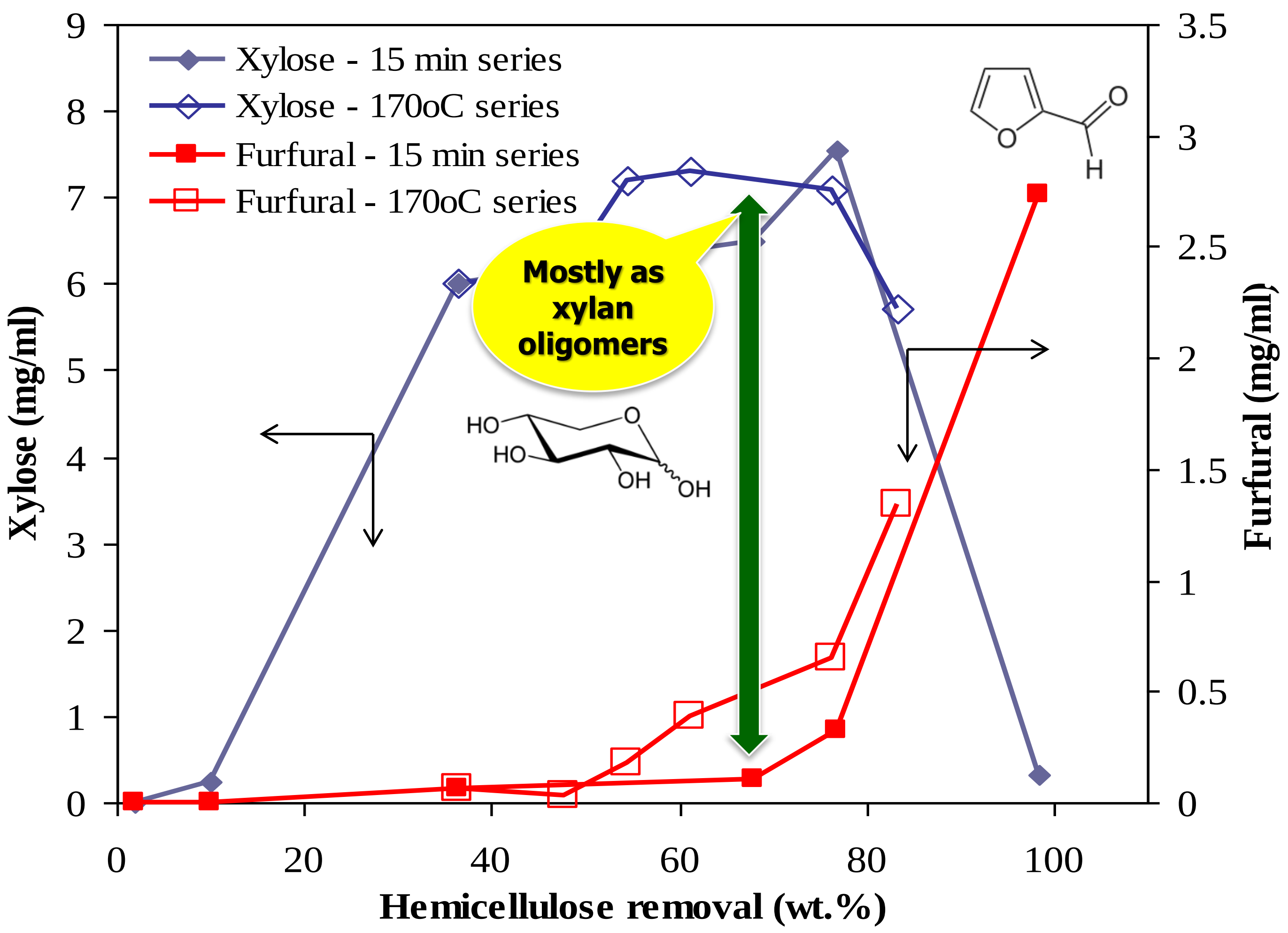


Evolution of main structural components in hydrothermally treated solids

(expressed as % of their initial content in parent biomass)



Xylose and furfural concentration in liquid products vs. % hemicellulose removal



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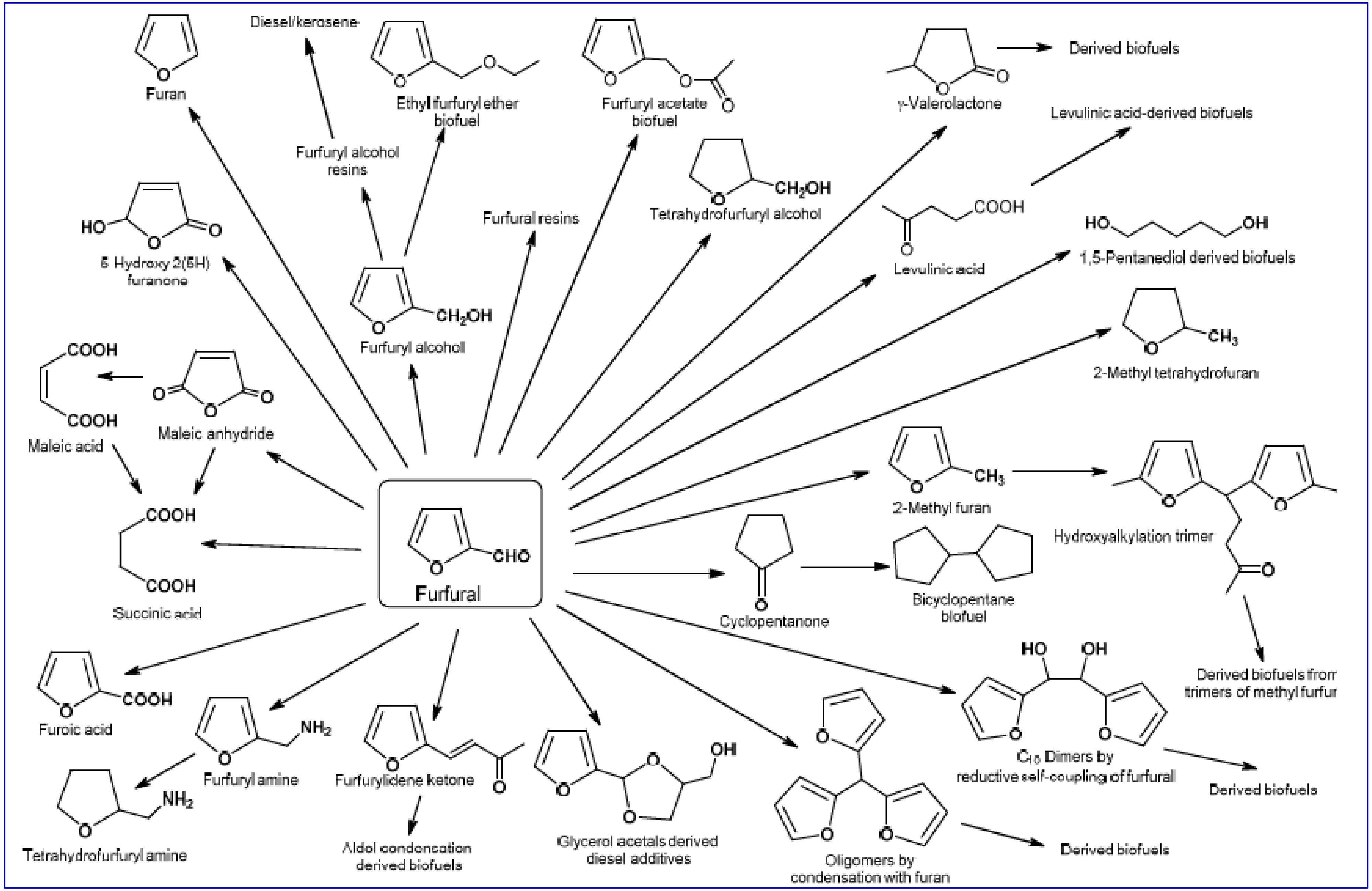
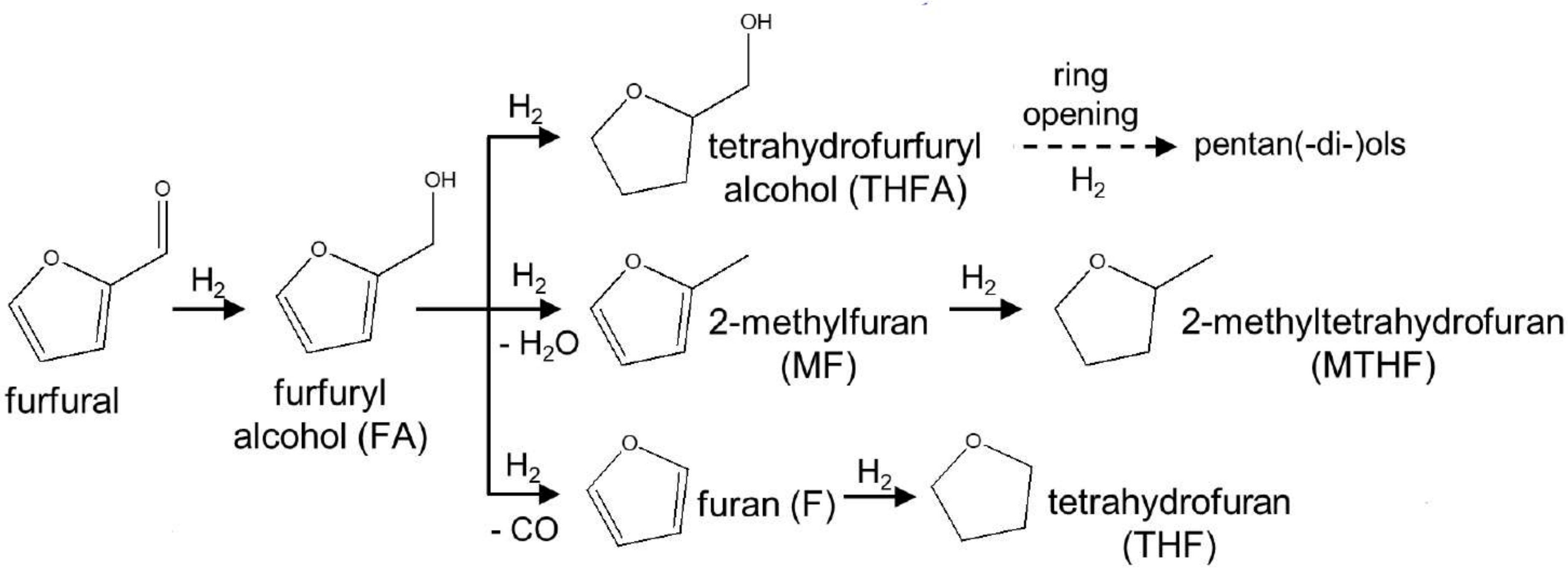
Furfural derived chemicals and fuels



R. Mariscal, P. Maireles-Torres, M. Ojeda, I. Sádaba, M. López Granados, Energy Environ. Sci., 2016,9, 1144-1189

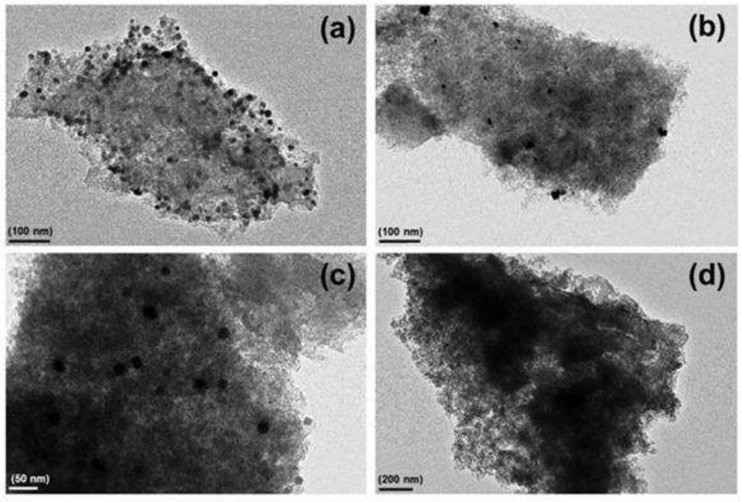
Catalytic hydrogenation of furfural: General reaction mechanism – possible routes



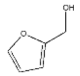
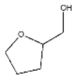
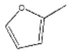
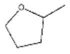
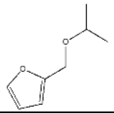
- **Dominant pathways/products depend on catalyst type, reaction parameters and solvent (acting or not as H-donor for inducing transfer hydrogenation)**

Y. Wang, P. Prinsen, K.S. Triantafyllidis, S.A. Karakoulia, A. Yepez, C. Len, R. Luque, ChemCatChem 2018, 10, 3459– 3468

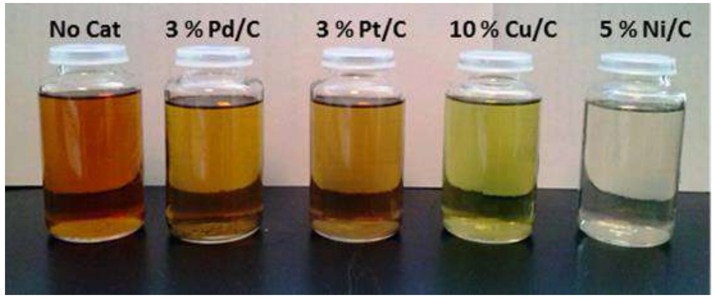
Catalytic transfer hydrogenation of furfural (solvent acting as hydrogen donor)



Ni, Cu, Pt, Pd on micro/mesoporous carbon

Entry	Catalyst	Conversion (%)	Yield (%)					Mass balance (%)
			FA 	THFA 	MF 	MTHF 	iPrOMF 	
1	-	2	6	0	0	0	0	104
2	10%Cu/AC	24	22	0	2	1	1	103
3	3%Pd/AC	47	21	1	5	2	5	87
4	3%Pt/AC	93	47	1	24	3	5	87
5	5%Ni/AC	85	6	1	66	2	3	93
6	5%Ni/AC[b]	10	10	1	1	0	0	102
7	5%Ni/AC[c]	95	20	1	50	1	1	78
8	5%Ni/AC[d]	87	13	1	9	2	0	38[e]
9	5% Ni/AC[f]	67	38	1	17	1	13	103

[a] 200 °C, 5 h, 0.35 M furfural in 60 mL isopropanol, 30 bars H_2, [b] 0 bar H_2/200 ºC, [c] 0 bar H_2/260 ºC, [d] In methanol, [e] Unknown compound eluting at 3.8 min in GC analysis, not included (48 % of total peak area), [f] Spent catalyst recovered after the experiment in entry 5



An example of the successful collaboration between Greece, France and Spain, involving training/exchange of young scientists within the frame of European COST Action "LIGNOVAL"

COST EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

LIGNOVAL

Y. Wang, P. Prinsen, K.S. Triantafyllidis, S.A. Karakoulia, P.N. Trikalitis, A. Yepez, C. Len, R. Luque, ACS Sustainable Chem. Eng. 2018, 6, 9831–9844

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2nd Generation Bioethanol process scheme

Byproducts

Forestry waste

Wheat straw

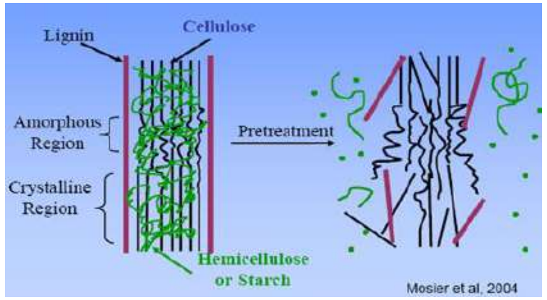
Energy crops

Mischanthus

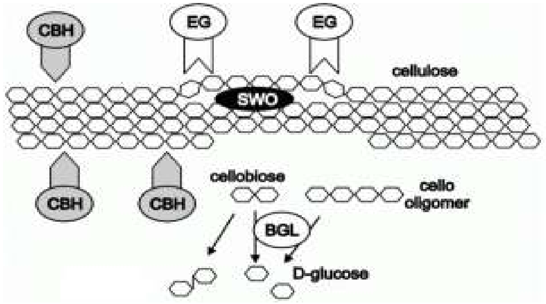
Switchgrass

Lignocellulose

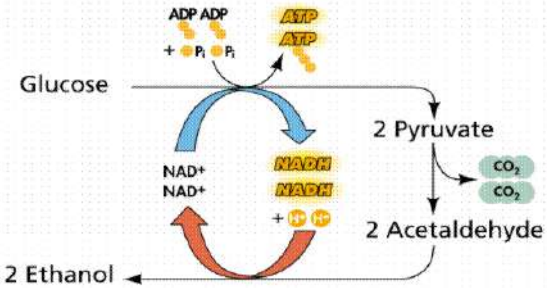
Pretreatment
Disruption of Lignocellulose structure



Hydrolysis
Conversion of cellulose to glucose using enzyme or acid catalyst



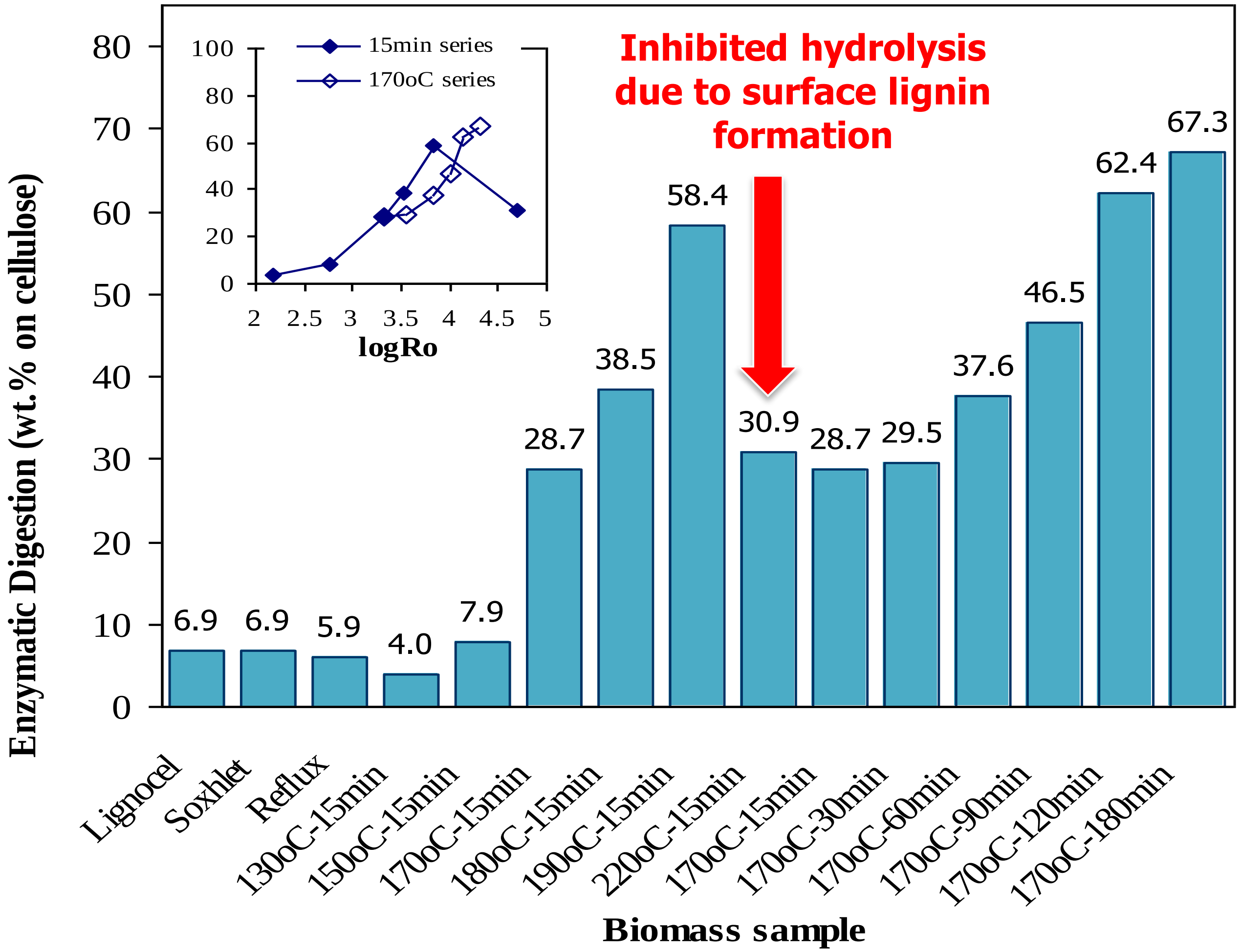
Fermentation
Conversion of sugars into ethanol



Distillation/ Dehydration
Separation and rectification of alcohol

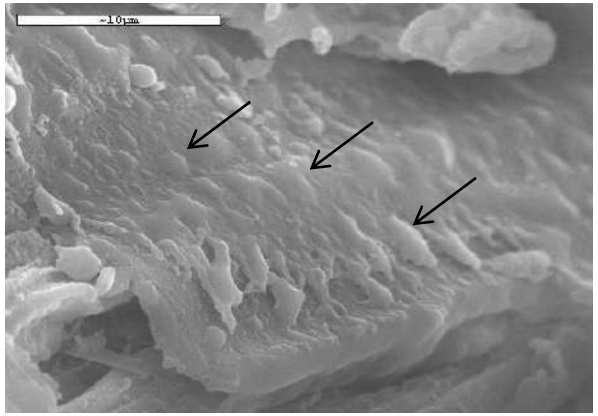
Ethanol
Density: 0,795 kg/l
Energy content: 26,8 MJ/kg
Purity: 99,7% m/m (min.)
Water content: 0,3% m/m (max.)
European Norm: EN 15376

Enzymatic hydrolysis of pretreated biomass



Maximum enzymatic digestibility test

1% w/v cellulose, 60 FPU/g cellulose, 50 °C, pH 4.8, 68 rpm, 168 h.

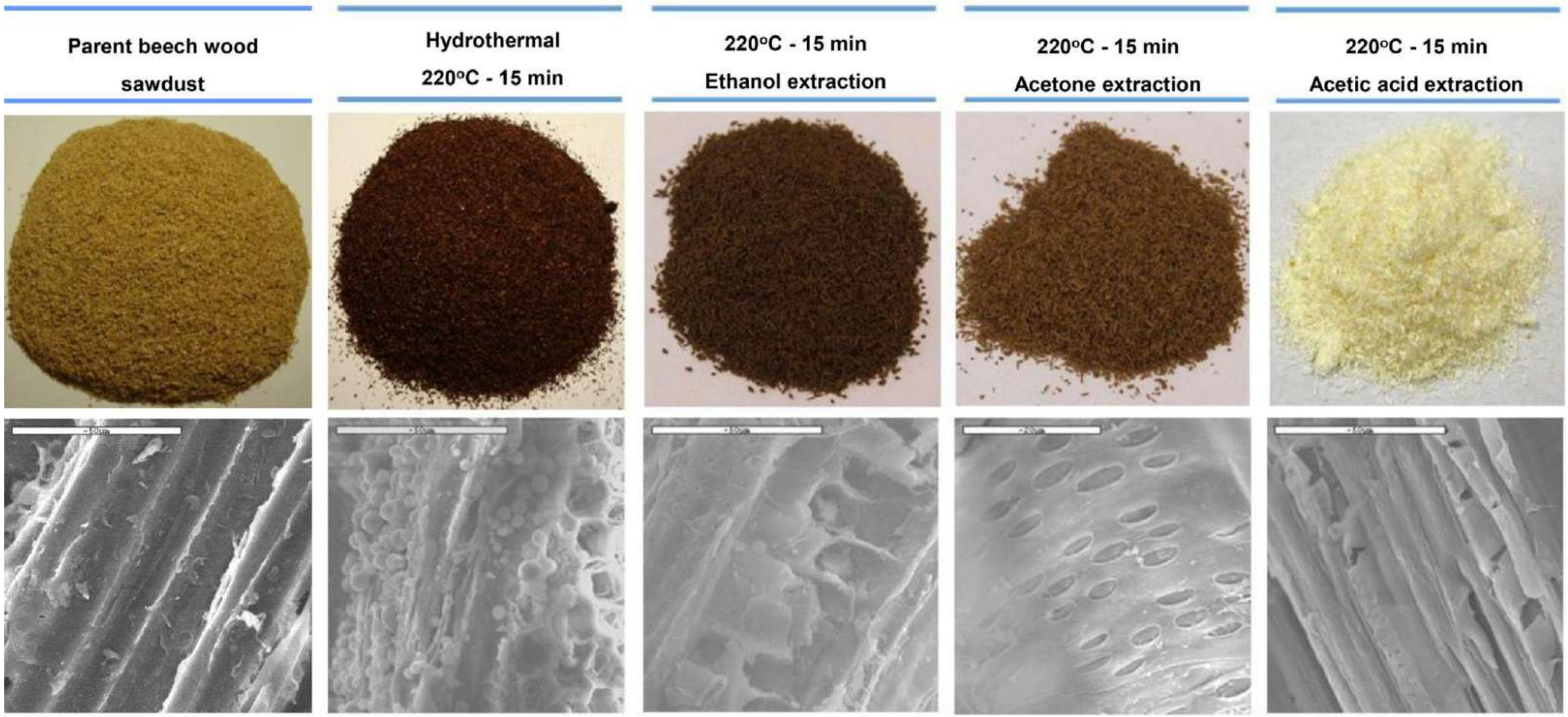


- Lignin layer protects cellulose from enzymatic hydrolysis

Lignin can be removed by mild extraction prior to enzymatic hydrolysis

- **Increase of logRo improves enzymatic hydrolysis**
- **170 °C, 180 min (logRo 4,31):** 67% cellulose conversion
- ***220 °C , 15 min (logRo 4,69):*** conversion reduction to 31%

Extraction & recovery of lignin from hydrothermally pretreated biomass



C. K. Nitsos, P. A. Lazaridis, A. Mach-Aigner, K. A. Matis, & K. S. Triantafyllidis, ChemSusChem (2019) 12 (6): 1179

Hydrothermal pretreatment in neat water → recovery of xylan & lignin → enchanced cellulose enzymatic hydrolysis

Hydrothermal Pretreatment
220 °C, 15 min

Extraction of "surface" lignin

Enzymatic hydrolysis
+
wet ball milling

Beech wood (Lignocel)
Cellulose 42,1%

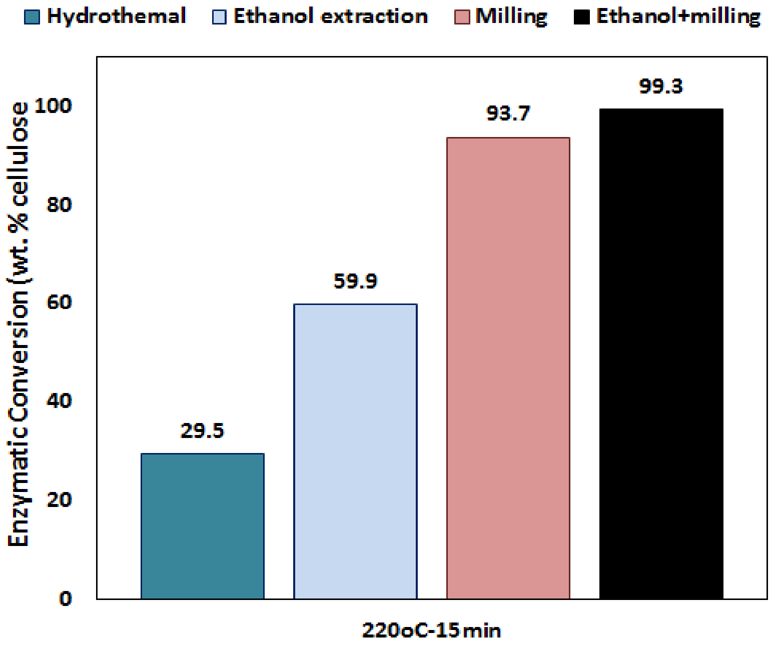
→ **Pretreated solid**
Cellulose 63,2%

→ **Extracted solid**
Cellulose 77,0%

→ **Liquid hydrolyzate**
Glucose 99,3%

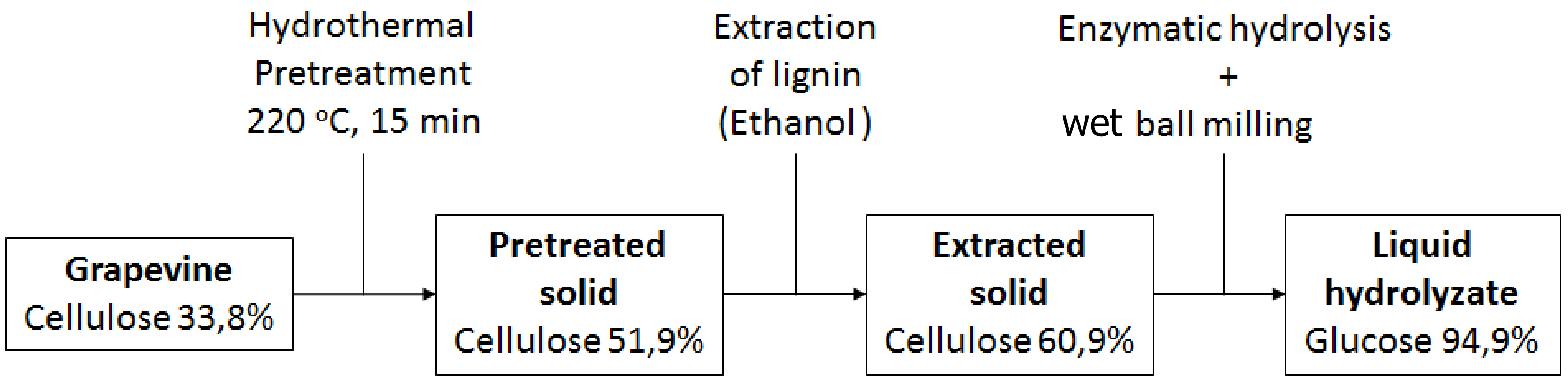
Cellulose recovery > 85%

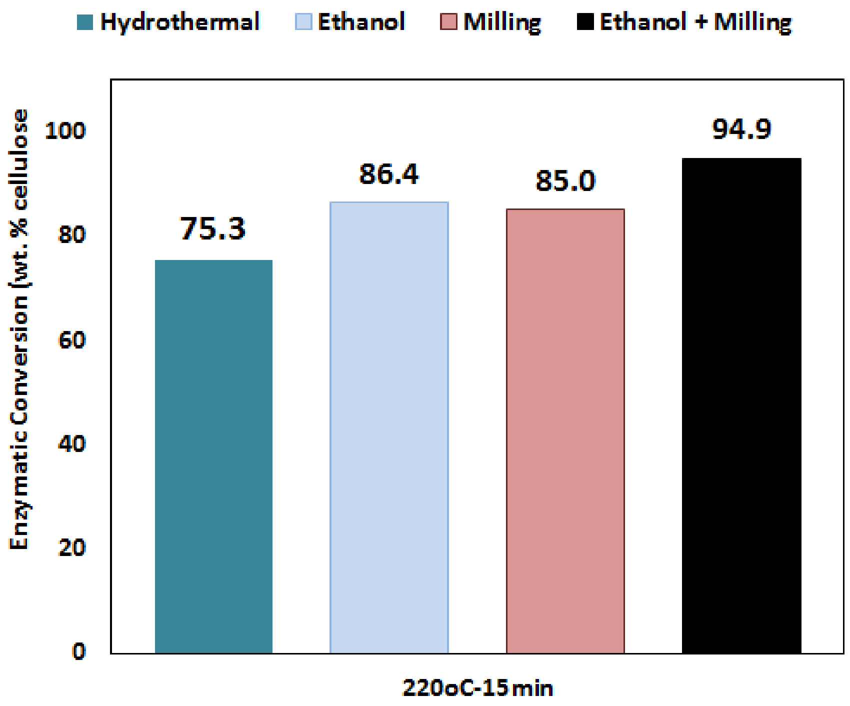
Furfural – rich
Xylose
Acetic acid



C.K. Nitsos, K.A. Matis, K.S. Triantafyllidis, ChemSusChem, 6 (2013) 110
C.K. Nitsos, et al. ACS Sust. Chem. & Engin. 4 (2016) 4529-4544
C. K. Nitsos, et al. ChemSusChem (2019) 12 (6): 1179

Enzymatic hydrolysis optimization (grapevine trimmings)





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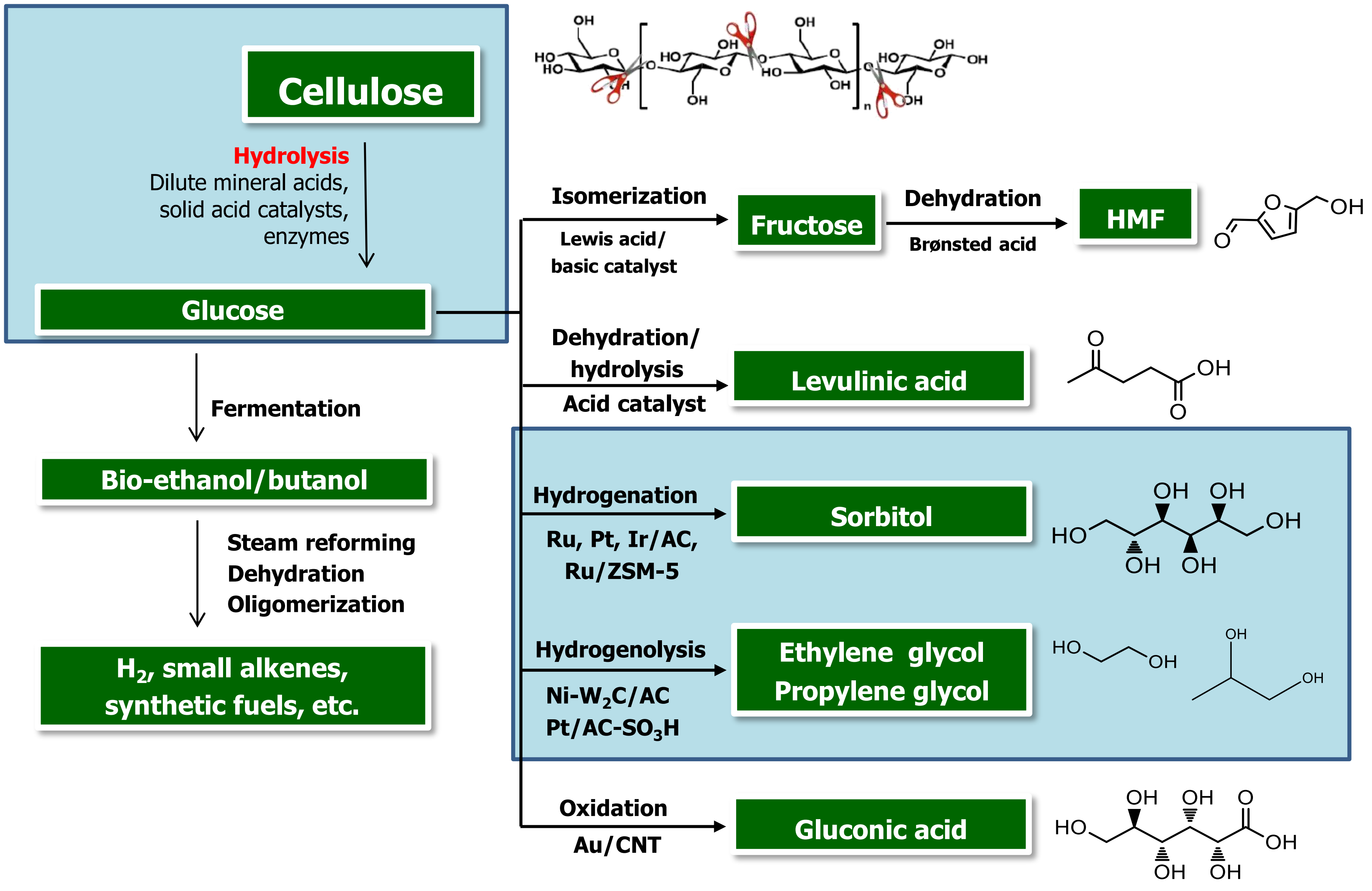
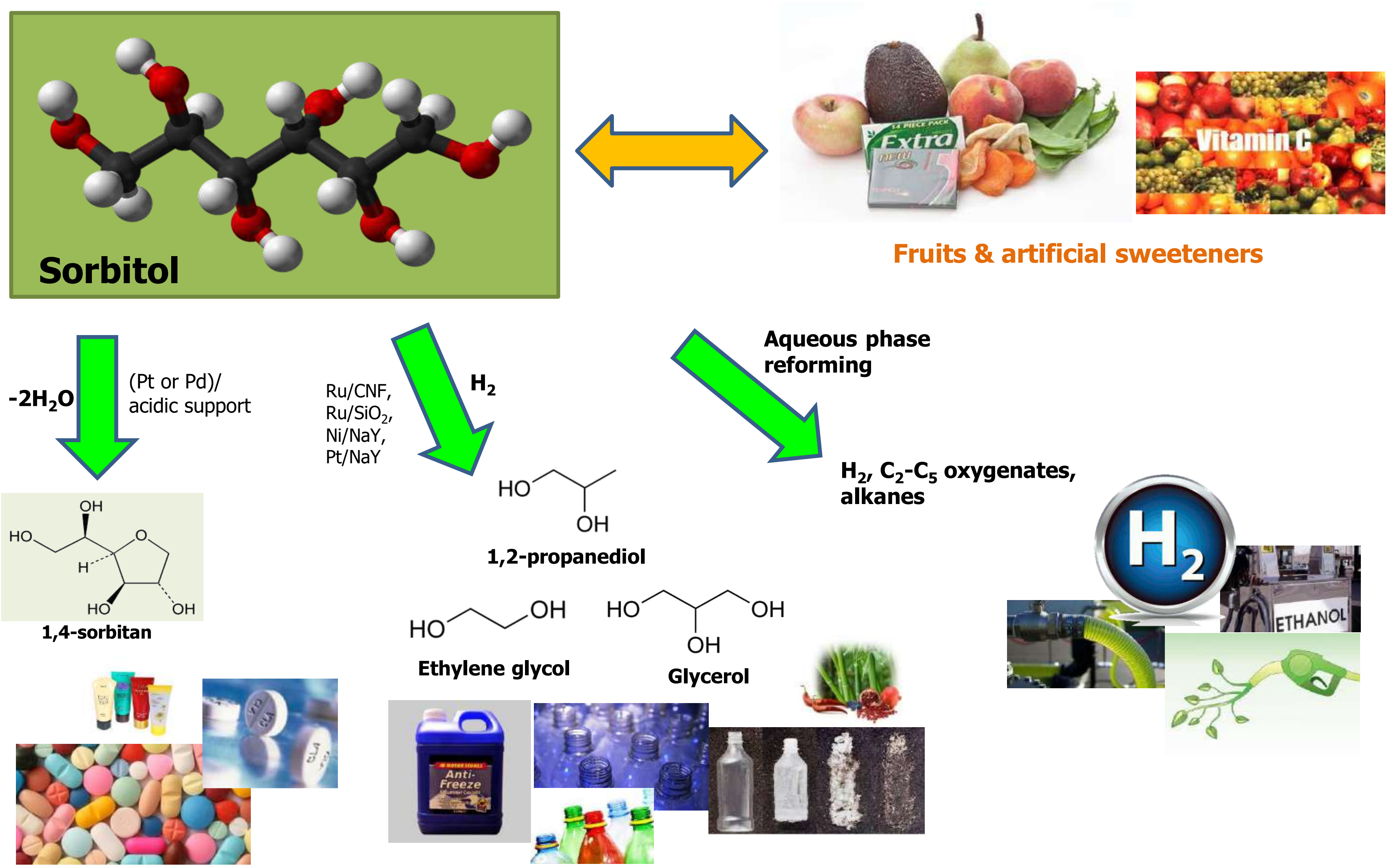
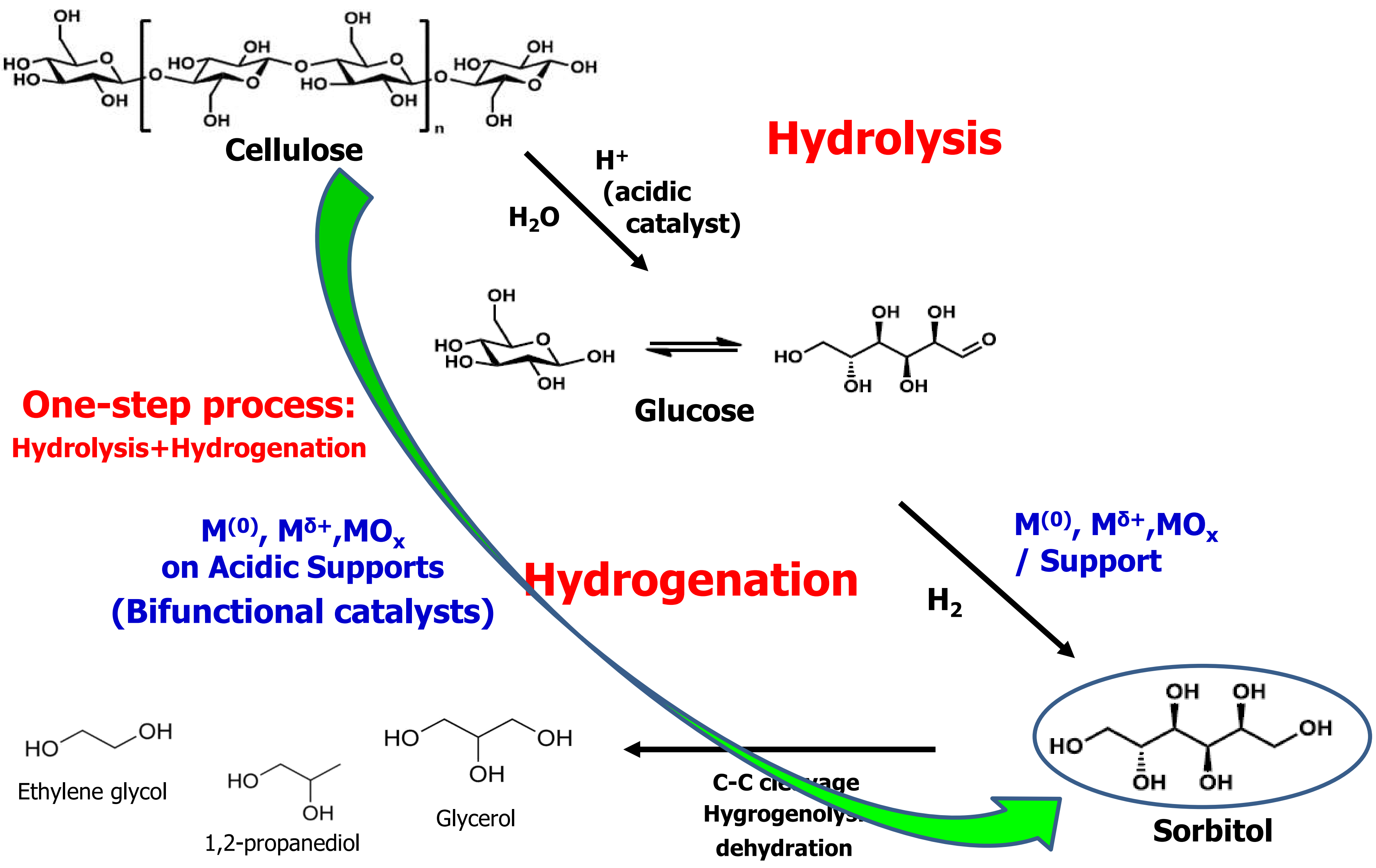
Conversion of Cellulose into chemicals: Role of catalysis



Why sugar alcohols?

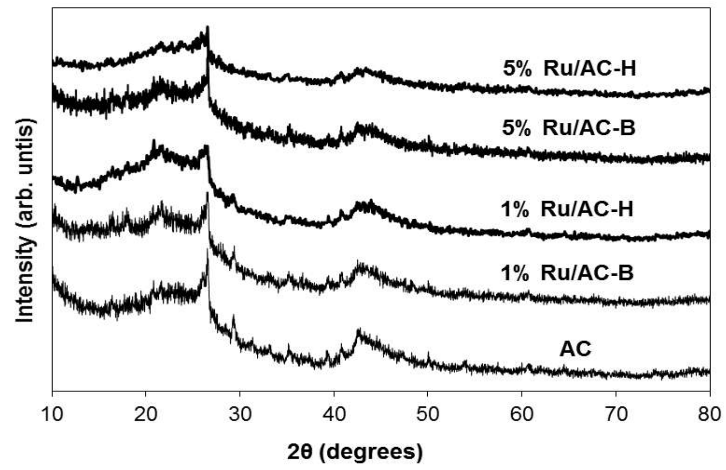


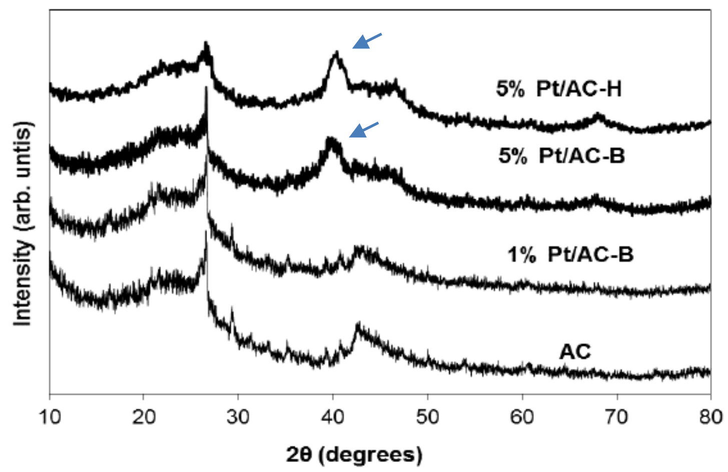
Catalytic Conversion of Cellulose into sugar alcohols

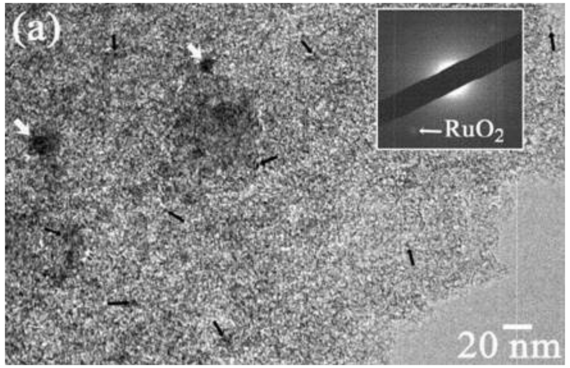


Catalysts: Ru and Pt supported on micro/mesoporous Activated Carbon

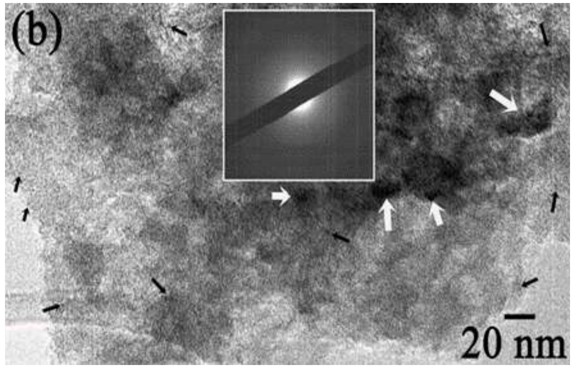
Crystallinity and dispersion of nanometals (XRD & HRTEM)



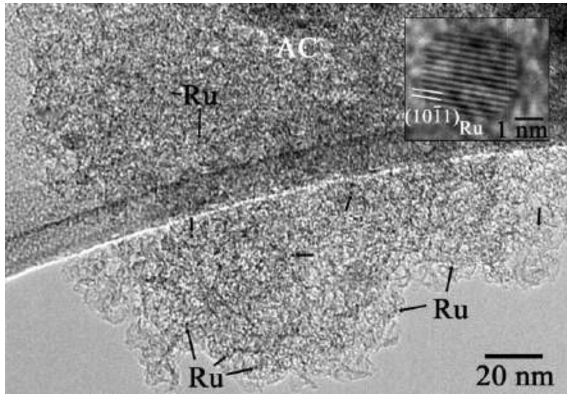




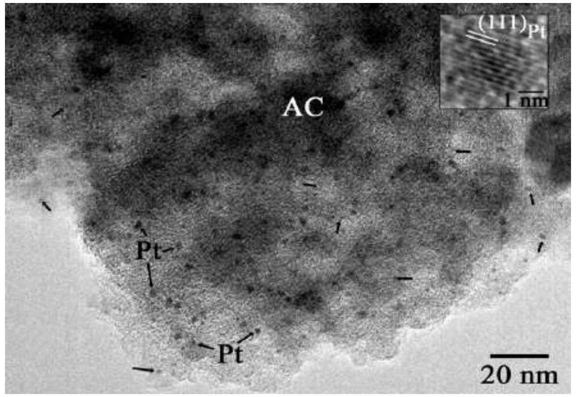
1% Ru/AC-B



5% Ru/AC-B



3% Ru/AC-H



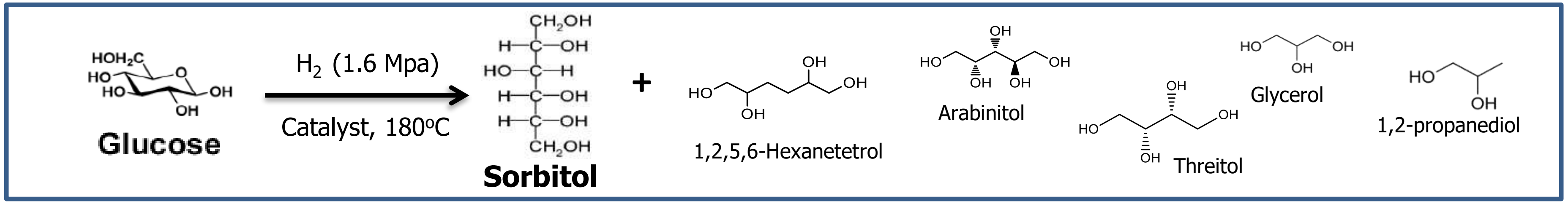
3% Pt/AC-H

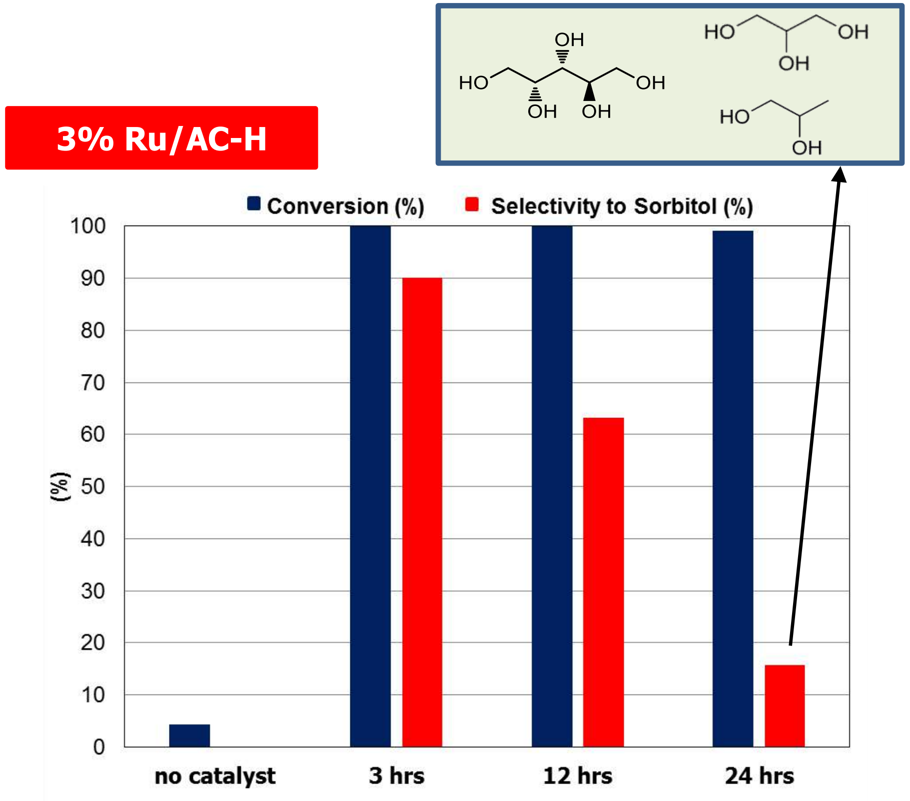
XRD patterns

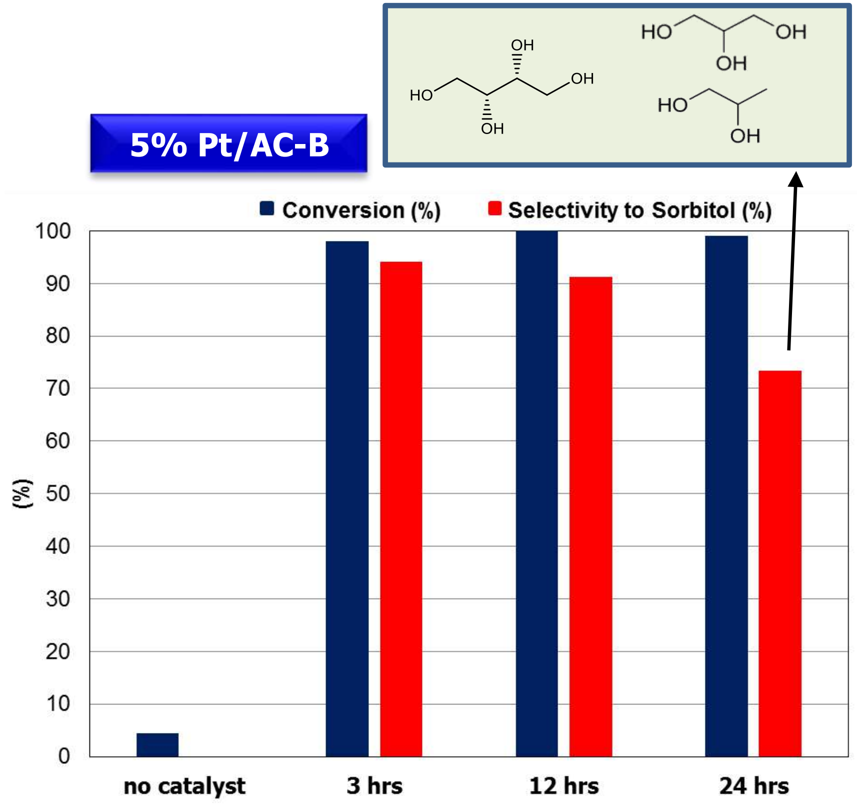
TEM images

P. Lazaridis et al. , Catalysis Today, 257, 281-290, 2015

Hydrogenation of D-glucose to hexitols and glycols

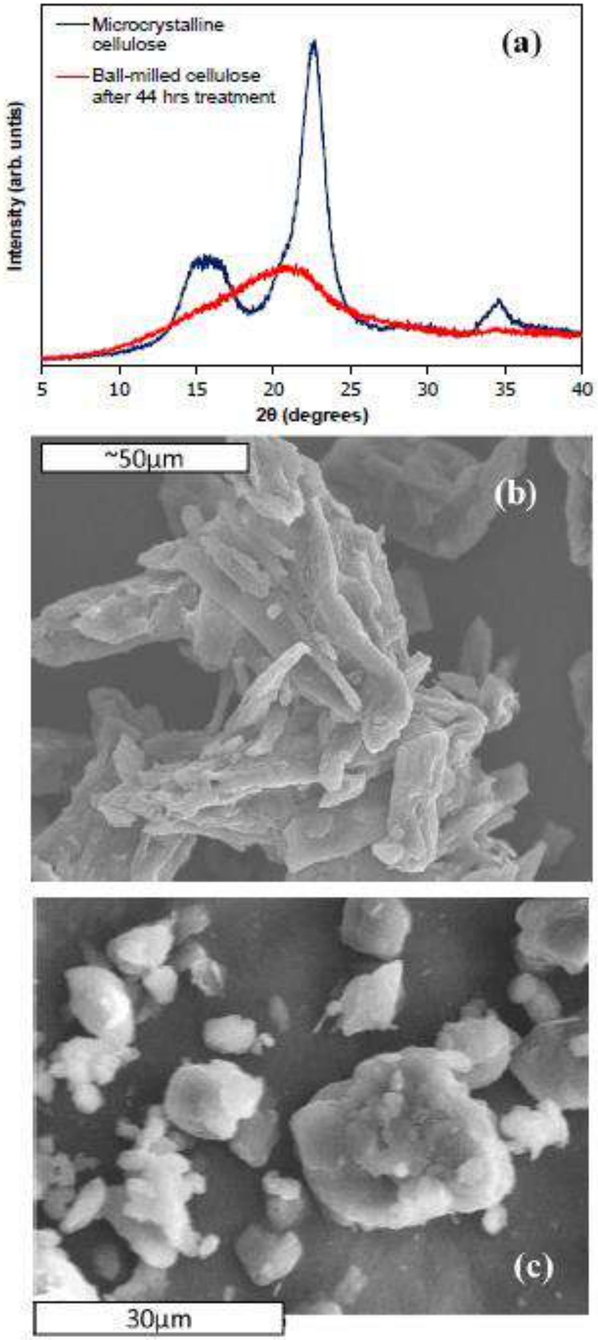


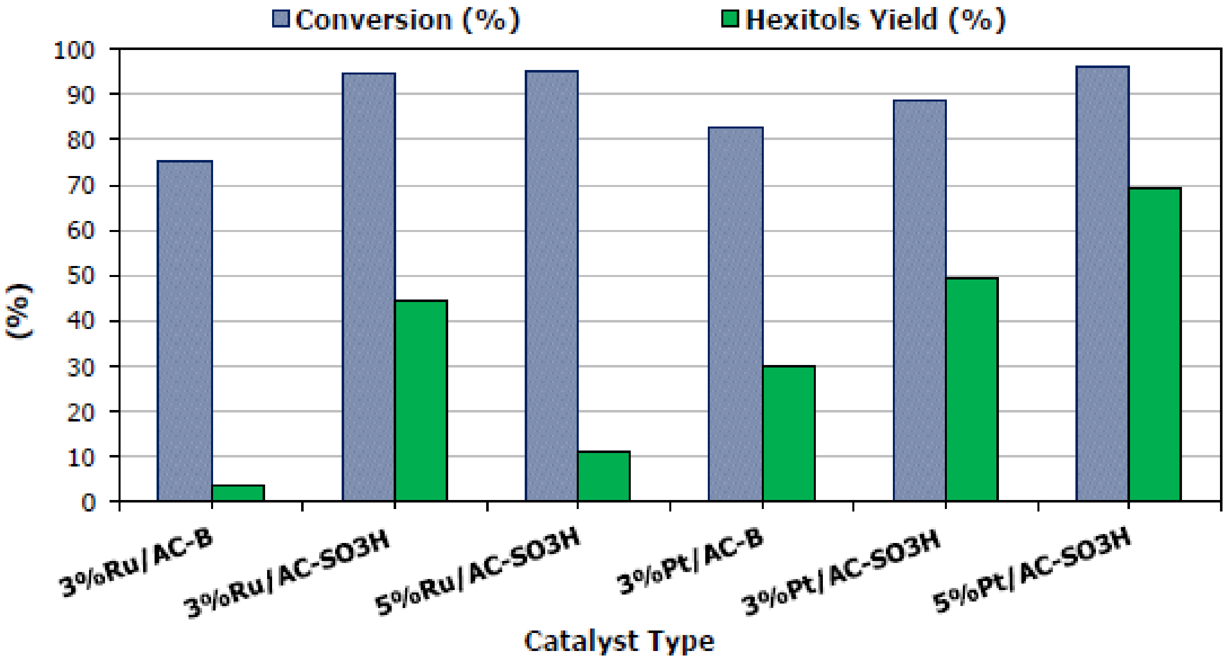




✓ **Pt/AC catalysts are more selective towards hexitols (sorbitol, mannitol) under intense reaction conditions compared to Ru/AC catalysts**

Hydrogenolysis of ball-milled (amorphous) cellulose to hexitols and glycols



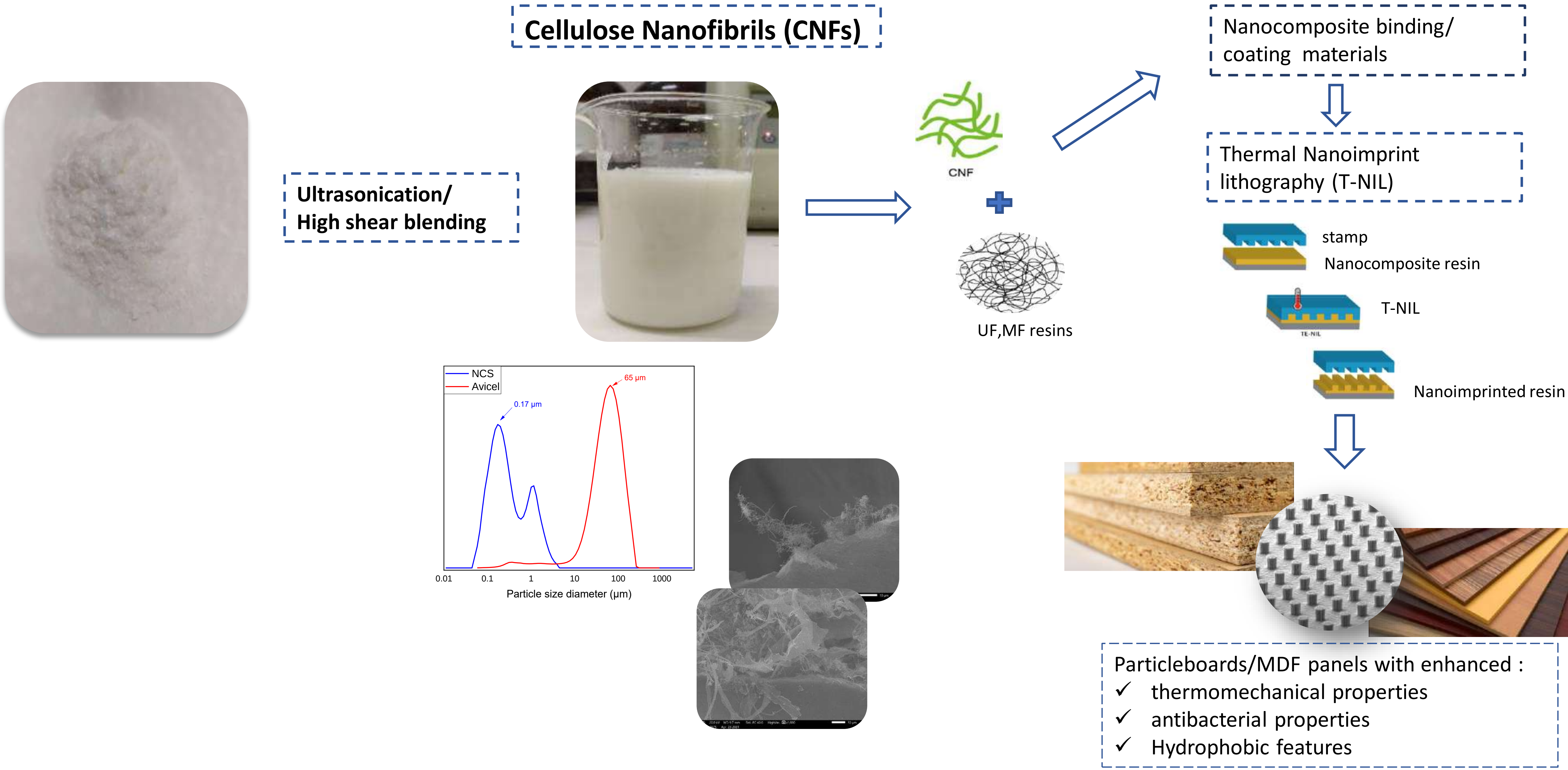


Lazaridis et. al., Applied Catalysis B: Environmental 214 (2017) 1–14

Topics for today

- ❑ Hydrothermal pretreatment/fractionation of biomass
- ❑ Catalytic hydrogenation of furfural to furfuryl alcohol and 2-MF/2-MTHF
- ❑ Enzymatic hydrolysis of hydrothermally pretreated biomass
- ❑ Hydrolytic hydrogenation of cellulose to sugar alcohols
- ❑ (Nano)cellulose as reactive additive in resins/polymers
- ❑ Catalytic fast pyrolysis of lignin to phenolics, aromatics & fuels
- ❑ (Nano)lignin and phenolics for bio-based polymers and composites

Nanocellulose reinforced nanoimprinted resins



Topics for today

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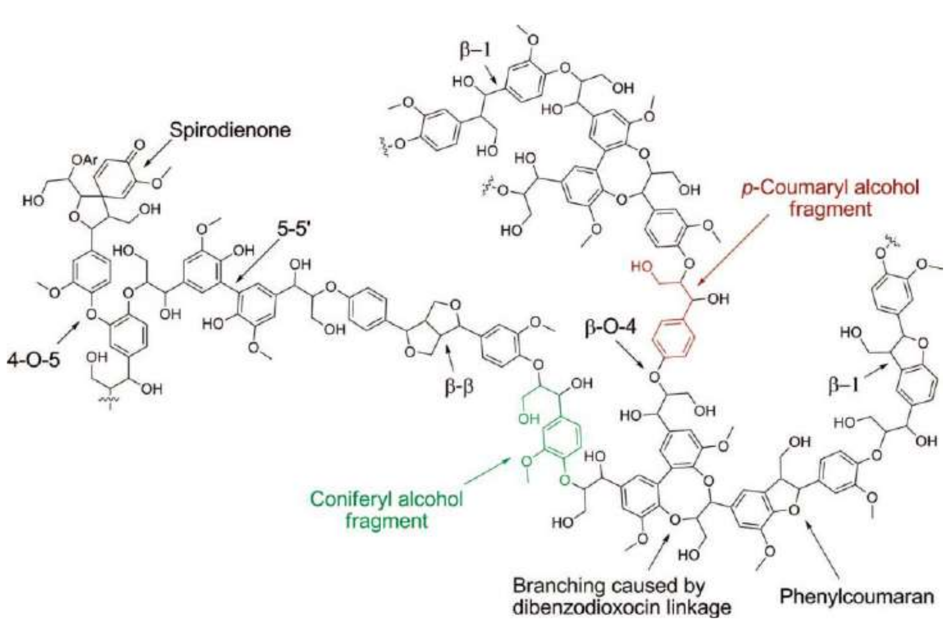
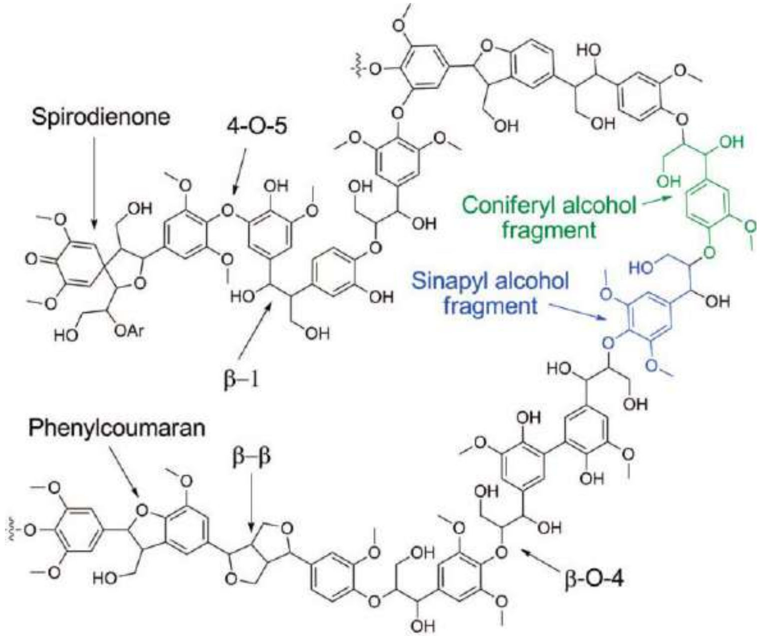
- ❑ Glucose isomerization to fructose
- ❑ (Nano)cellulose as reactive additive in resins/polymers
- ❑ Catalytic fast pyrolysis of lignin to phenolics, aromatics & fuels
- ❑ (Nano)lignin and phenolics for bio-based polymers and composites

Representative lignin structure and composition



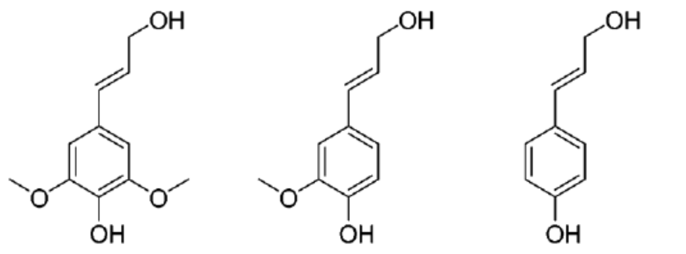
Representative softwood lignin structure



Representative hardwood lignin structure

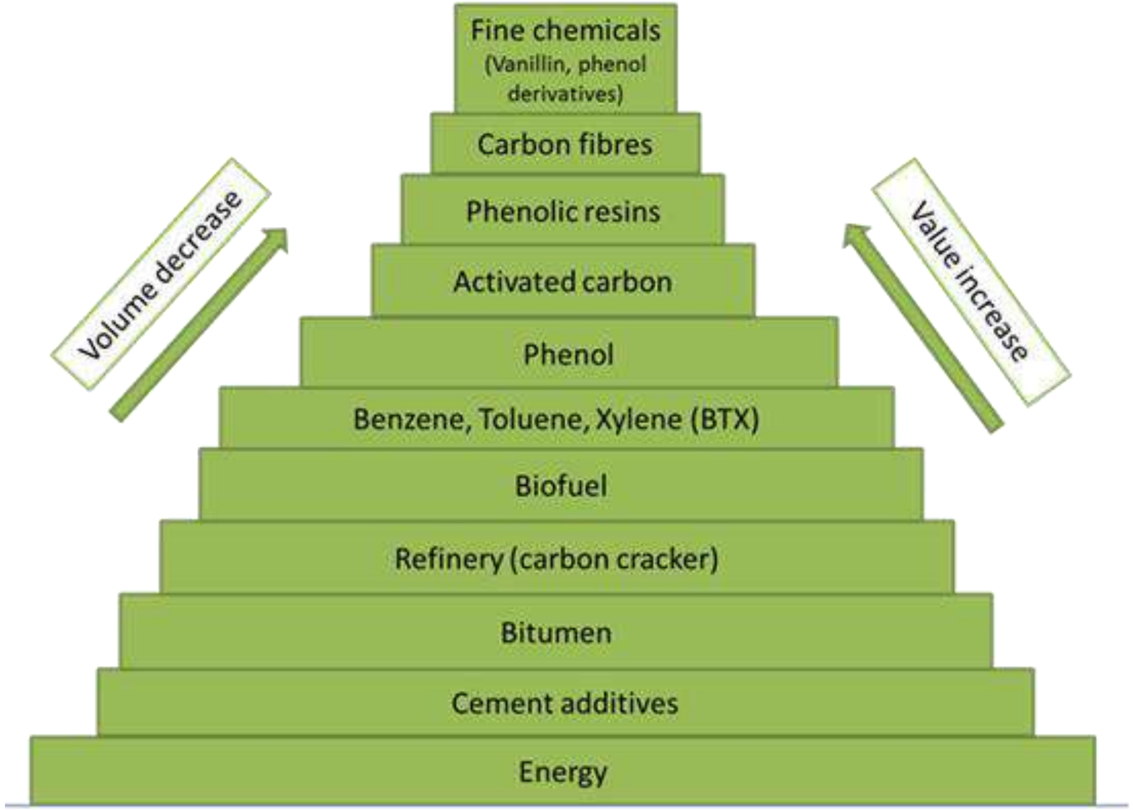
J. Zakzeski et al., *Chem. Rev.* **2010,** *110,* 3552–3599

	Lignin (wt.%)	Phenylpropane unit (%)		
		Coumaryl	Coniferyl	Sinapyl
Softwood	**27-33**	**0.5-3.4**	**90-95**	**Very low**
Hardwood	**18-25**	**trace**	**25-50**	**50-75**
Grasses	**17-24**	**10-25**	**25-50**	**25-50**



P. Azadi et al., Ren and Sus Energy Reviews 21,**2013**, 506–523
C. Li et al., Chem. Rev., 115 (21), **2015**, 11559–11624

Lignin applications (value vs. volume)



Gosselink R.J.A. 2011

Biomass fractionation & Lignin recovery processes

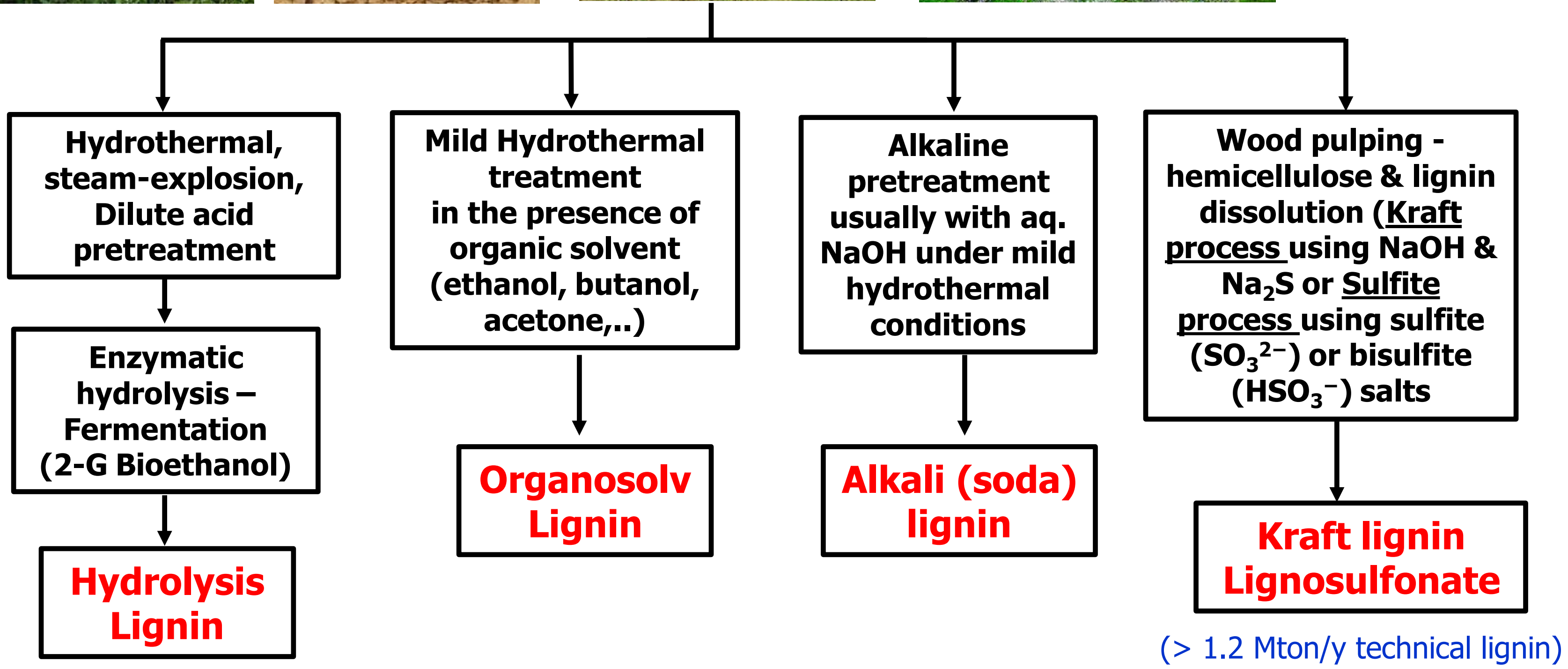
Kenaf

Beechwood

Vine prunings

Olive prunings

Almond shells



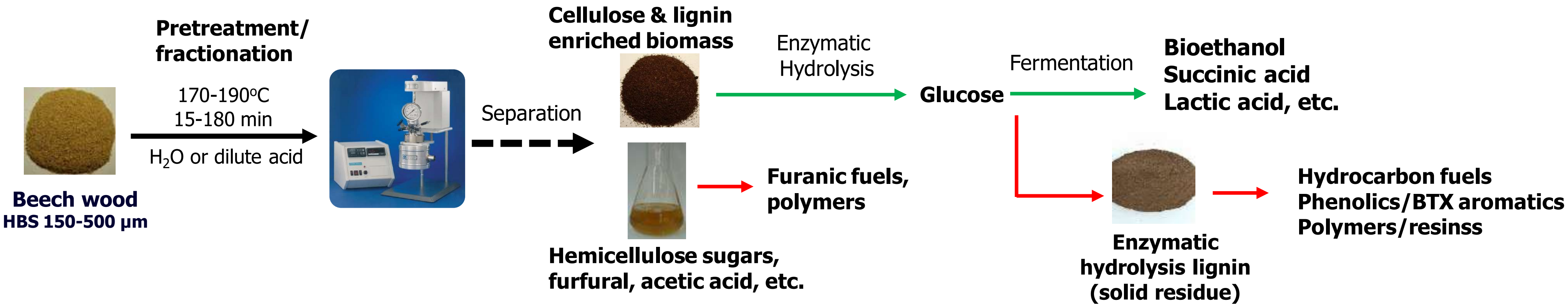
C.K. Nitsos, K.A. Matis, K.S. Triantafyllidis, ChemSusChem, 6 (2013) 110 – 122

C.K. Nitsos, T. Choli-Papadopoulou, K.A. Matis, K.S. Triantafyllidis, ACS Sust. Chem. & Engin. 4 (2016) 4529-4544

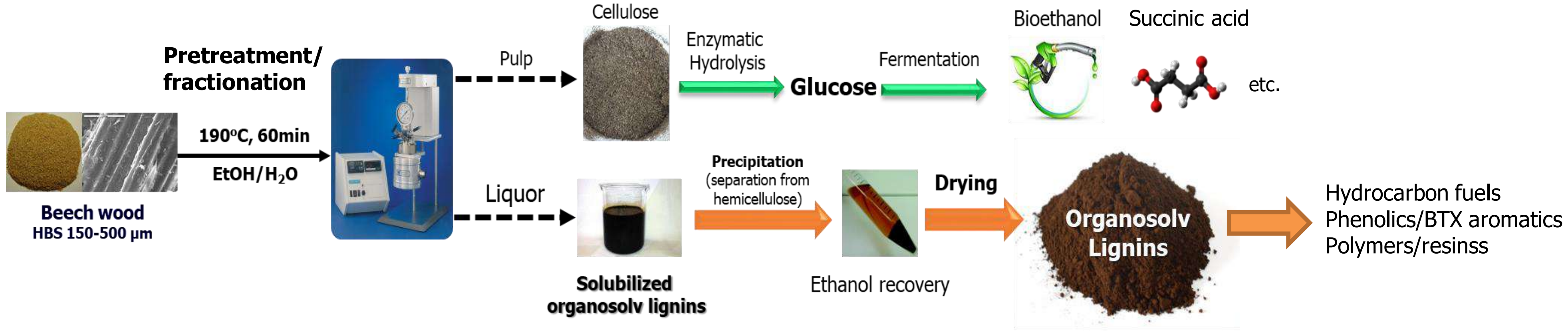
C. K. Nitsos, P. A. Lazaridis, A. Mach-Aigner, K. A. Matis, & K. S. Triantafyllidis, ChemSusChem (2019) 12 (6): 1179

Biorefinery processes: Hydrolysis & Organosolv lignins (S-free)

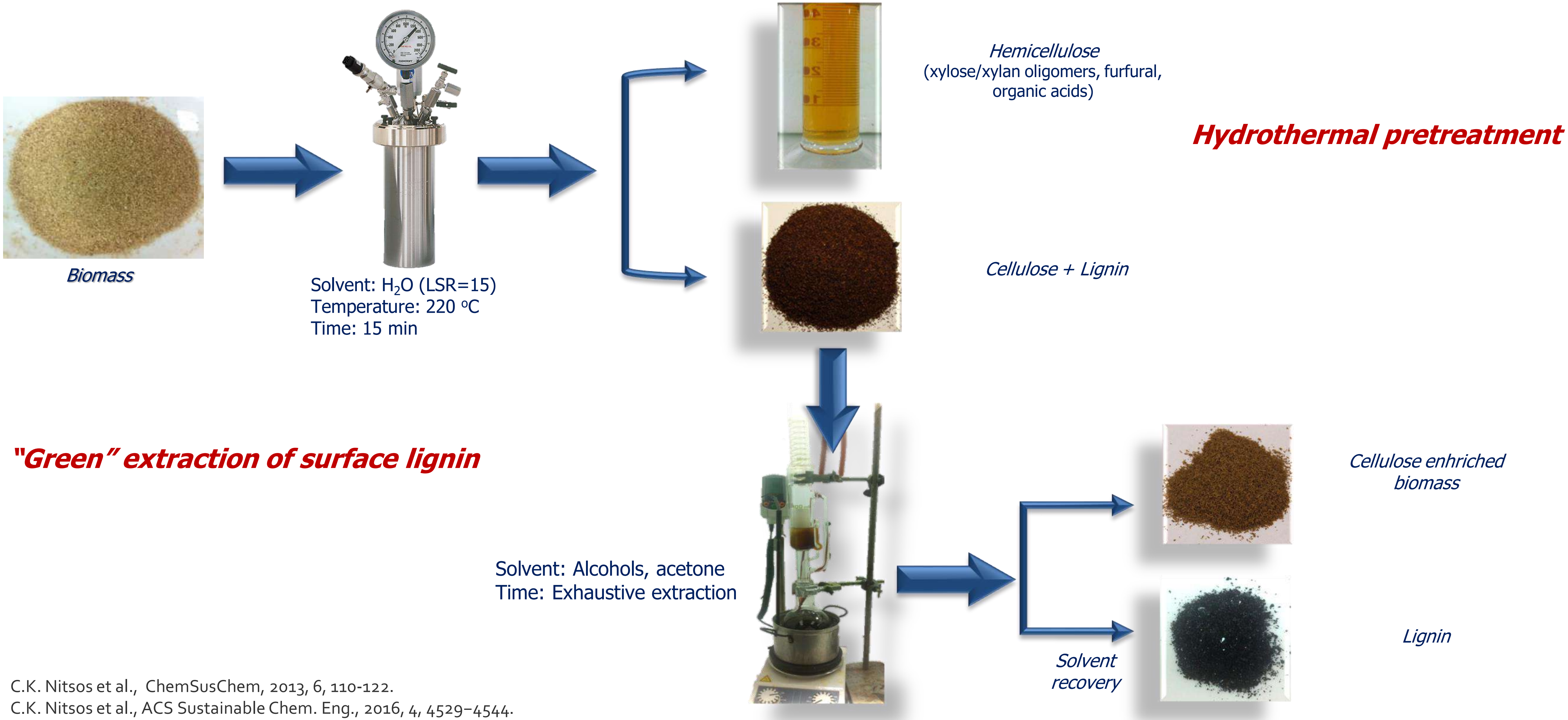
❑ Acid / enzymatic hydrolysis lignin



❑ Organosolv lignin

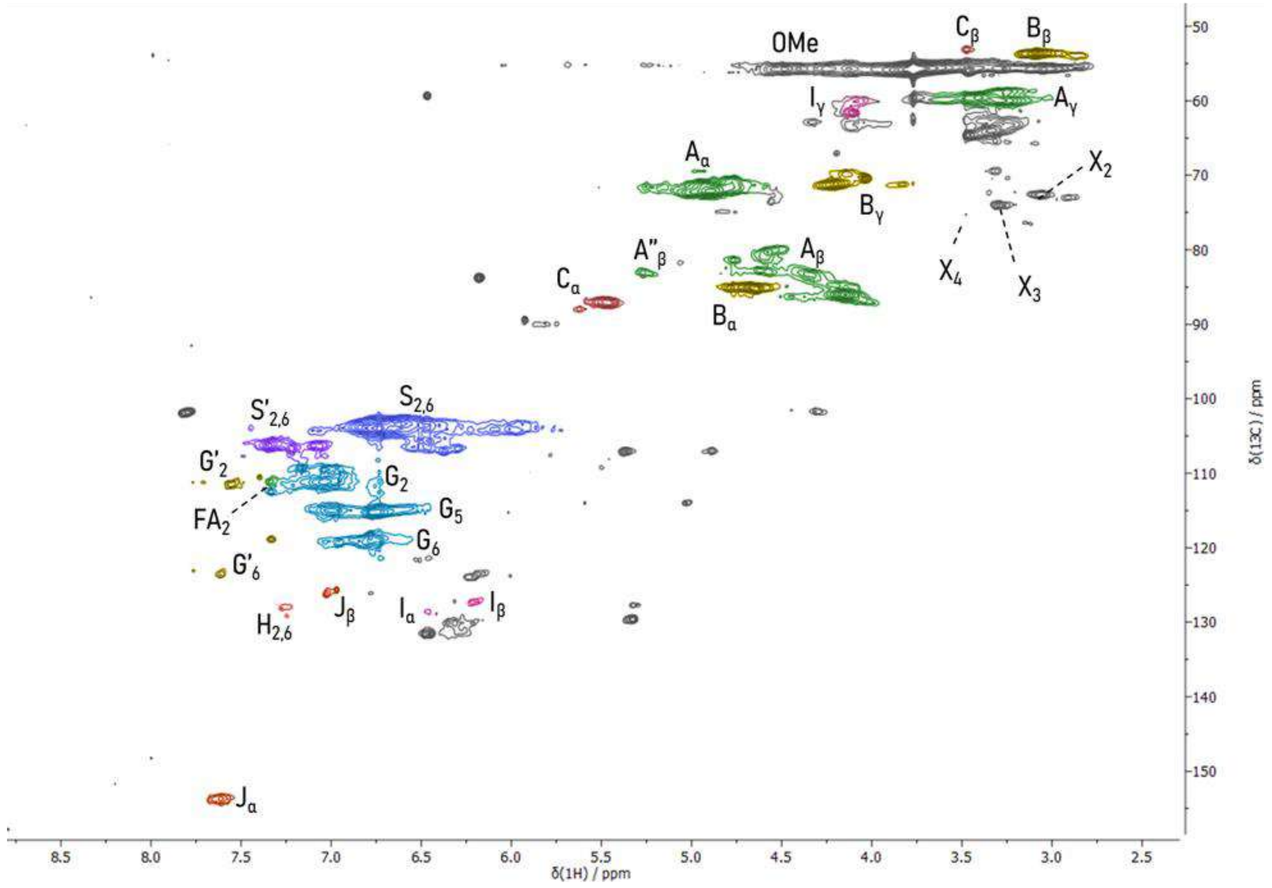


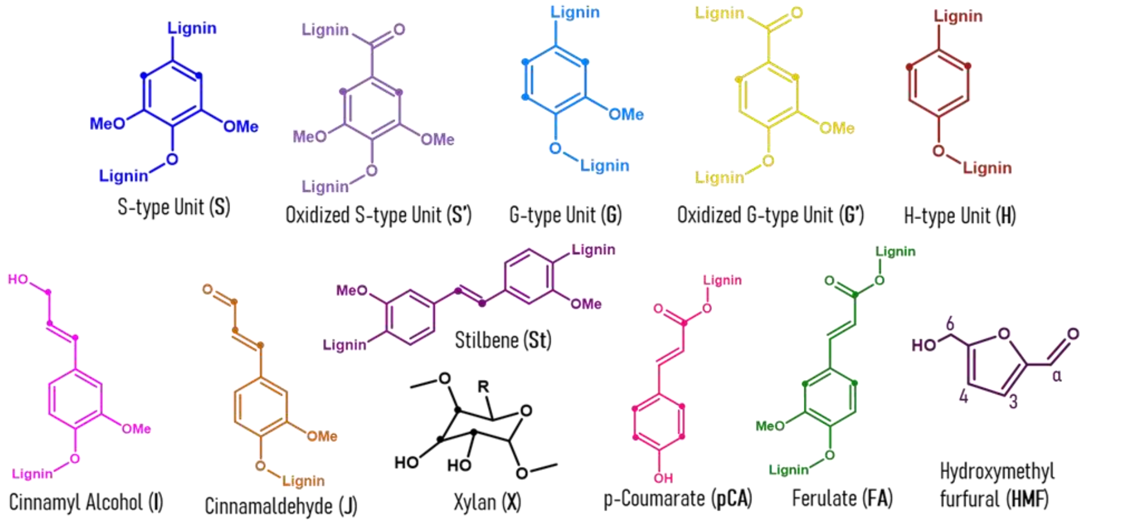
Biorefinery processes: "Surface" lignin extraction

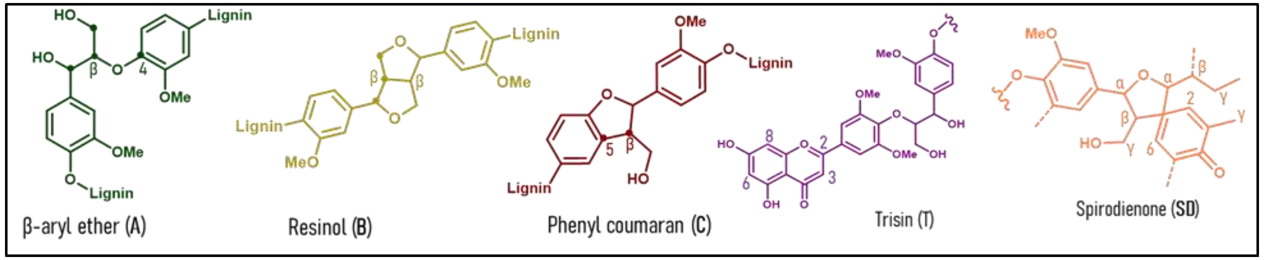


C.K. Nitsos et al., ChemSusChem, 2013, 6, 110-122.
C.K. Nitsos et al., ACS Sustainable Chem. Eng., 2016, 4, 4529–4544.
C.K. Nitsos et al., ChemSusChem, 2019, 12, 1179-1195.

2D HSQC NMR of lignins



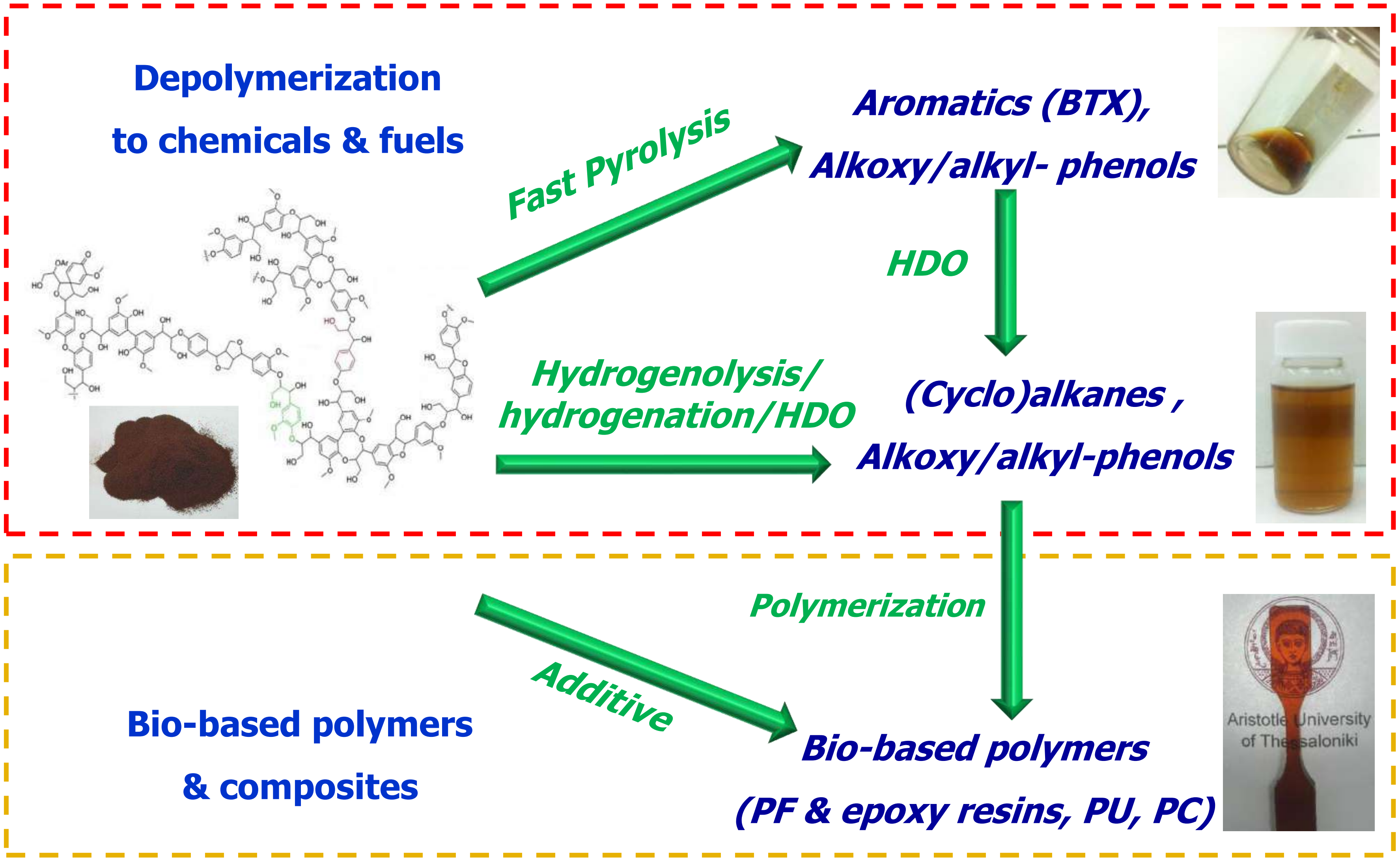




Lignin	Kraft	Organosolv
Origin	Spruce-Softwood	Beech-Hardwood
% Aromatic units S/G/H	-/90.1/9.9	60.9/37.8/1.3
Inter-unit linkages (/100 Ar)		
β-O-4	23.7	62.4
β-β	11.6	18.3
β-5	7.9	10.7

Lignin in DMSO-d6
(512 increments, 32 scans, 5 s interscan delay)

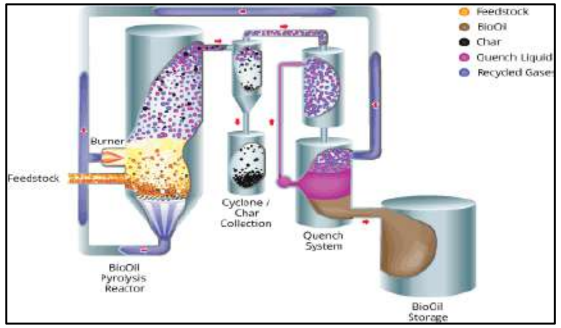
Lignin valorization processes (in our group)



Biomass Fast Pyrolysis (BFP)

Main process characteristics:

- small particles of biomass (< 3 mm)
- inert solid heat carriers (silica sand) & inert carrier gas (i.e. N_2)
- atmospheric pressure
- high heating rates and moderate temperatures (400-600^{o}C)
- low residence time (0.5 – 2 sec)
- rapid cooling of pyrolysis vapours to enhance bio-oil production



Bubbling or circulating-riser fluidized-bed reactors

BFP products:

Pyrolyis oil (bio-oil)	up to 75 wt.% (including water, 15-30 %)
Gases	10-25 wt.%, CO, CO_2; also H_2,C_1-C_6
Char/ coke	10-20 wt.%

Pilot unit Circulating Fluidized Bed reactor (1 kg/h) CPERI/CERTH, Greece

Additional process characteristics:

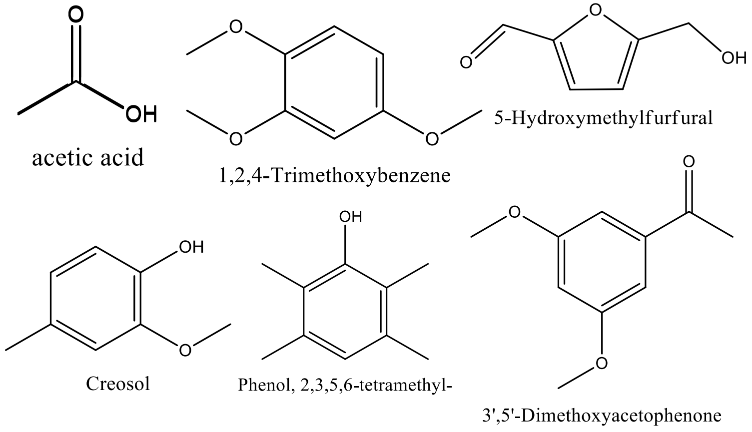
- Flexibility with regard to biomass feedstock
- Autothermal (gas & solid/char products can cover energy requirements)

E. Iliopoulou, S. Stefanidis, K. Kalogiannis, A. Psarras, A. Delimitis, K. Triantafyllidis, A. Lappas, Green Chem. 16 (2014) 662–674.

Characteristics of fast pyrolysis oil (bio-oil)

✓ **Dark brown, low viscosity, relatively acidic with 15-30 wt.% water**

Composition	Origin
Acetic acid	Hemicellulose
Ketones	Hemicellulose, cellulose & lignin
Ethers	Hemicellulose, lignin
Furans	Hemicellulose & cellulose
Phenolics	Lignin & hemicellulose
Minor: Esters, aldehydes, alcohols, sugars, N-comp, heavy	



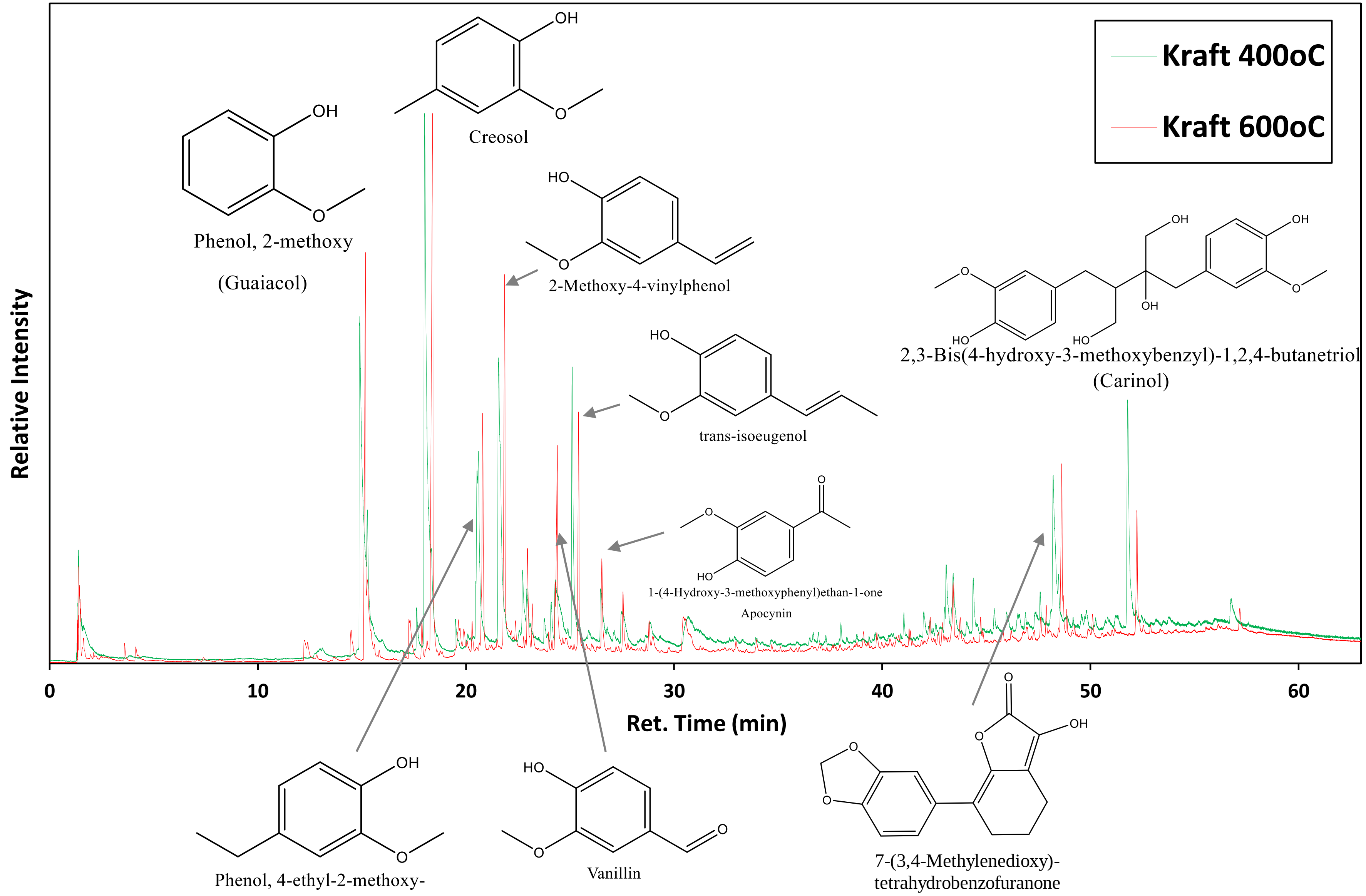
Bio-oil characteristics (e.g. from wood pyrolysis):	
Density	1150 - 1250 kg/m^3
Energy density	**15-25 GJ/m^3** ***(biomass: 9 GJ/m^3)***
Water content	15 - 30 wt.%
Acidity	(pH) 2.5 - 3
Viscosity	25 - 1000 cP
Ash	< 0.1 wt.%

Undesirable properties:

- **Acidic - corrosive**
- **Unstable (polymerizes)**
- **Not miscible with petroleum fuels**
- **Low Higher heating value (HHV)**

Lignin Fast Pyrolysis bio-oil

(Kraft Lignin, spruce - Py/GC-MS spectra)

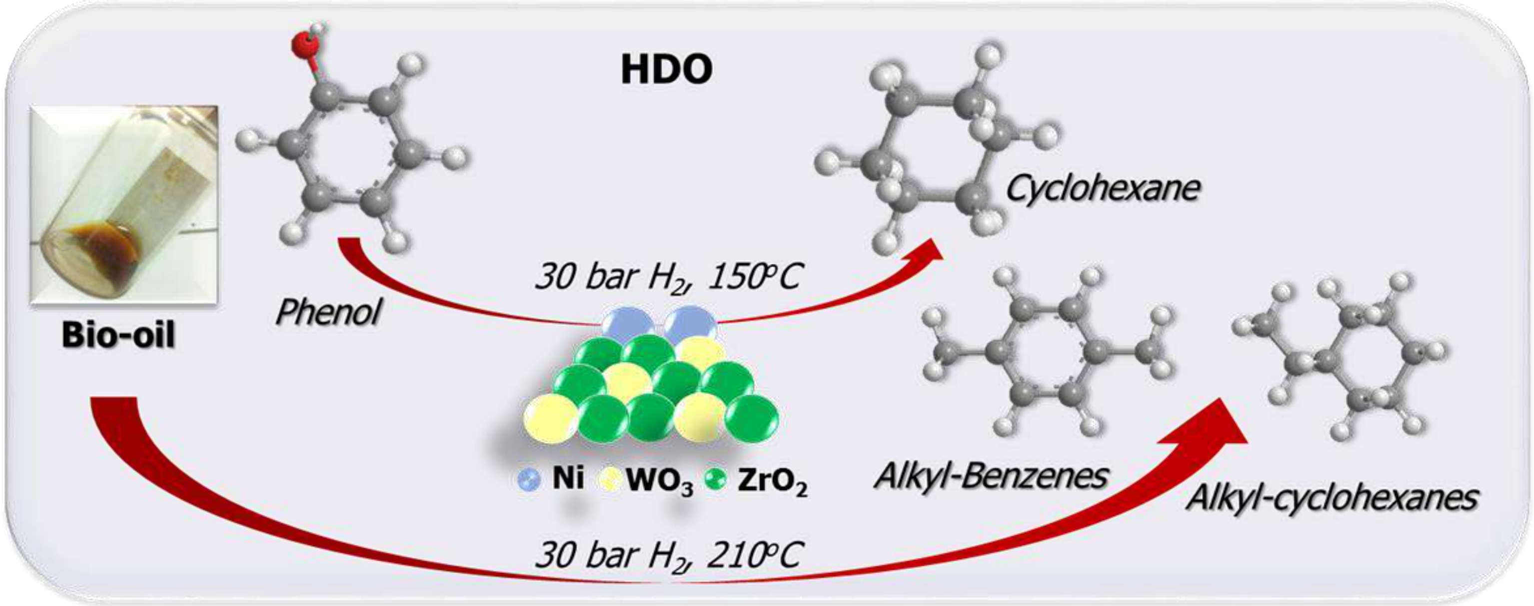


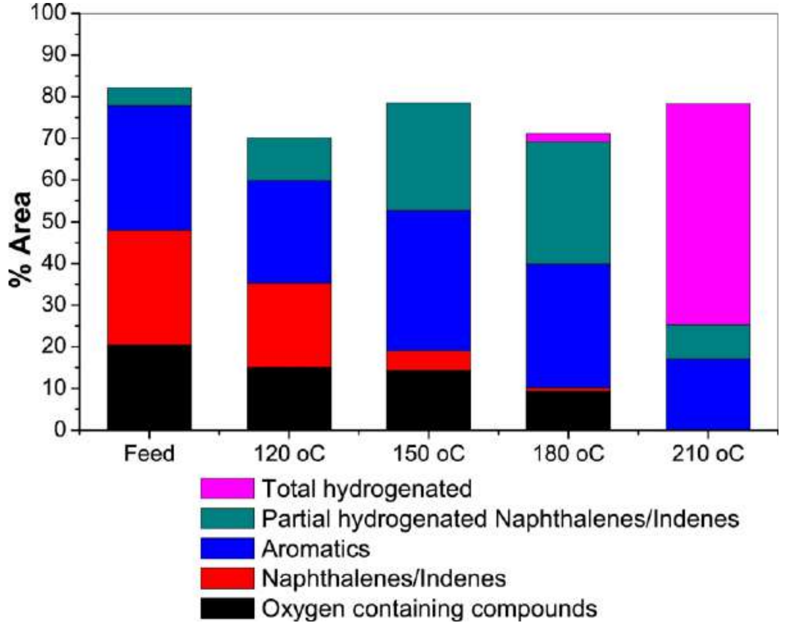
Lignin derived bio-oil : Homogeneous mixture of alkoxy-phenolics

- Utilization in phenol-formaldehyde resins replacing petroleum phenol
- Homogeneous substrate for catalytic upgrading

Valorization of lignin derived bio-oils as fuels

Hydrodeoxygeaniton of bio-oils towards cyclohexanes (drop-in fuels)





C. Zerva, et al., Catalysis Today (2020)

FLEXI-GREEN FUELS

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 101007130.

AUTH main objective:

Development of non-sulfided catalyst for HDO of lignin bio-oils towards aviation and shipping hydrocarbon fuels

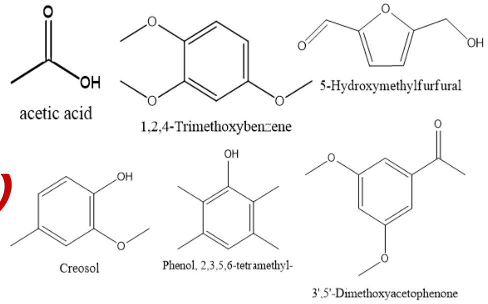
In situ upgrading of bio-oil via Catalytic Fast Pyrolysis (CFP)

Lignocellulosic biomass/lignin

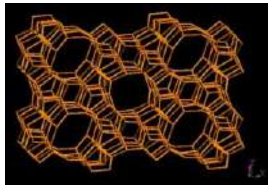
Initial degradation reactions: thermal / non-catalytic

Depolymerization, Hydrolysis, Dehydration, Decarbonylation, Decarboxylation, C-O cleavage

Smaller oligomers and monomers
(non-catalytic biomass pyrolysis vapours)



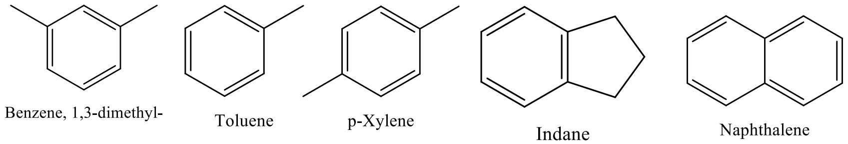
Catalytic Effect:
Porosity
morphology
active sites



MFI (ZSM-5)
5.1x5.5 & 5.3x5.6 Å

dehydration, decarbonylation, decarboxylation, ketonization, esterification, cracking, aromatization, condensation, coke formation

De-oxygenated, aromatic bio-oil



Gaseous products: **CO, CO_2, H_2, light hydrocarbons**
Solid products: **Char and reaction-coke on catalyst**

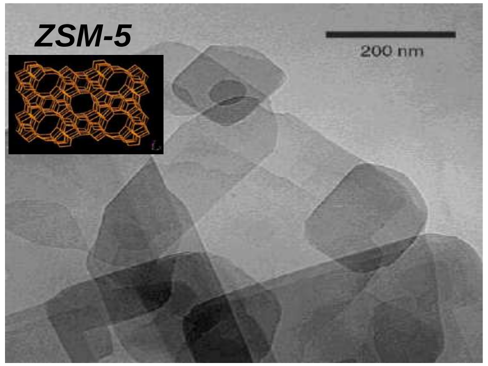
E.F. Iliopoulou, S.D. Stefanidis, K.G. Kalogiannis, A. Delimitis, A.A. Lappas, K.S. Triantafyllidis, Appl. Catal. B: Environ. 127 (2012) 281–290.
E.F. Iliopoulou, S. Stefanidis, K. Kalogiannis, A.C. Psarras, A. Delimitis, K.S. Triantafyllidis, A.A. Lappas, Green Chem. 16 (2014) 662–674.
S. Stefanidis, S. Karakoulia, K. Kalogiannis, E.Iliopoulou, A. Delimitis, H. Yiannoulakis, T. Zampetakis, A.Lappas, K. Triantafyllidis, Appli.Catal.B: Environ. 196 (2016) 155–173

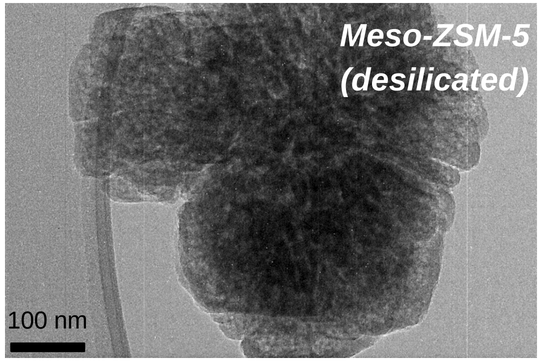
Conventional & Hierarchical ZSM-5 zeolite catalysts

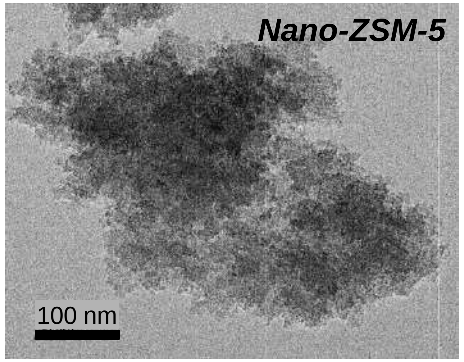
Catalyst	Total SSA [a] (m^2/g)	Micropore area [b] (m^2/g)	Meso/macropore and external area[c] (ml/g)	Average mesopore diameter [e] (nm)	Chemical composition		Acidity FT-IR/pyridine (μmol Pyr/g)		
					Al	Na	Brønsted	Lewis	B/L
					(wt.%)				
ZSM-5 (40)	437	332	105	-	0.91	0.03	190	26	7.3
ZSM-5 (11.5)	424	349	75	-	3.20	0.06	430	123	3.5
Meso-ZSM-5 (9nm)	560	259	301	~ 9 & 90	0.82	0.05	192	21	9.1
Meso-ZSM-5 (45nm)	556	289	267	~ 45	3.00	0.09	385	76	5.0
Nano-ZSM-5	524	343	181[d]	macropores	0.86	0.08	100	53	1.9

[a] Multi-point BET method; [b] t-plot method; [c] Difference of total SSA minus micropore area; [d] Attributed mainly to macropores and external surface area; [e] BJH analysis using adsorption data.

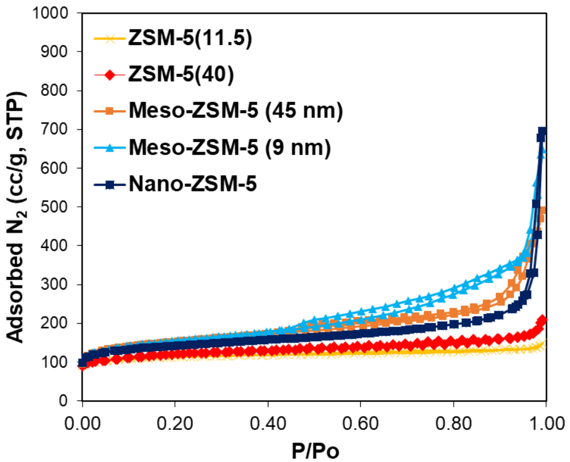
TEM images

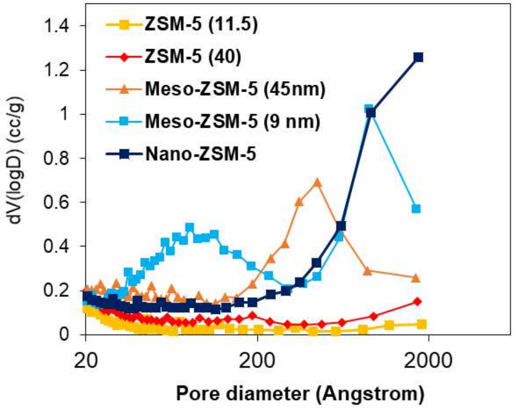




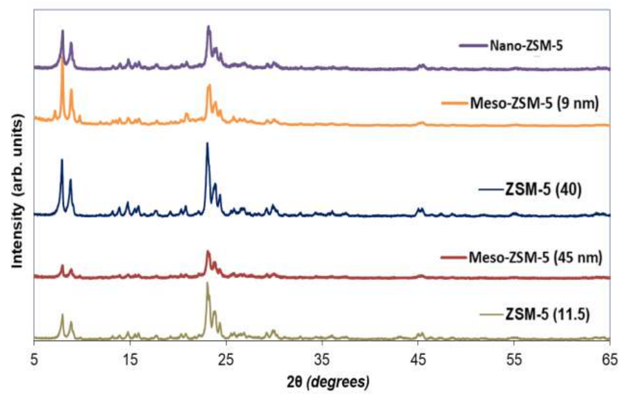


N_2 isotherms & BJH pore size distribution



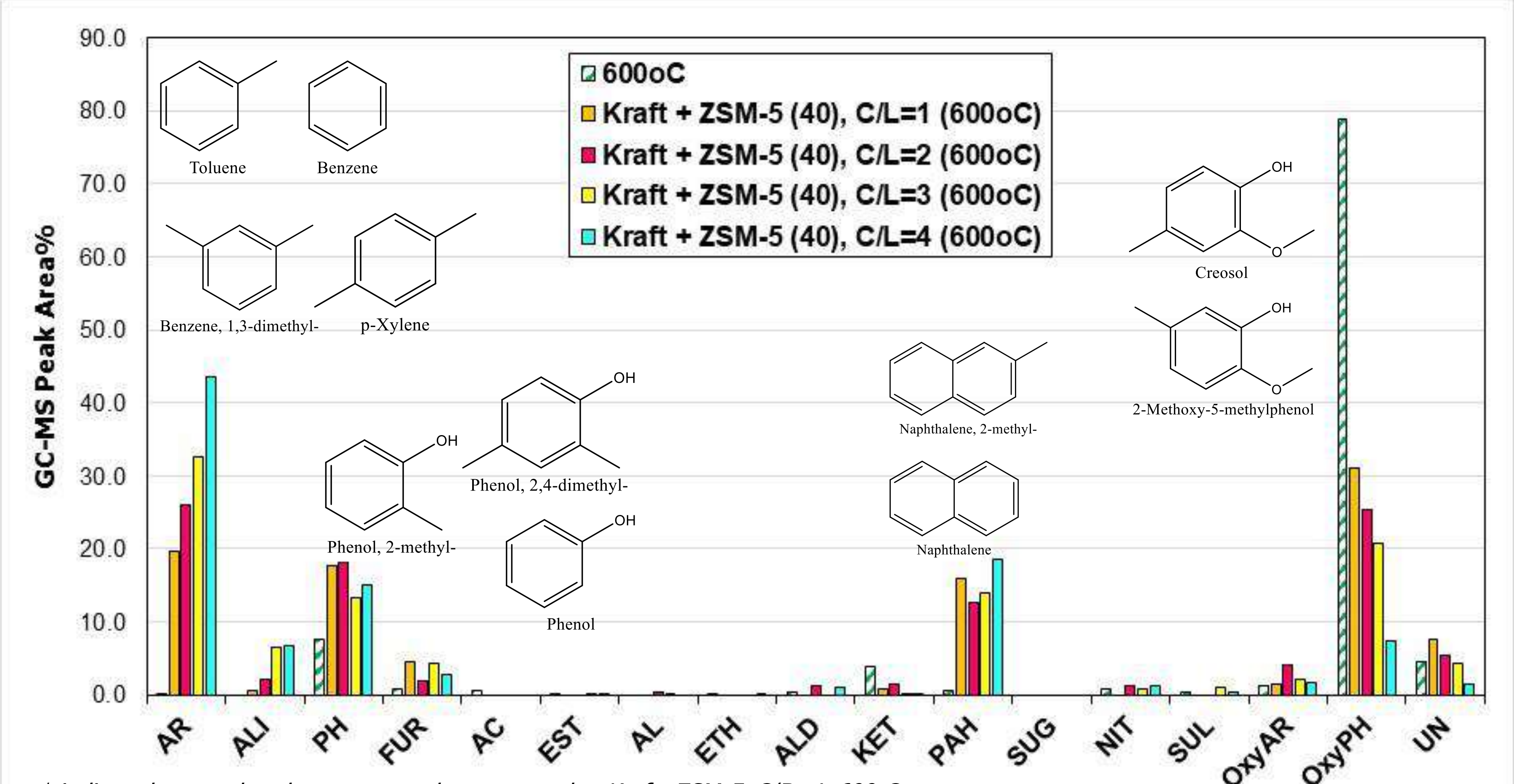


XRD patterns



CFP of Kraft lignin with ZSM-5 to BTX aromatics, naphthalenes and alkyl-phenols:

Effect of Catalyst to lignin ratio (C/B) at 600°C

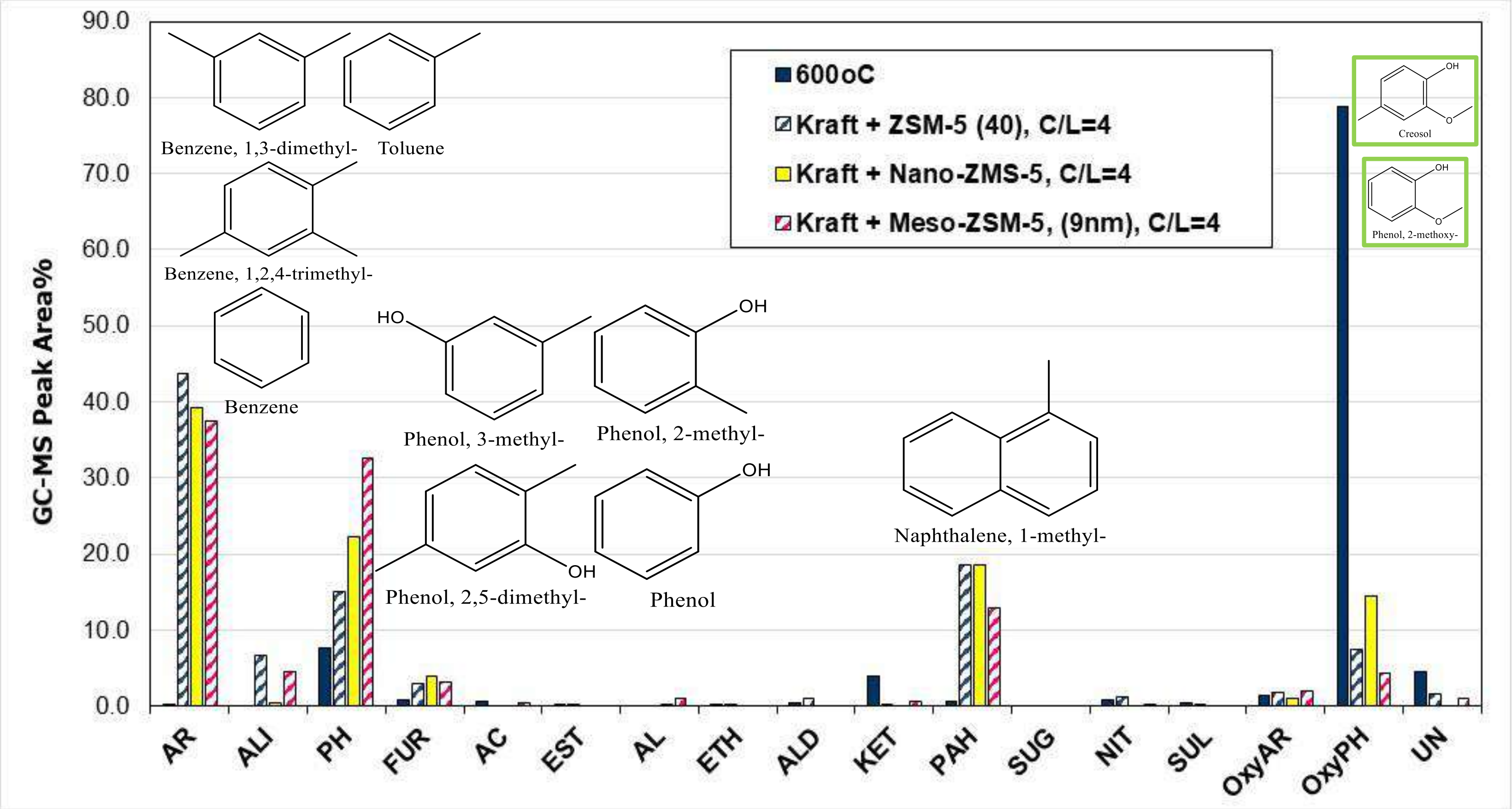


** indicated most abundant compounds correspond to Kraft+ZSM-5, C/B=4, 600°C*

- ZSM-5 induced reactions: dealkoxylation (C-O breaking), dehydroxylation, cracking (C-C breaking), aromatization-condensation

V.B. F. Custodis et al. ChemSusChem, 2016, 9, 1134– 1145
P. Lazaridis et al., Frontiers in Chemistry, 6:295. 2018. doi: 10.3389/fchem.2018.00295

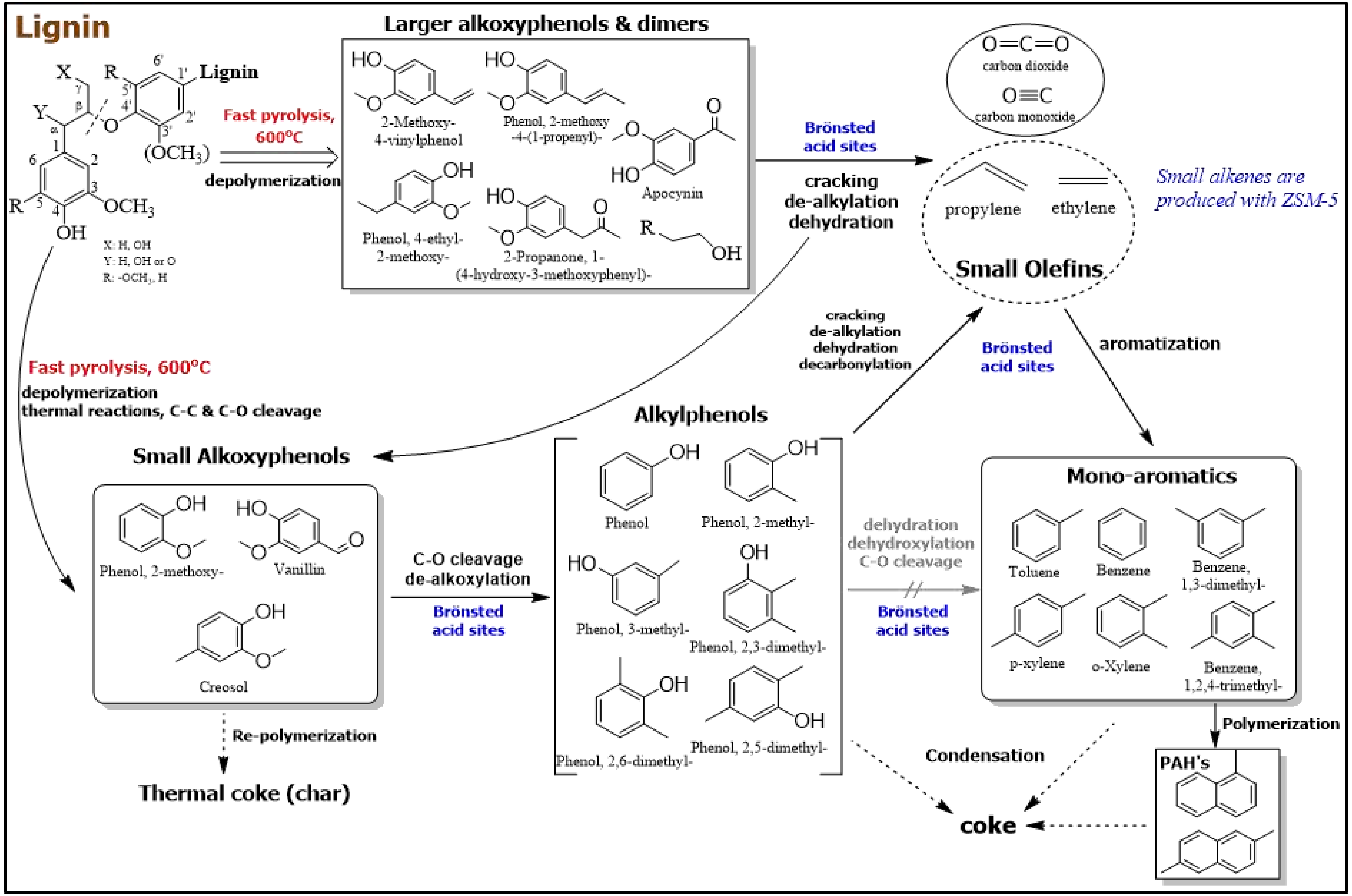
CFP of Kraft lignin with conventional, mesoporous and nanosized ZSM-5 zeolite (600°C)



** Indicated abundant compounds correspond to Kraft + Meso-ZSM-5 (9nm), C/B=4, 600°C*

- Nano-ZSM-5 (half acidity): very reactive towards aromatics and phenols
- Meso-ZSM-5: simultaneous high selectivity to aromatics and alkyl-phenols

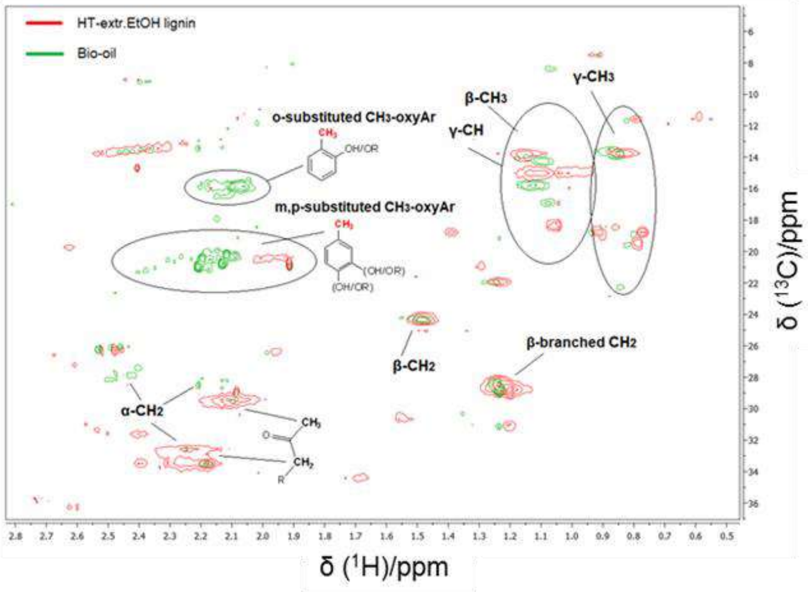
Suggested reaction pathways



P. Lazaridis et al., Frontiers in Chemistry, 6:295. 2018. doi: 10.3389/fchem.2018.00295

2D HSQC NMR of lignin (non-catalytic) bio-oil

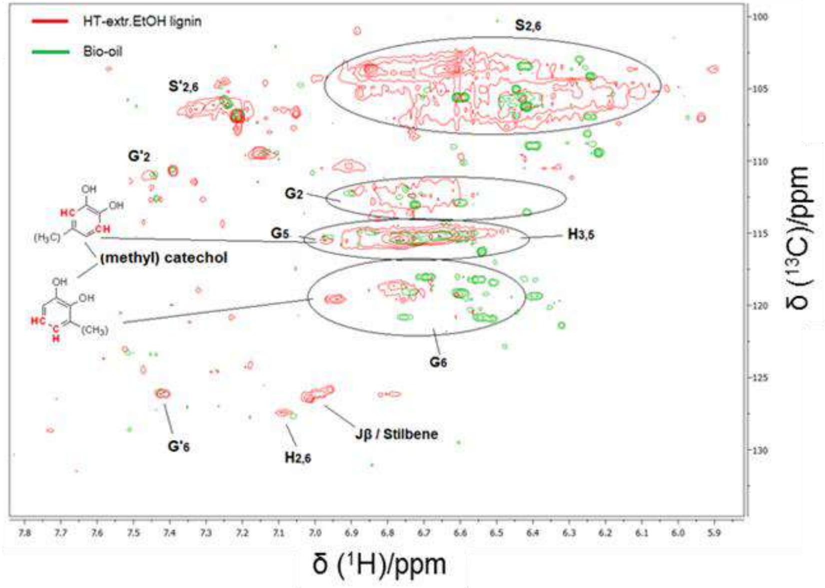
Aliphatic region



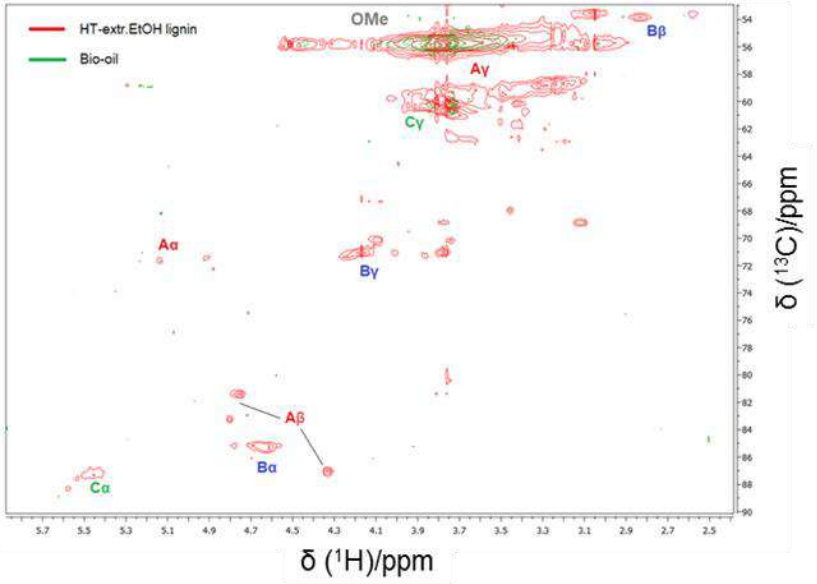
- *Lignin consists of longer aliphatic chains and more β, γ C atoms than the non-catalytic bio-oil*
- *Bio-oil exhibits cross-peaks corresponding to oxyphenols or oxyaromatics*

2D HSQC NMR analysis

Aromatic region



Linkages region



Type A (β-O-4), B (β-β′), C (β-5′) linkages have been cleaved and they are not present in the bio-oil

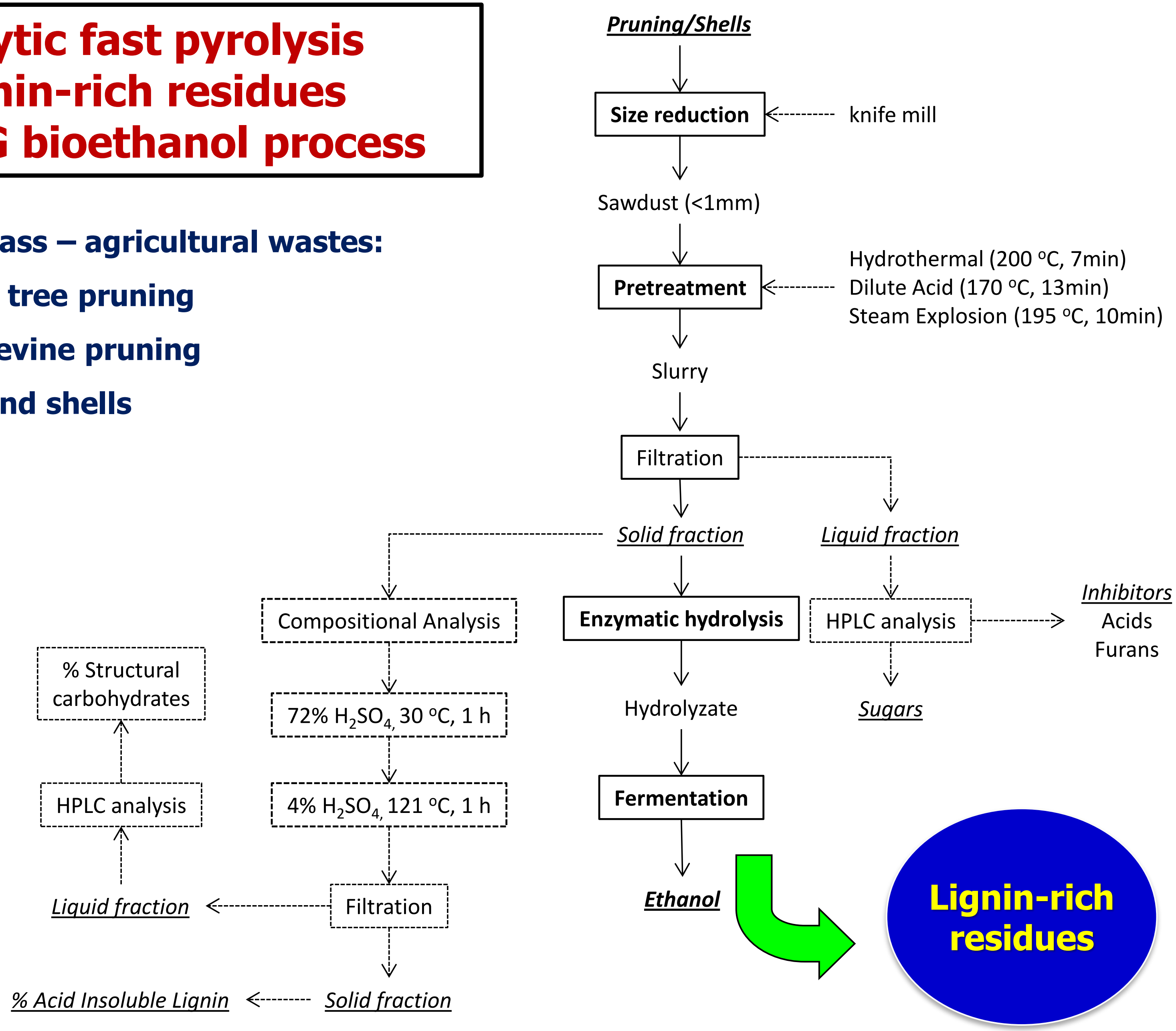
- *Signals of S and G aromatic units in bio-oil have lower intensity compared to those for the initial lignin.*
- *S/G ratio in bio-oil is 63/36, slightly lower compared to lignin with S/G=72/27.*
- *Demethoxylation of S, G units indicates the presence of (methyl)catechols and/or hydroxyphenyl (H) units.*

A. G. Margellou et al. Appl. Catal. A (2021) 623,118298

Catalytic fast pyrolysis of lignin-rich residues in 2-G bioethanol process

- **Biomass – agricultural wastes:**
  - **Olive tree pruning**
  - **Grapevine pruning**
  - **Almond shells**

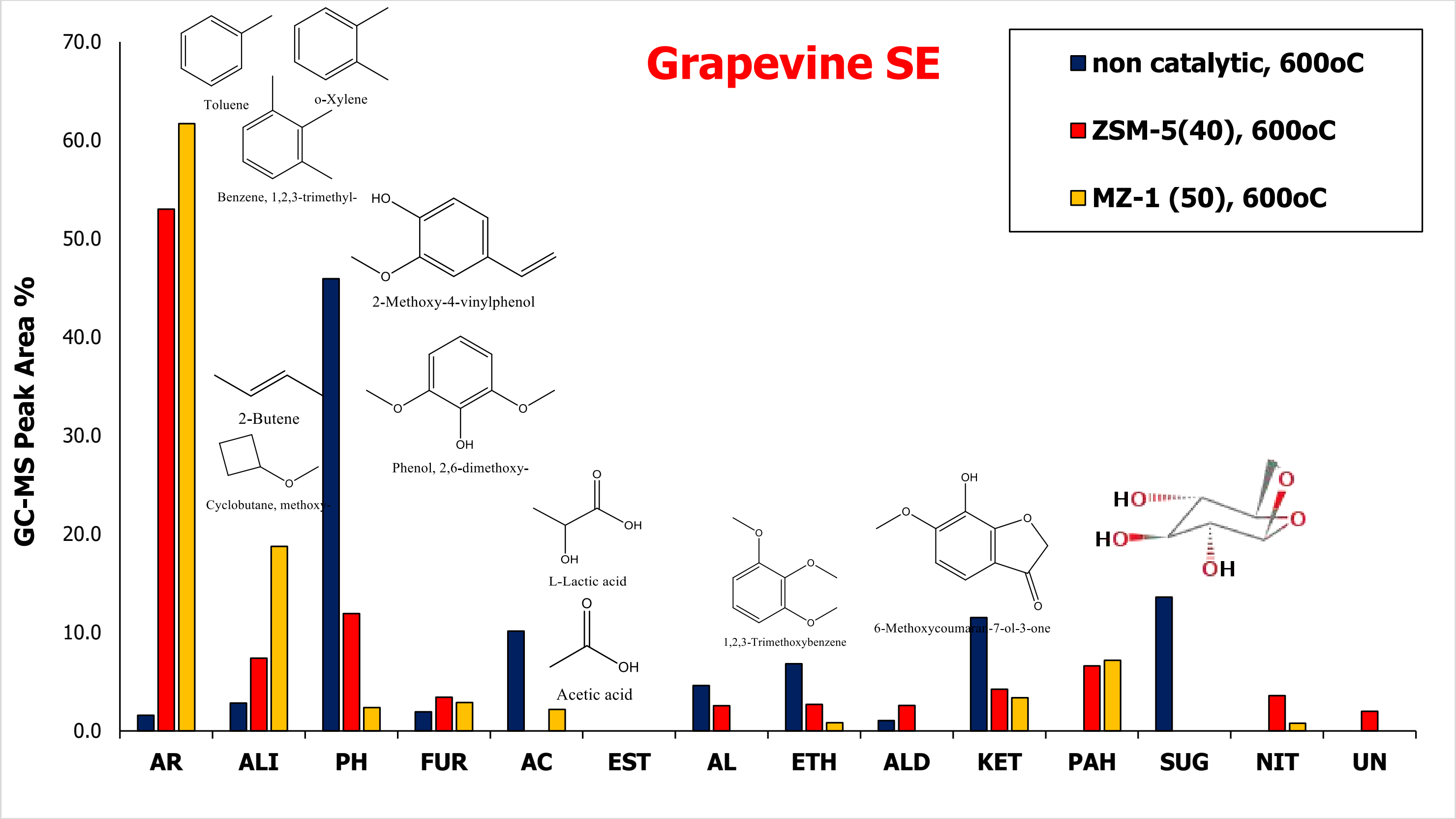
Collaboration with LTU, Sweden



C. Nitsos, L. Matsakas, K. Triantafyllidis, U. Rova, P. Christakopoulos , BIOFUELS, 2017, doi.org/10.1080/17597269.2017.1378988

Catalytic pyrolysis of Lignin-rich residues in 2G bioethanol process

(Feedstock: grapevine pruning)



❖ **Carbohydrates "remainings" in the lignin-rich residues after enzymatic/fermentation are converted to aromatics by catalytic fast pyrolysis**

Topics for today

- ❑ Hydrothermal pretreatment/fractionation of biomass
- ❑ Catalytic hydrogenation of furfural to furfuryl alcohol and 2-MF/2-MTHF
- ❑ Enzymatic hydrolysis of hydrothermally pretreated biomass
- ❑ Hydrolytic hydrogenation of cellulose to sugar alcohols
- ❑ Glucose isomerization to fructose
- ❑ (Nano)cellulose as reactive additive in resins/polymers
- ❑ Catalytic fast pyrolysis of lignin to phenolics, aromatics & fuels
- ❑ (Nano)lignin and phenolics for bio-based polymers and composites

Lignin or lignin biooils for biobased polymers

Polymer Additive

- **Thermoplastic** (PS, PET, PP, PVC, PVA, PBS, PE)
- **Thermosetting** (epoxies, phenol-formaldehyde)
- **Rubbers, Foams** (poly-urethanes)

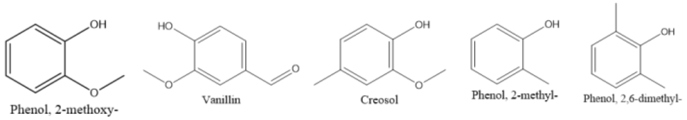
Copolymers

- **poly(N-isopropyl-acrylamide)**
- **poly(lactic acid)**
- **polystyrene**
- **poly(methyl methacrylate)**
- **poly(vinyl acetate)**
- **polybutadiene diisocyanate**
- **methylene diphenyl diisocyanate**

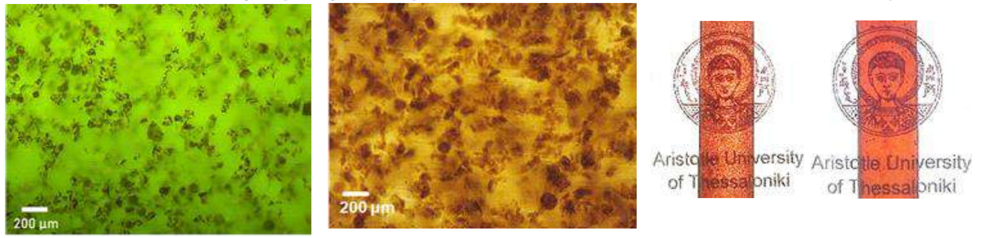
Polymer Blends

- **PEG, PBS**
- **PP, PE, PS, LD/HD-PE,**
- **PVC, PET, PMMA, PLA**
- **PEO, PVA, EVOH**

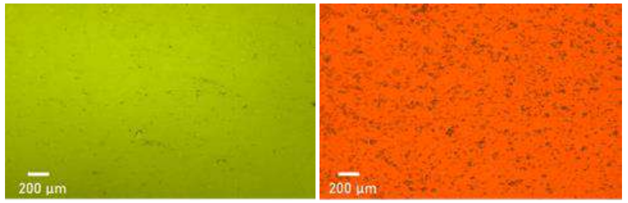
Depolymerization via solvolysis, hydrogenolysis, fast pyrolysis to reactive alkoxy- and alkyl-phenols monomers



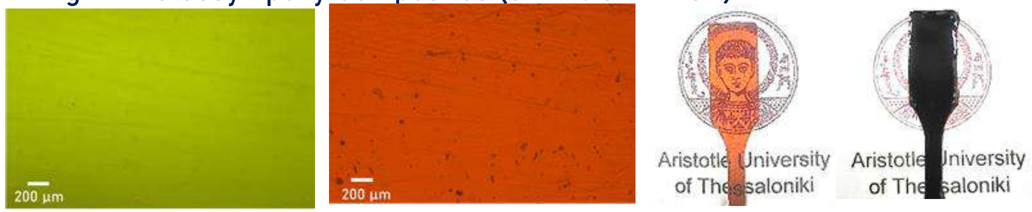
Lignin – Glassy Epoxy Composites (3 wt.% kraft)



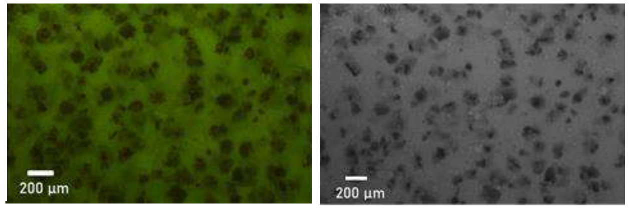
Lignin – Glassy Epoxy Composites (3 wt.% nano-lignin, kraft)



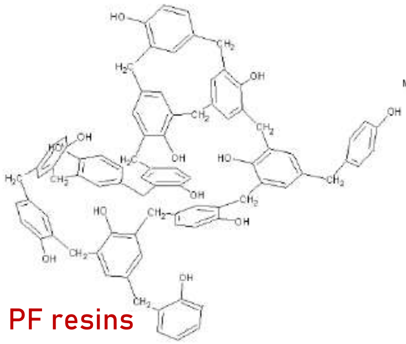
Lignin – Glassy Epoxy Composites (3 wt.% GKL kraft)

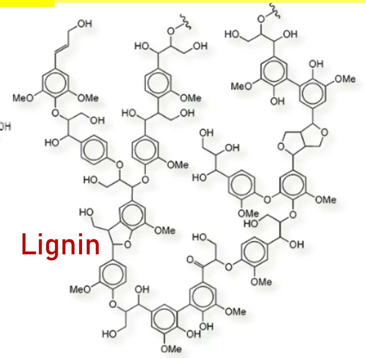


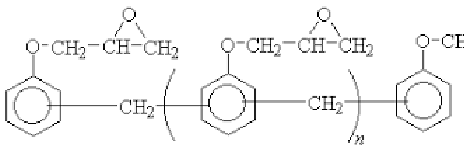
Lignin – Glassy Epoxy Composites (16 wt.% kraft)



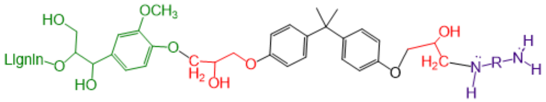
Chemical similarity & affinity between lignin and phenolic/epoxy resins







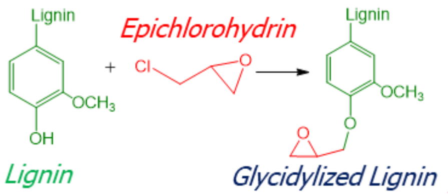
Curing Mechanism of epoxy resins and lignin

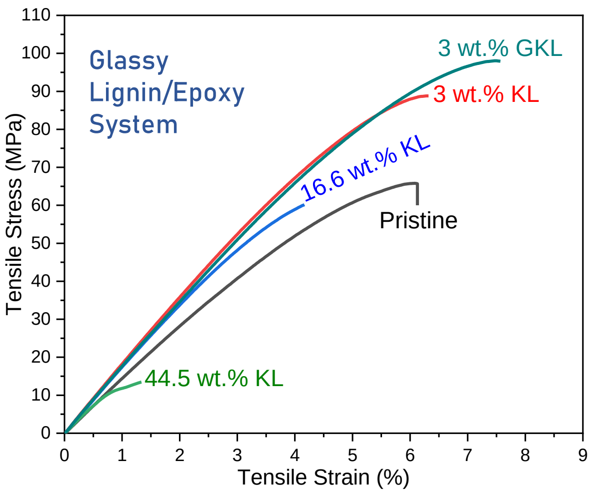


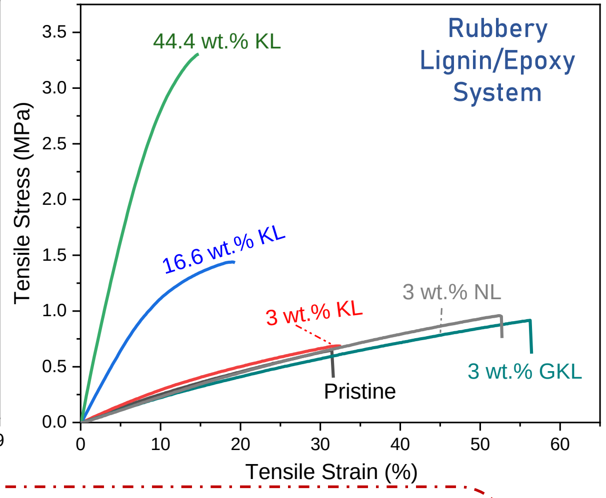
DGEBA crosslinked with –OH of lignin

DGEBA crosslinked with amine curing agent

Functionalization with glycidylation





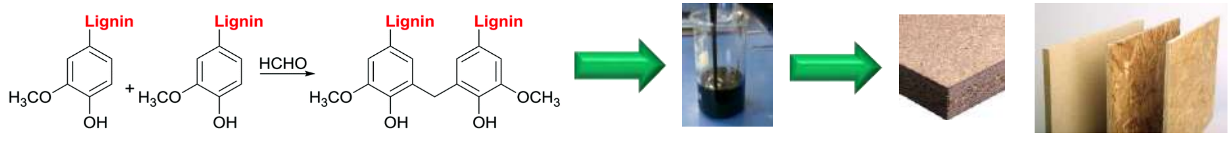


Lignin functionalization:

- Glycidylation
- Etherification
- Amination
- Phenolation
- Esterification
- Hydroxyalkylation
- Nitration

Lignin-induced properties & applications:

- Improved mechanical properties
- Antimicrobial/Antibacterial agent/Drug delivery - Biomedical Applications
- Antioxidant – Packaging material
- Flame retardancy – Wood pellets
- Thermal stability - Batteries
- UV-absorbing - Cosmetics

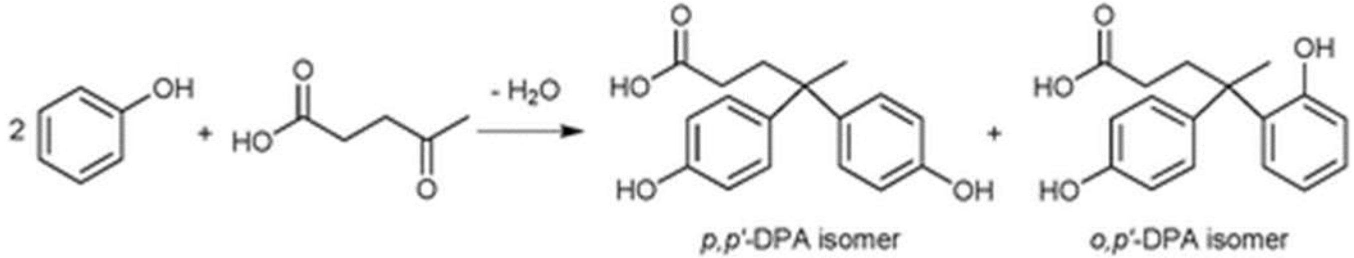


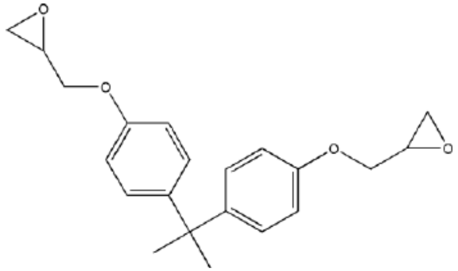
Today: ~ 50 wt.% replacement of petroleum phenol by lignin in Phenol-formaldehyde resins

Valorization of lignin derived bio-oils in resins production

Condensation of lignin derived phenolics with levulinic acid towards diphenolic acid -

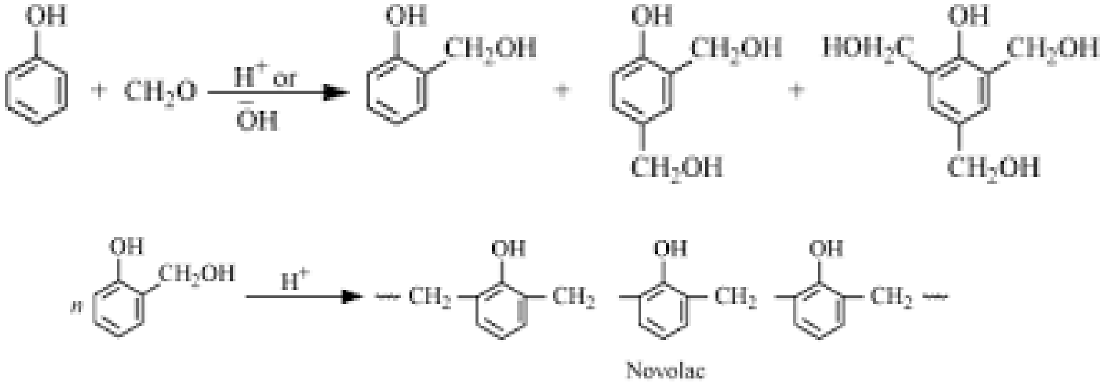
A substitute of petroleum bisphenol A





Epoxy resins

Production of "phenol"-formaldehyde resins replacing petroleum phenol



Phenol-formaldehyde resins

Particle boards & plywoods

BIOBASED NANOMATERIALS COMMUNITY

BIOMAC: European Sustainable BIO-based nanoMAterials Community
Horizon2020 project that will establish an Open Innovation Test Bed (OITB)

BIOMAC

33 partners

B I O M A C

Project Coordinator

ARISTOTLE UNIVERSITY OF THESSALONIKI

www.biomac-oitb.eu

biomac@chem.auth.gr

Project details

Start date: JANUARY 2021
Duration: 4 YEARS
EU contribution: 16.5 mil €

Open Innovation Test Bed for concept development in the field of nano-enabled bio-based materials and products

BIOMASS → 17 PILOT PLANTS → CHEMICALS & NANOPARTICLES → NOVEL NANO-ENABLED BIOPOLYMER CONCEPTS ↔ VALIDATION / MARKET UPTAKE / VALUE CHAIN

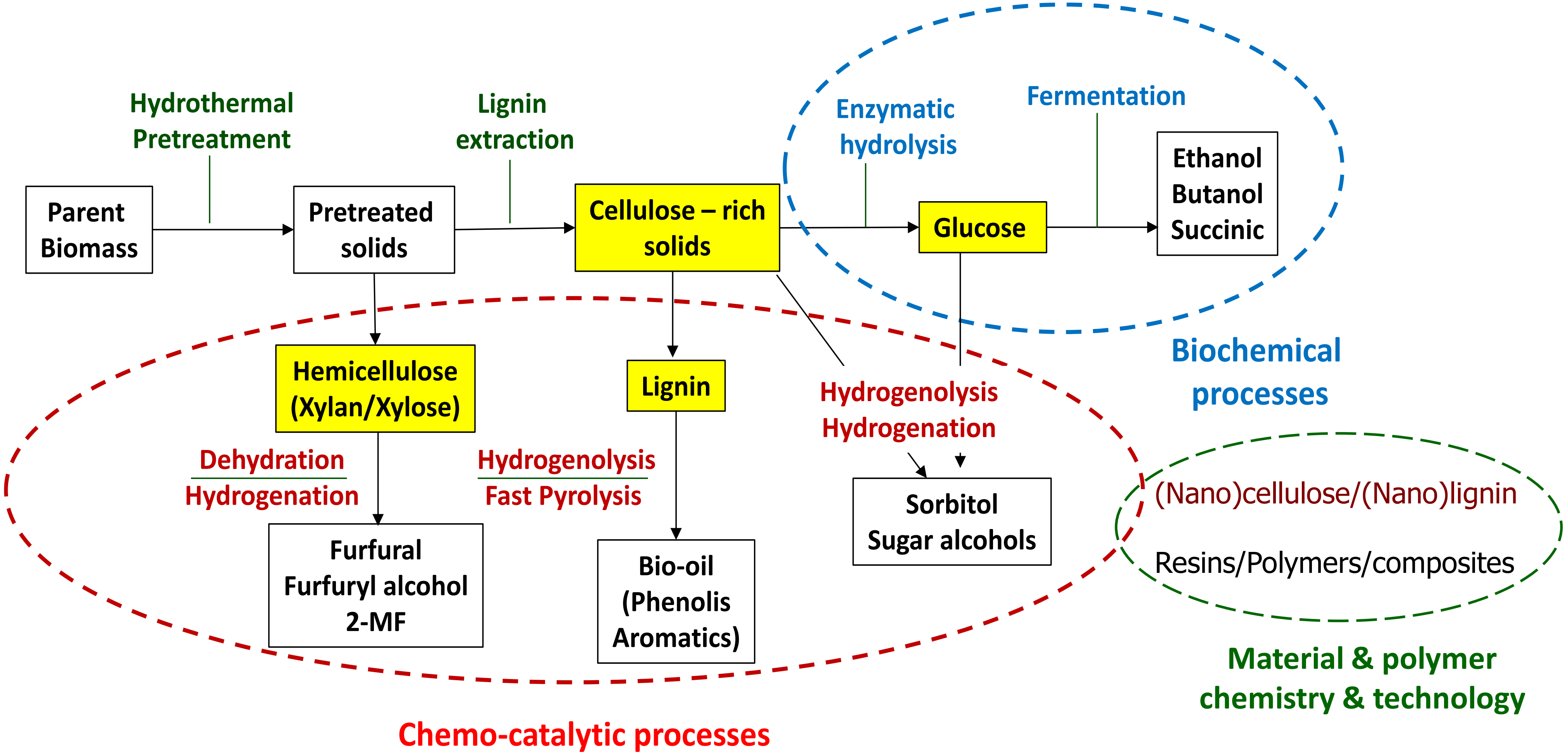
Demonstration of 5 test cases

- Automotive
- Construction
- Agriculture
- Printed electronics
- Food packaging

Open call for upscaling biomaterial concepts

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 952941

“Whole biomass” valorization scheme at AUTH



A synergy between thermochemical pretreatment, chemo- and bio-catalysis, and materials/polymer chemistry is necessary for more efficient biomass valorization

Acknowledgements

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Thank you for your attention!
Thessaloniki, April 2015

Pretreatment of biomass as a key step for bioproducts development

Universidad de Jaén

CEACTEMA

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Dept. Chemical, Environmental and Materials Engineering
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Workshop "***Sustainable Production of Biobased Products in the Bioeconomy Era***"

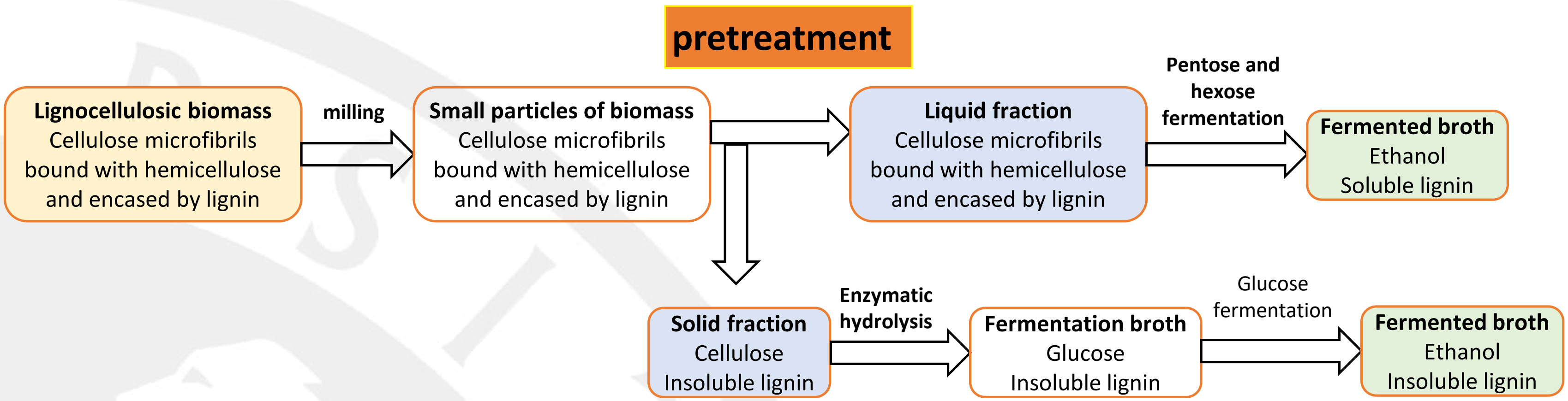
Athens, 10 Nov 2021

Universidad de Jaén

Pretreatment of biomass as a key step for bioproducts development

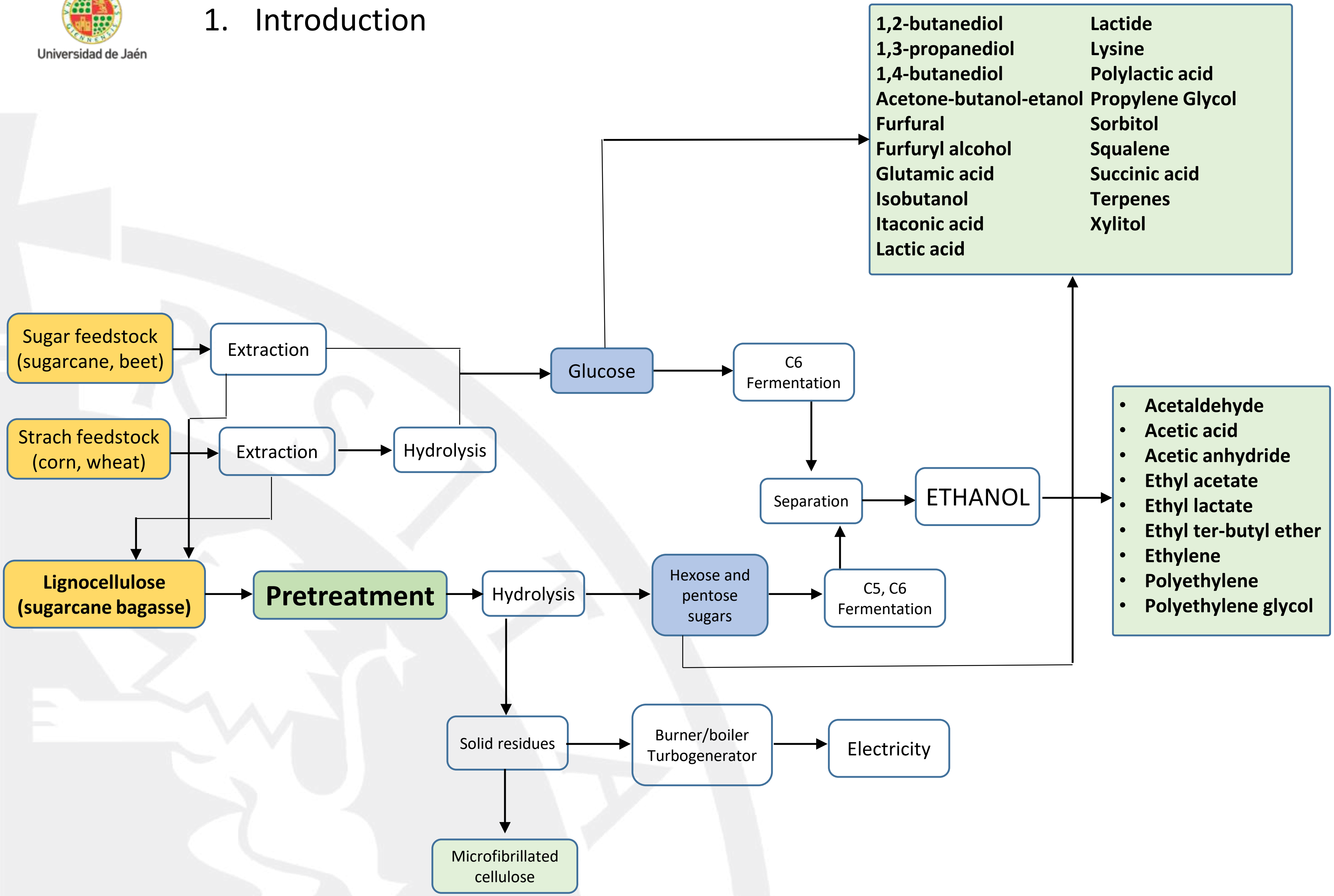
1. Introduction
2. Biomass composition
3. Pretreatment of biomass
4. Some relevant aspects of pretreatment
   - Severity factor
   - Combined severity factor
   - Neutralizing capacity of the biomass
   - Inhibitors generation
   - Image analysis
   - Compositional analysis
   - Crystallinity
5. Pretreatment of olive derived biomass
6. Conclusions

1. Introduction



Abdul Waheed Bhutto et al. (2018). Insight into progress in pre-treatment of lignocellulosic biomass. Energy 122, 724-745

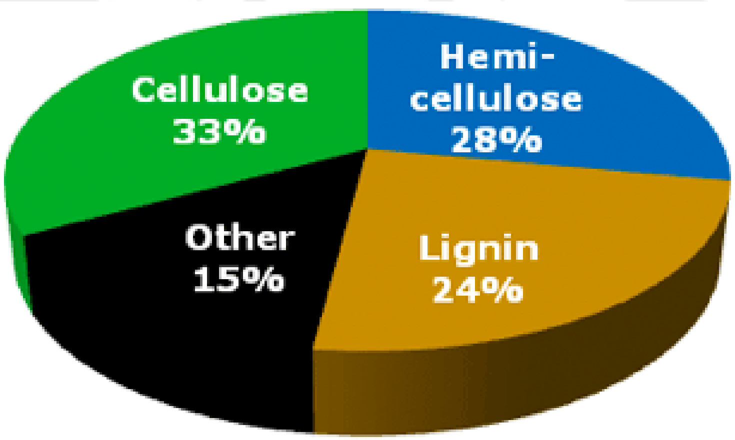
1. Introduction

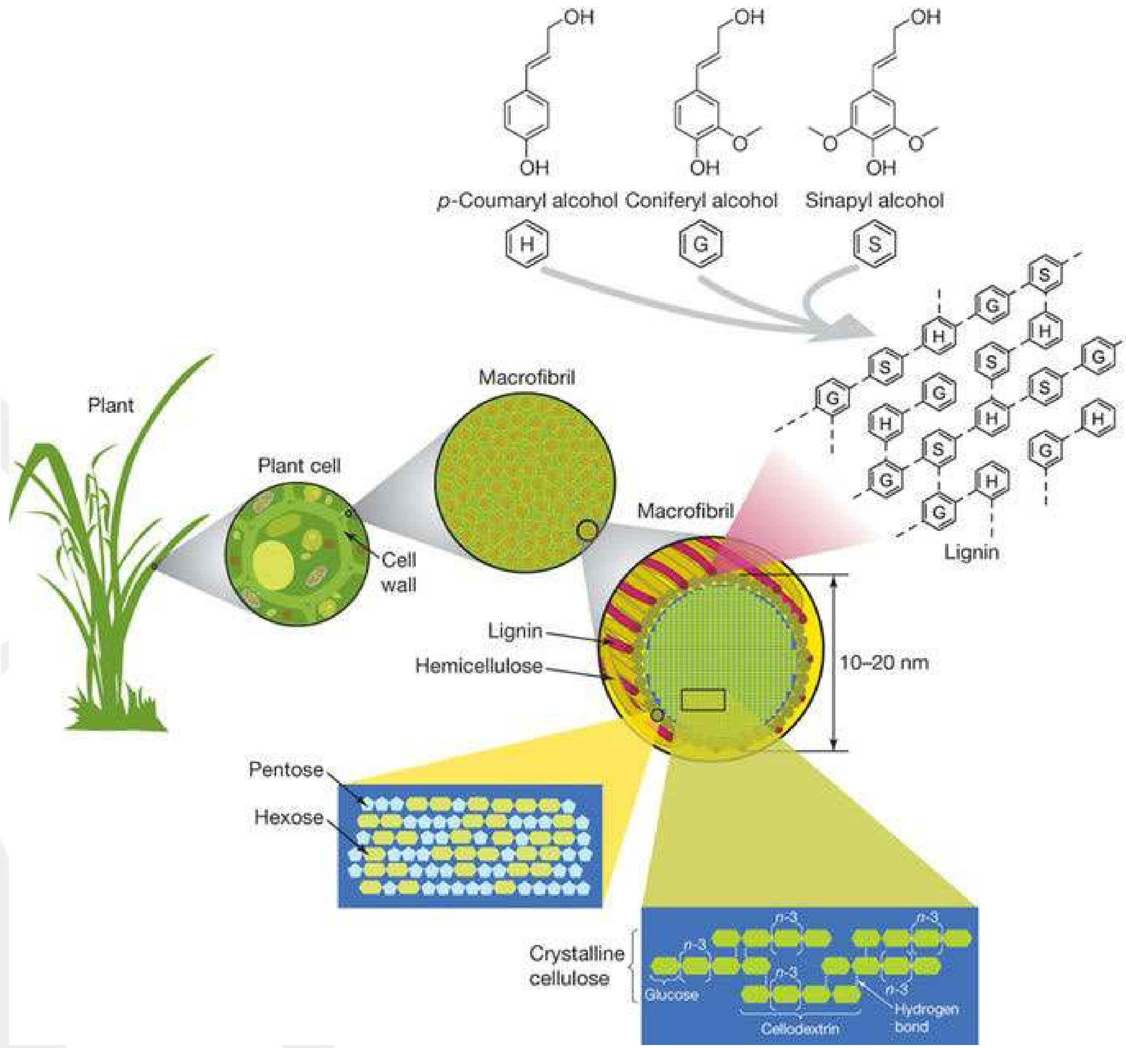


Rosales-Calderon, O., Arantes, V. A review on commercial-scale high-value products that can be produced alongside cellulosic ethanol. *Biotechnol Biofuels* **12,** 240 (2019).

2. Biomass composition

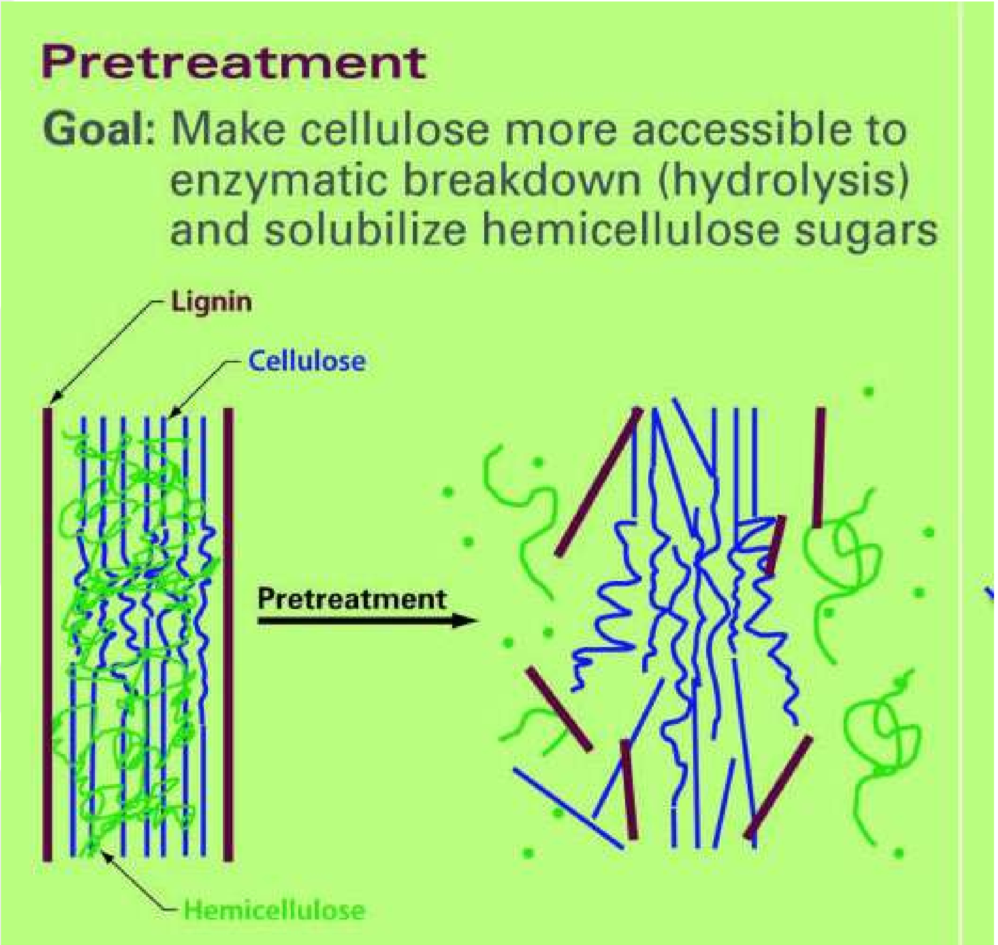
COMPOSITION OF BIOMASS





E.M. Rubin (2008). Genomics of cellulosic biomass. Nature 454 (7206), 841-845

3. Pretreatment of biomass



U.S. Department of Energy Genomic Science program and the website http://genomicscience.energy.gov

3. Pretreatment of biomass

EFFECTS OF BIOMASS PRETREATMENT

- ❑ Reduces cellulose cristallinity
- ❑ Increases accesible surface area
- ❑ Removes and modifies lignin
- ❑ Partially solubilises hemicelluloses

↓

- ❑ Increases accesibility to enzymes

↓

- ❑ Improvement of yields

EFFICIENT BIOMASS PRETREATMENTS

(1) improve the formation of sugars or the ability to subsequently form sugars by hydrolysis
(2) avoid the degradation or loss of carbohydrate
(3) avoid the formation of byproducts that are inhibitory to the subsequent hydrolysis and fermentation processes
(4) are cost-effective

BALANCED PRETREATMENTS

- ⇨ Hard enough to open up/break down lignocellulose matrix, facilitating enzyme access
- ⇨ Smooth enough to avoid degradation of sugars/lignin derivatives

3. Pretreatment of biomass

Attribute	Remarks
Cost	Effective pretreatment process should have **low capital and operational cost.** The use of expensive materials (catalyst, reagents, solvents, feedstock) during pretreatment and subsequent neutralization should be avoided
Energy performance	Should have a **low energy demand**. Pretreatment technologies that require size reduction to small size to are undesirable. Technologies that process feedstock of large dimension give better energy performance and the overall process efficiency.
Operating environment	**Do not** use highly corrosive chemicals or performing the operation at high operating pressure requiring exotic materials of construction.
Presence of inhibitors	The **use of chemicals should be reduced** as much as possible or even totally avoided, since they interfere during hydrolysis and fermentation.
A cost-effectiveness	Should result in the **recovery of most the lignocellulosic components** in a useable form in separate fractions. Pretreatment should yield high fermentable cellulose and hemicellulose sugars. Improve the formation of sugars in the subsequent phase of enzymatic hydrolysis, reducing the degradation of the carbohydrates, and the formation of inhibitors for hydrolysis and fermentation.
Process integration and intensification	Pretreatment should be **effective on a wide range and loading of lignocellulosic material**. It is highly desirable to eliminate conditioning to reduce costs and to reduce yield losses. The concentration of sugars from the coupled operations of pre-treatment and enzymatic hydrolysis should be above 10% to ensure that ethanol concentrations are adequate to keep recovery and other downstream costs manageable.

Abdul Waheed Bhutto et al. (2018). Insight into progress in pre-treatment of lignocellulosic biomass. Energy 122, 724-745

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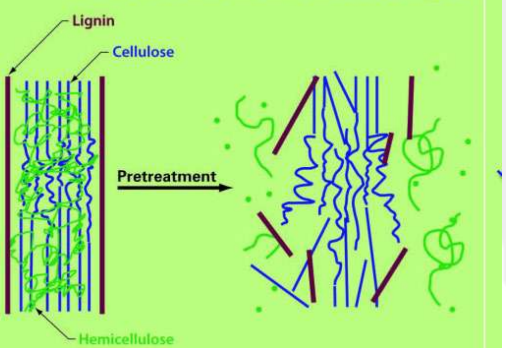
3. Pretreatment of biomass

- **Physical**
  - Milling
  - Extrusion
  - Irradiation
- **Physicochemical**
  - Steam explosion
  - Liquid hot water
  - AFEX
  - Wet oxidation
  - Supercritical fluids
  - CO_2 explosion
- **Chemical**
  - Alkali
  - Acid
  - Ozonolysis
  - Organosolv
  - Ionic liquid
  - Metal salts
- **Biological**
  - Fungi treatment

Pretreatment

Goal: Make cellulose more accessible to enzymatic breakdown (hydrolysis) and solubilize hemicellulose sugars



U.S. Department of Energy Genomic Science program and the website http://genomicscience.energy.gov

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3. Pretreatment of biomass

Pretreatment	Major advantages	Major disadvantages
Milling	Increases accessible specific surface area and reduces crystallinity	High energy consumption
Alkali	Reduces the content of lignin and hemicellulose; low cost	High pollution and high chemical recovery cost
Acid	Reduces the content of hemicelluloses; low cost	Chemical recovery problem
Oxidative	Efficient removal of lignin	High cost of bleaching agents
Organosolv	Separation and recovery of high quality lignin	High price of organic solvents and operation
Ionic Liquids	Reduces the crystallinity of cellulose efficiently	High price of ionic liquids and operation
Steam explosion	Solubilizes hemicelluloses, alters the structure of lignin; cost effective	High equipment cost and generation of inhibitors
Hydrothermal	Solubilizes hemicelluloses; recovers sugars from hemicelluloses	High equipment cost
AFEX®	Causes cellulose swell and increases accessible specific surface area	High equipment and ammonia costs
Supercritical CO2	Increases accessible specific surface area	High equipment cost
Biological	Degrades lignin and hemicelluloses	Long treatment period

Chiaramonti et al., 2012. Biomass Bioenergy 46:25-35.
Mood et al., 2013. Renewable and Sustainable Energy Reviews 27:77–93.
Sunet al., 2016. Bioresource Technology 199:49-58.

4. Some relevant aspects of pretreatment

LEADING ANALYSES

Compositional analysis; Imaging analysis; Cristallinity; Enzymes adsorption/desorption; Degree of polymerization; Accesability

Is the pretreatment effective?
What has happened to the structure?

Native lignocellulose → **PRETREATMENT** → Pretreated lignocellulose →

Improved process for
- Ethanol
- Biogas
- Fermentable sugars
- Biochemicals
- Animal feed
- Composting
- Ensiling

Pretreatment effects

- ❑ Reduces cellulose cristallinity
- ❑ Increases accesible surface area
- ❑ Removes and modifies lignin
- ❑ Partially solubilises hemicelluloses
- ❑ Increases accesibility to enzymes

Keikhosro Karimi and Mohammad J. Taherzadeh (2016). A critical review of analytical methods in pretreatment of lignocelluloses: Composition, imaging, and crystallinity. Bioresource Technology 200, 1008-1018

4. Some relevant aspects of pretreatment

Relevant pretreatment factors/parameters/variables

- Time
- Temperature
- pH
- Liquid to solid ratio
- Inhibitors generation
- Technology selection according to initial composition and final products
- Neutralising capacity of the biomass
- Downstream operations
- Liquid and solid fractions after pretreatment or slurry

4. Some relevant aspects of pretreatment

Relevant pretreatment factors/parameters/variables

Severity factor

$$Ro = t \cdot exp\left(\frac{T - 100}{14.75}\right)$$

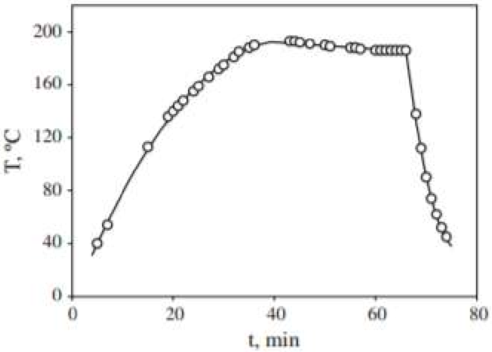
Overend, R.P., Chornet, E., 1987. Fractionation of lignocellulosics by steam-aqueous pretreatments. Philos. Trans. R. Soc. London A 321, 523–536.

$$Ro = \int_0^t \left(\frac{T(t) - 100}{14.75}\right) dt$$

Abatzoglou, N., Chornet, E., Belkacemi, K., Overend, R.P., 1992. Phenomenological kinetics of complex systems. The development of a generalized severity parameter and its application to lignocellulosics fractionation. Chem. Eng. Sci. 47, 1109–1122.

Severity

$$S_o = log\ Ro$$



Díaz MJ, Cara C, Ruiz E, Romero I, Moya M, Castro E. (2010). Hydrothermal pre-treatment of rapeseed straw. Bioresource Technology 101, 2428-2435

Heat to 200ºC, hold for 20 min and then cooling down to room temperature ⇔ Heat to 200ºC, hold for 27.11 min provided instantaneous heating and cooling

Castro E, Díaz MJ, Cara C, Ruiz E, Romero I, Moya M. (2011). Dilute acid pretreatment of rapeseed straw for fermentable sugar generation. Bioresource Technology 102, 1270-1276.

Combined Severity factor

$$\log CS = \log R_o - pH$$

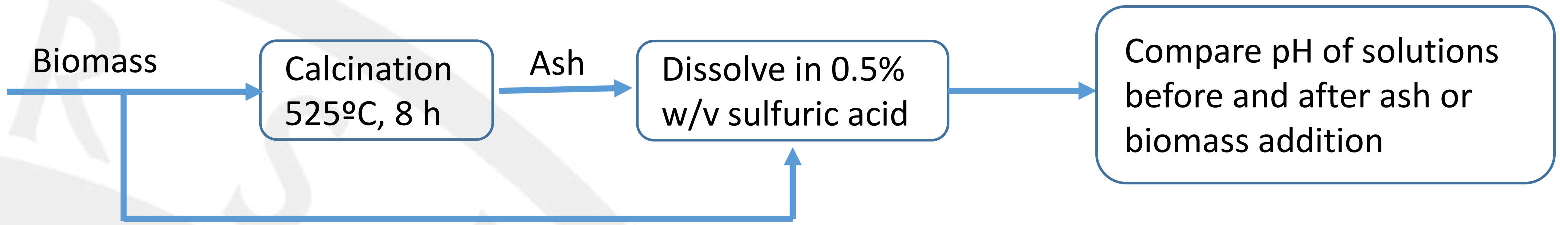
Chum, H.L., Johnson, D.K., Black, S.K., Overend, R.P., 1990.Organosolv pretreatment for enzymic hydrolysis of poplars. 2.Catalyst effects and the combined severity parameter. Appl.Biochem. Biotechnol. 24/25 (1–14)

4. Some relevant aspects of pretreatment

Relevant pretreatment factors/parameters/variables

Neutralizing capacity of biomass

Suspensions of lignocellulosic materials have been shown to partially neutralize acid solutions due to the presence of basic cations in the lignocellulosic matrix.

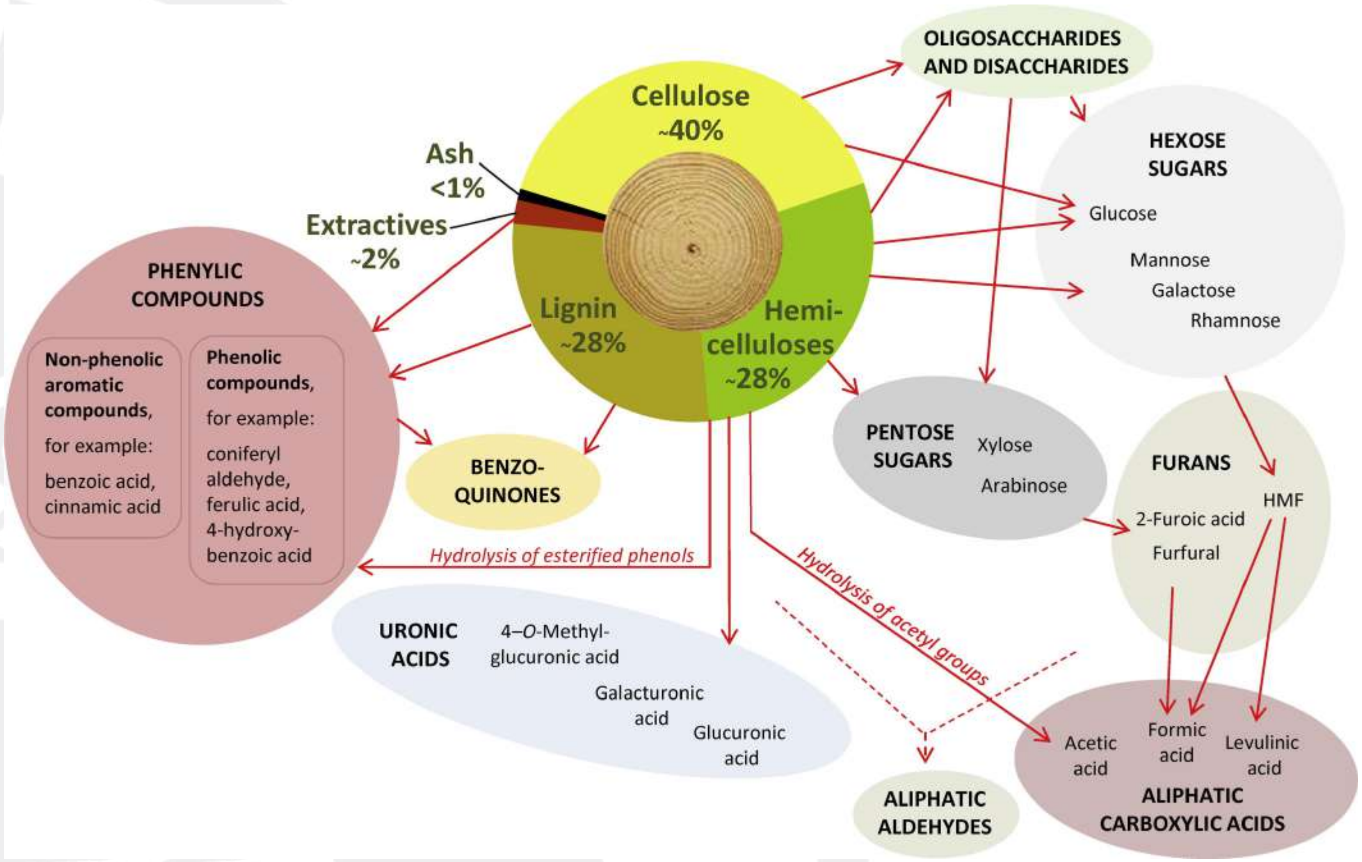


Biomass	Neutralizing capacity g H_2SO_4/g biomass	Reference
Rapeseed straw	19.7	Castro et al. (2011). Bioresour. Technol. 102, 1270-1276.
Corn stover	43.7	Esteghlalian et al. (1997). Bioresour. Technol. 59, 129–136
Poplar	25.8	
Switchgrass	16-7	
Corn stover	17.3	Lloyd and Wyman (2005). Bioresour. Technol. 96, 1967–1977.

4. Some relevant aspects of pretreatment

Inhibitors Where do inhibitors come from?

- Solubilisation and degradation of lignin and hemicelluloses (as a consequence of pretreatment conditions, e.g., temperature, pressure, acidity…)
- Extractives and cellulose unintentionally affected by pretreatment



L.J. Jönsson and Carlos Martín (2016). Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. Bioresour Technol 199, 103-112.

4. Some relevant aspects of pretreatment

Inhibitors

Hydrolysate	Acetic Acid (g/L)	Formic Acid (g/L)	Levulinic acid (g/L)	Furfural (g/L)	HMF (g/L)
Agave bagasse	7.8–8.3	2.0–3.6	N.D	N.D	N.D
Aspen hardwood	8.2–10.1	0.0	0.0	2.1–3.5	1.3–6.8
Bark	0.0–6.2	N.D	N.D	0.5–1.0	0.4–4.3
Biowaste	2.0	N.D	N.D	0.0	N.D
Birch hardwood	2.0–11.5	4.6	0.0	0.2–4.6	0.1–5.8
Corn cob	6.0	N.D	N.D	0.4	N.D
Corn stover	0.0–2.2	0.0–6.8	0.0–2.2	0.6–11.0	0.1–5.3
Pine	0.0–3.7	0.0	0.0	0.7–6.9	1.0–8.6
Rice husks	1.8–2.1	2.5–2.7	N.D	N.D	N.D
Rice Straw	2.3	N.D	N.D	0.1	0.3
Sorghum bagasse	1.0	0.16	0.2	0.0	1.6
Spruce	0.0.–4.7	0.6–3.1	0.2–3.2	0.2–1.4	0.5–8.4
Sugar cane bagasse	N.D.–4.9	N.D.–2.5	N.D.–2.7	0.1–3.1	0.1–3.0
Wheat Straw	3.0–7.0	0.0–1.3	0.0	0.4–1.4	0.1–0.3

Different pretreatment methods and references

Vanmarcke, G., Demeke, M.M., Foulquié-Moreno, M.R. et al. Identification of the major fermentation inhibitors of recombinant 2G yeasts in diverse lignocellulose hydrolysates. Biotechnol Biofuels 14, 92 (2021).

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4. Some relevant aspects of pretreatment

Inhibitors Strategies to circumvent problems with soluble lignocellulose-derived inhibitors formed during pretreatment under acidic conditions

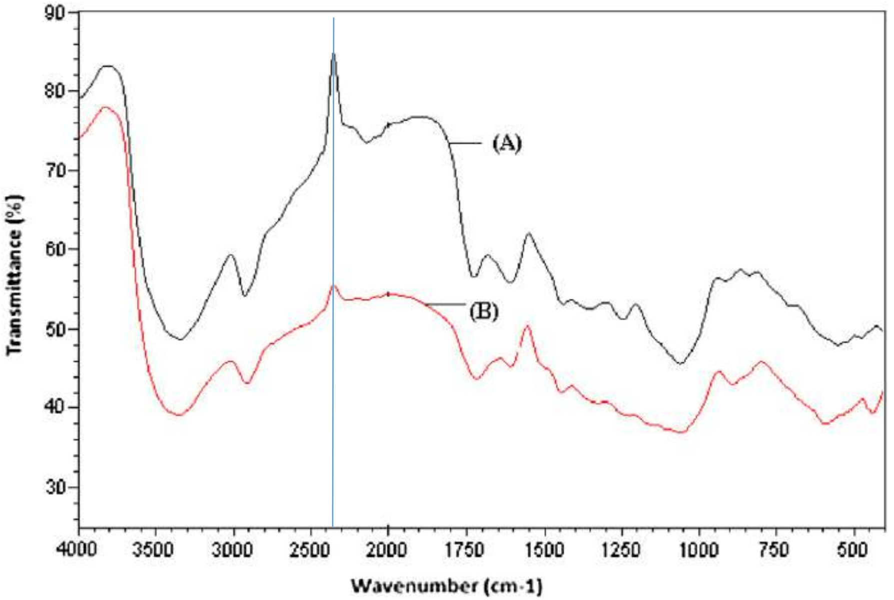
Strategy	Approach (examples)	Considerations/potential drawbacks
Feedstock selection and engineering	Using less recalcitrant feedstocks and feedstocks that generate less inhibitors during pretreatment	Desirable to use broad range of feedstocks; short-rotation crops
Detoxification/ conditioning	Chemical additives, e.g., alkaline treatment, reducing agents, polymers	More chemicals needed; some methods require additional process step
Bioabatement	Microbial treatment	Could be time-consuming and affect sugar content
Culturing schemes	SSF/CBP decrease feed-back inhibition by sugars; use large inoculum size	Effects on productivity and product yield; inoculum adds to cost of an industrial process
Selection of microorganism	Screening of microbial collections from natural or industrial environments	Selection to be made primarily on basis of specific productivity and product yields
Evolutionary engineering	Adaptive evolution using specific inhibitors and lignocellulosic hydrolysates	Cause of inhibition problems varies depending on feedstock, pretreatment conditions
Genetic/metabolic engineering	Engineering of resistance to phenolics, furfural, and carboxylic acids	GMM-based process

L.J. Jönsson and Carlos Martín (2016). Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. Bioresour Technol 199, 103-112.

4. Some relevant aspects of pretreatment

Pretreated solid characterization. Identified peaks in FT-IR spectra for sweet sorghum bagasse

Peak (cm^{-1})	Assignment
3400	H-bonded OH stretching associated with the breakage of H bonds in cellulose
3010	-C-H stretching associated with cellulose
2350	CH_2 stretching associated with amorphous cellulose
1710	-C-O-C ether bonds associated with lignin
1650	-C=O carbonyl group with intra-molecular hydrogen bonds associated with lignin
1600	Aromatic skeletal vibrations in lignin
1425	-O-CH_3 methoxide group present in lignin and hemicellulose
1350	Phenolic hydroxyl groups associated with the structure of lignin
1250	-C-O-C ether associated with lignin-carbohydrate complexes
1050	-C-OH bending in hemicellulose and lignin
885	β-Glucosidic linkages in cellulose and hemicellulose
675	Characteristic feature of ligno-sulphates
<500	Inorganic content in biomass

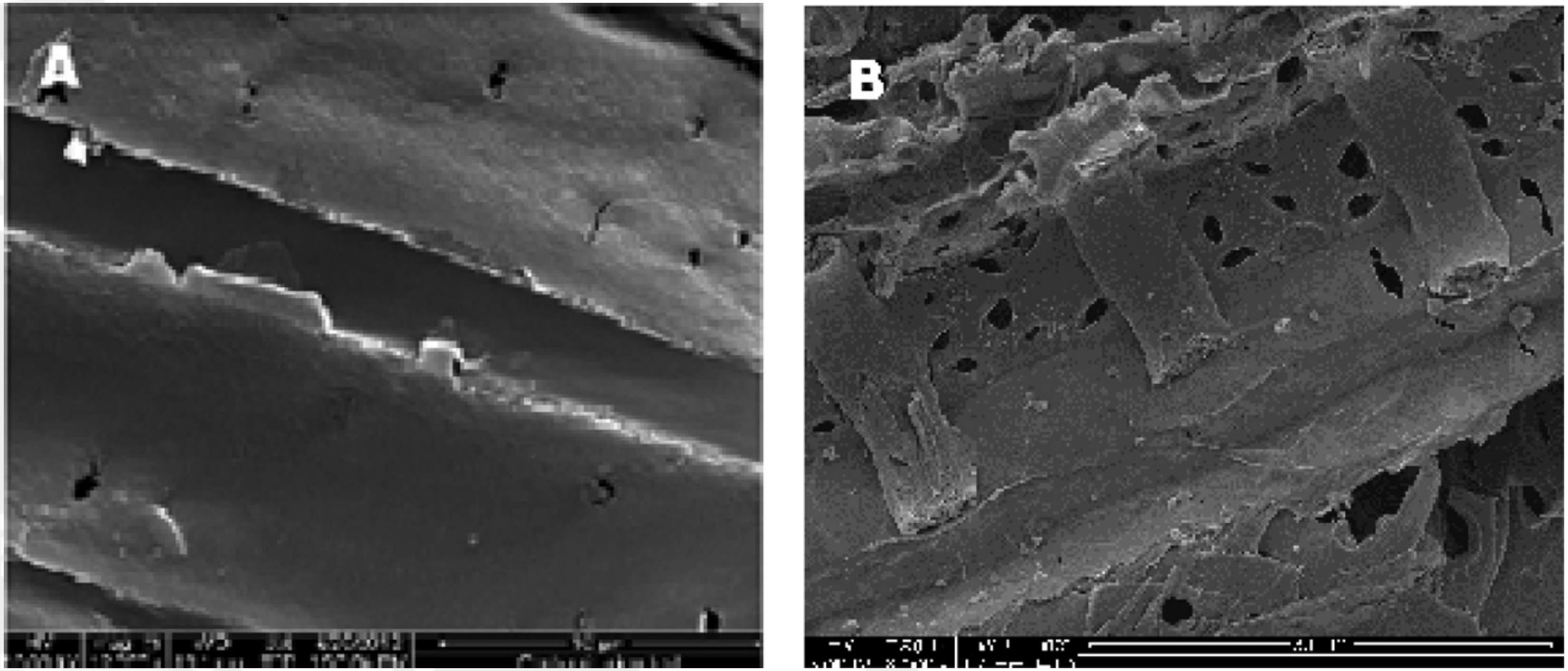


The broad absorption at 3350-3500 cm-1 which is the O-H stretching band of the hydroxyl group associated with the breakage of hydrogen bonds in cellulose. The peak at 2350 cm-1 is due to CH2 stretching associated with amorphous cellulose and which is unaffected by the change in crystallinity of the biomass. This peak is sharper for the untreated bagasse (line A) and almost disappears after pretreatment (line B), showing that this portion of the cellulose was converted by the treatment.

S. Marx et al. (2014). Fuel ethanol production from sweet sorghum bagasse using microwave irradiation. Biomass Bioener 65, 145-150.

4. Some relevant aspects of pretreatment

Pretreated solid characterization. SEM images



SEM images of untreated (A) and 50 g kg^{-1} sulphuric acid in water microwave treated at 43.2 kJ g^{-1} power input (B) sweets sorghum bagasse.

S. Marx et al. (2014). Fuel ethanol production from sweet sorghum bagasse using microwave irradiation. Biomass Bioener 65, 145-150.

The SEM image (B) shows that almost all the cellulose parts of the plant structure had been removed; exposing the some hemicellulose still present as well as some lignin fibrils.

4. Some relevant aspects of pretreatment

Pretreated solid characterization. SEM images

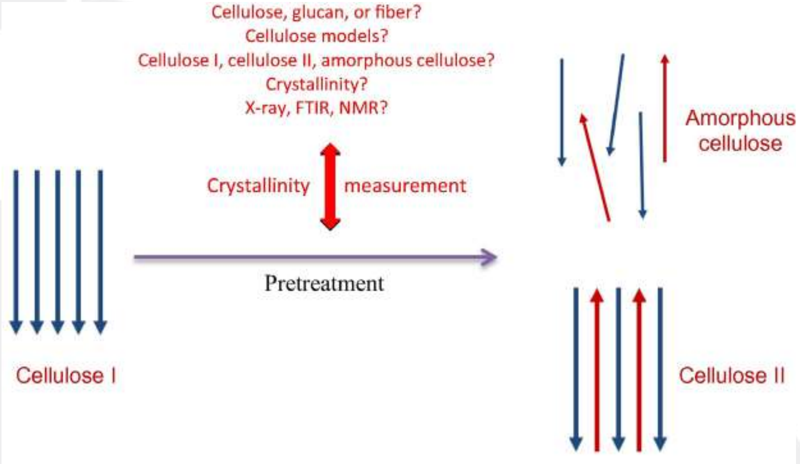
Reported consequences of pretreatment on pretreated solids according to SEM images

- Segregation of fiber bundles and isolation of the fiber cells
- Weakening of the particle mechanical integrity
- Increased fragmentation creating smaller clusters of cells and individual fibers
- Loosened and segregated particles
- Low density structure is observed as lighter areas within the cell wall
- Severe deconstruction by intracell wall nanofibrillation and delamination
- Reduced contrast of the cell walls, indicating lower lignin content
- The smooth cell wall surfaces become extremely irregular from the apparent deposition of the re-localized cell wall matrix
- Lignin re-localization into lignin-rich globules, apparent at high magnifications (20,000×)-
- A homogeneous surface texture in the treated sample, creating an even relocation and removal of lignin
- Color changes from light brown to white, indicating lignin removal
- Particle fragmentation and cell separation by pretreatment may be attributed to the lignin removal and depolymerization from the shared compound in the middle lamella
- Structural deformation of the fiber cells by pretreatment

Karimi and Taherzadeh (2016). A critical review of analytical methods in pretreatment of lignocelluloses: Composition, imaging, and crystallinity. Bioresource Technology 200, 1008-1018

4. Some relevant aspects of pretreatment

Crystallinity



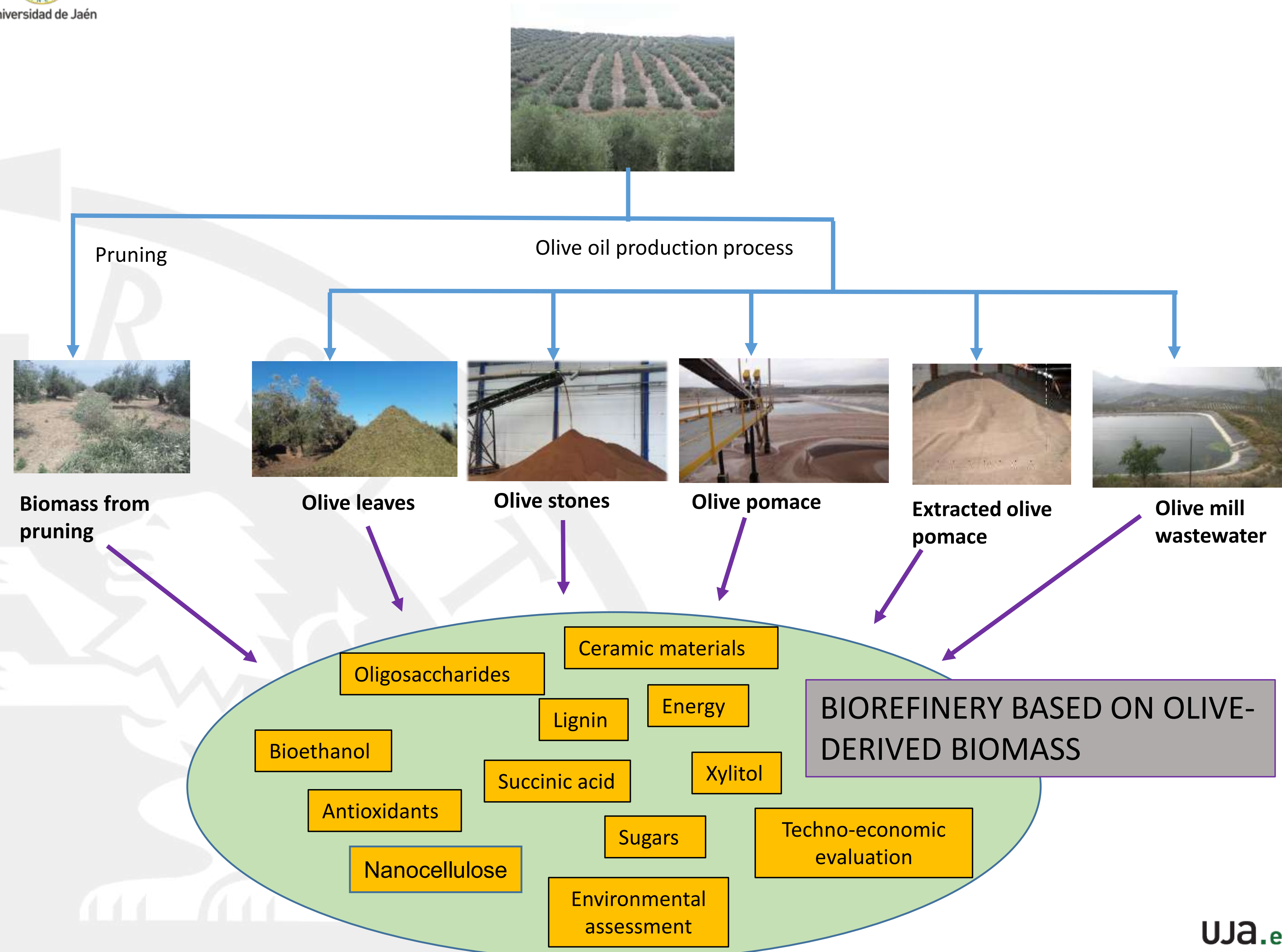
Karimi and Taherzadeh (2016). Bioresource Technology 200, 1008-1018

- Unlike starch or hemicelluloses, cellulose has a crystalline structure
- Crystallinity is among the parameters that is widely measured and related to the bioconversion of the lignocelluloses
- Neither compositional nor imaging can be used for investigating the crystallinity of the lignocelluloses
- Higher rates of lignocellulose bioconversion are not always found following crystallinity reduction

Cristallinity index, CrI. Relative amount of the crystalline (ordered) and amorphous (less ordered) regions of a cellulosic structure.

X-ray diffraction (XRD) as a common method for CrI determinations

5. Pretreatment of olive derived biomass



5. Pretreatment of olive derived biomass

Estimated yearly generation of residual biomass from olive tree cultivation, table olive and olive oil production in Spain

Residual Biomass	Localization	Estimated production, per year
Biomass from tree pruning	Cultivation fields	3-6 million tons
Olive stones or pits	Olive oil mills and table olive factories	1.5 million tons
Olive leaves	Olive oil mills	1 million ton
Olive pomace	Olive oil mills	5 million tons
Pomace dry residues	Pomace oil extraction plants	2 million tons
Wastewater	Aerial ponds and lagoons	

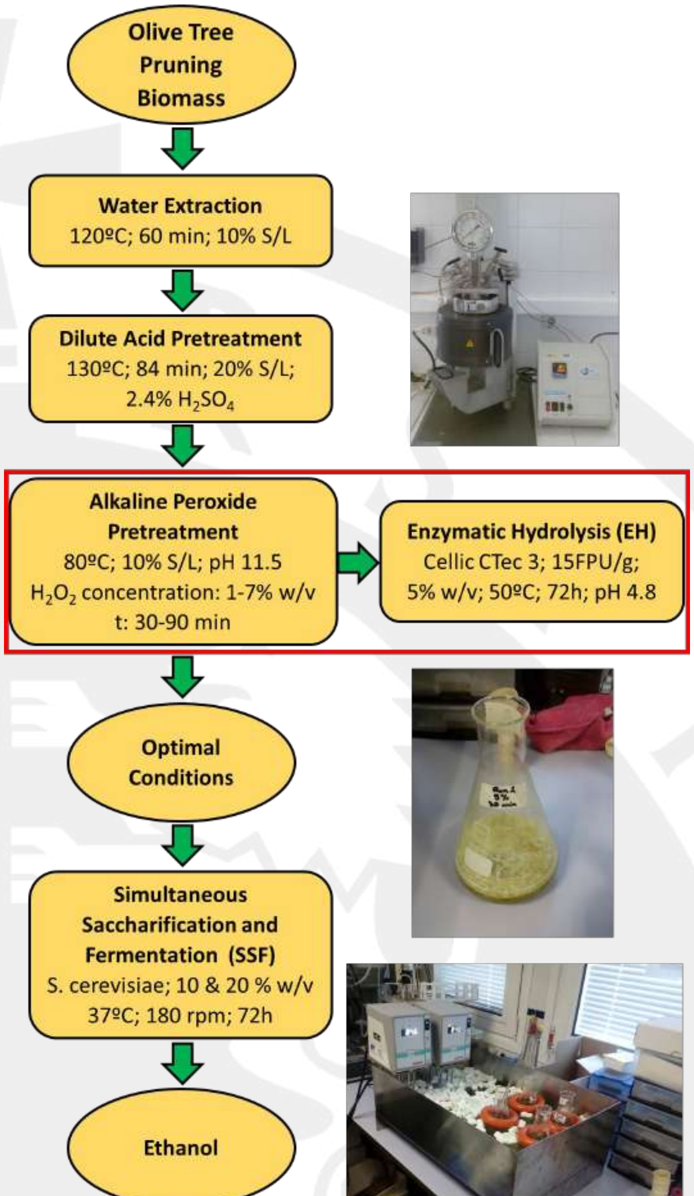
5. Pretreatment of olive derived biomass

Pretreatments assayed on olive-derived biomass

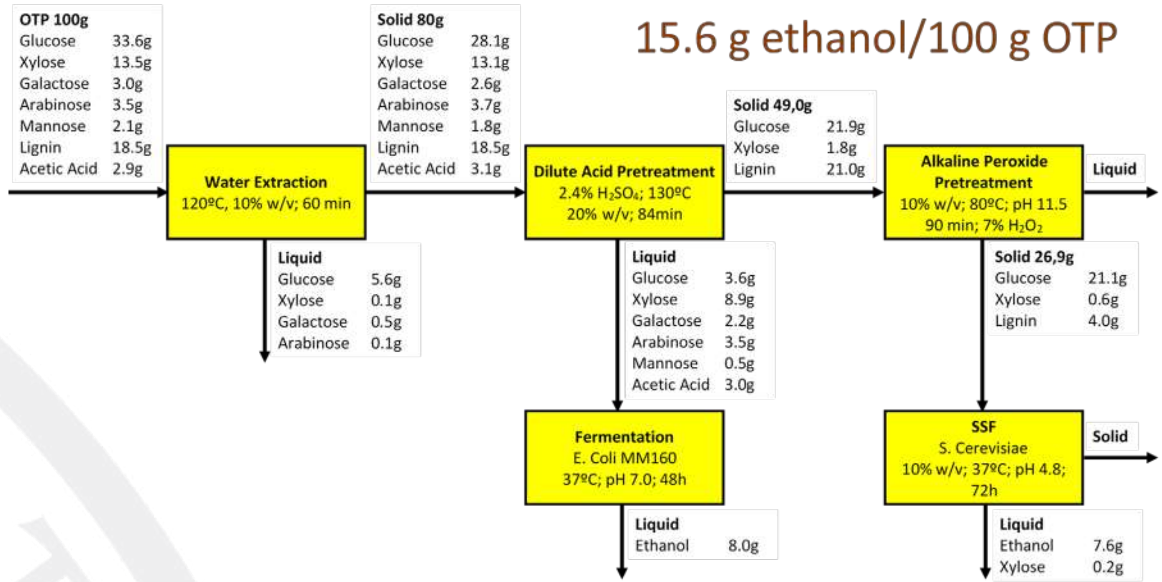
Pretreatment	Specific feature	Main result
Liquid hot water	No chemicals (other than water) used. Temperature≈ 180-200ºC	Solubilisation of hemicellulosic sugars (oligomers)
Steam explosion	Sudden decompression of materials. Temperature≈ 180-200ºC. Lower residence time	Partial breakdown of lignocellulosic matrix
Dilute acid pretreatment	Higher hemicellulose and cellulose hydrolysis	Sugars (monomers). Sugar degradation product (furfural, HMF)
Organosolv	Use of ethanol as organic solvent	Fractionation of lignin, heemicellulose and cellulose
Alkaline extraction	Usually in combination with other methods	Partial solubilisation of lignin
Biological pretreatment	Long time required. Room conditions	No inhibitors. Partial solubilisation of lignin

5. Pretreatment of olive derived biomass

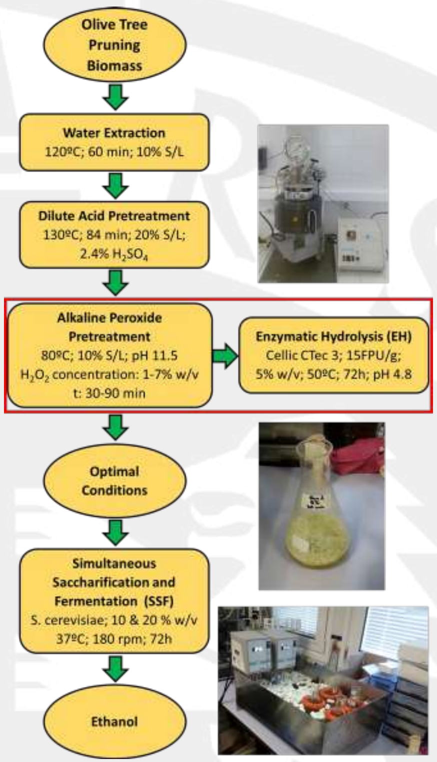
OLIVE TREE PRUNING BIOMASS



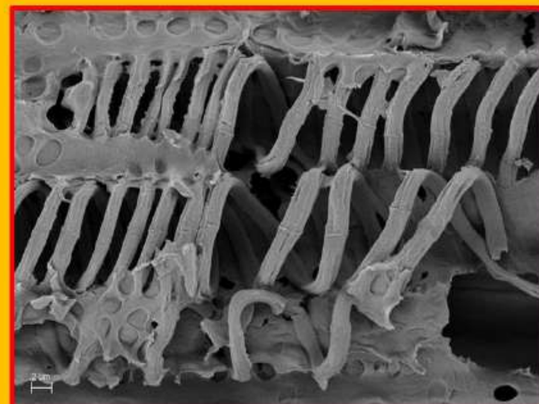
15.6 g ethanol/100 g OTP



5. Pretreatment of olive derived biomass

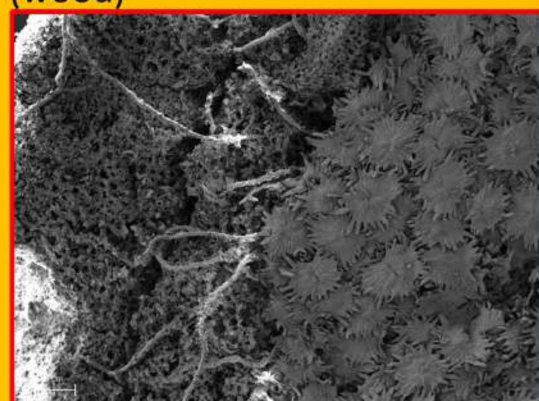


SEM images:



Delignified OTP 3% H_2O_2 (wood)

Delignified OTP 7% H_2O_2 (wood)



OTP (leaves)

Delignified OTP 3% H_2O_2 (leaves)

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OLIVE TREE PRUNING BIOMASS

Detoxification

Sugar and inhibitor concentration in liquor-1 (L-1) before and after detoxification.

Liquor L-1	Sugars (g/L)				
	Glucose	Xylose	Galactose	Arabinose	Mannose
Non detoxified	7.1 ± 0.2	17.3 ± 0.5	4.0 ± 0.1	6.3 ± 0.2	1.4 ± 0.1
Activated charcoal	7.1 ± 0.1	17.5 ± 0.2	4.0 ± 0.1	6.1 ± 0.1	1.5 ± 0.0
Ion-exchange resins	7.6 ± 0.1	18.5 ± 0.1	4.3 ± 0.0	6.7 ± 0.0	1.6 ± 0.1
Overliming	6.9 ± 0.1	16.9 ± 0.2	3.7 ± 0.1	6.1 ± 0.0	1.3 ± 0.0
	Inhibitors (g/L)				
	Formic acid	Acetic acid	HMF	Furfural	Phenols*
Non detoxified	2.2 ± 0.1	5.2 ± 0.1	0.3 ± 0.0	1.8 ± 0.1	4.0 ± 0.3
Activated charcoal	1.6 ± 0.0	5.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 0.2
Ion-exchange resins	1.8 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.8 ± 0.1
Overliming	1.6 ± 0.0	4.9 ± 0.1	0.1 ± 0.1	0.6 ± 0.1	2.0 ± 0.1

* expressed as g gallic acid/L.

José Carlos Martínez-Patiño, Encarnación Ruiz, Cristóbal Cara, Inmaculada Romero, Eulogio Castro (2018). **Advanced bioethanol production from olive tree biomass using different bioconversion schemes.** Biochemical Engineering Journal 137, 172-181

5. Pretreatment of olive derived biomass

OLIVE TREE PRUNING BIOMASS

Aplication of a combined fungal and diluted acid pretreatment on olive tree biomass

Combined pretreatment

OTB → Fungal pretreatment → Dilute acid pretreatment → Enzymatic hydrolysis

OTB → Dilute acid pretreatment → Fungal pretreatment → Enzymatic hydrolysis

Biological pretreatment

I. lacteus
28 days

Dilute acid pretreatment

130 ºC 2% H_2SO_4 90 min
20% S/L

	Glucose concentration (g/L)	EH yield (%)	Glucose recovery by EH (%)
OTB	2.4 ± 0.7	17.8 ± 4.2	17.8 ± 4.3
BP	3.1 ± 0.5	19.7 ± 3.0	19.6 ± 3.0
AP	7.4 ± 0.9	38.9 ± 4.2	31.2 ± 3.4
BP+AP	9.9 ± 0.1	50.1 ± 0.5	39.6 ± 0.4
AP+BP	5.1 ± 0.5	23.0 ± 2.4	19.4 ± 2.0

José Carlos Martínez-Patiño, Thelmo A. Lu-Chau, Beatriz Gullón, Encarnación Ruiz, Inmaculada Romero, Eulogio Castro, Juan M. Lema (2018), Application of a combined fungal and diluted acid pretreatment on olive tree biomass. Industrial Crops & Products 121, 10–17

Proposed biorefinery based on olive leaves

Steam explosion 180 °C, 8,3 min, whole leaves

NATURAL ANTIOXIDANTS
Total phenols: 387 mg AGE/100g
Oleuropein: 1576 mg/100g
Hydroxytyrosol: 341 mg/100g
Flavonoids: 122 mg/100g

OLIGOSACCHARIDES
11,9 g/100g

FERMENTABLE SUGARS
6,5 g/100g

PRETREATED SOLID
48 g/100g

Juan Miguel Romero-García, Antonio Lama-Muñoz, Guillermo Rodríguez-Gutiérrez, Manuel Moya, Encarnación Ruiz, Juan Fernández-Bolaños, Eulogio Castro (2016(. Obtaining sugars and natural antioxidants from olive leaves by steam-explosion Food Chemistry 210, 457-465

5. Pretreatment of olive derived biomass

OLIVE PITS

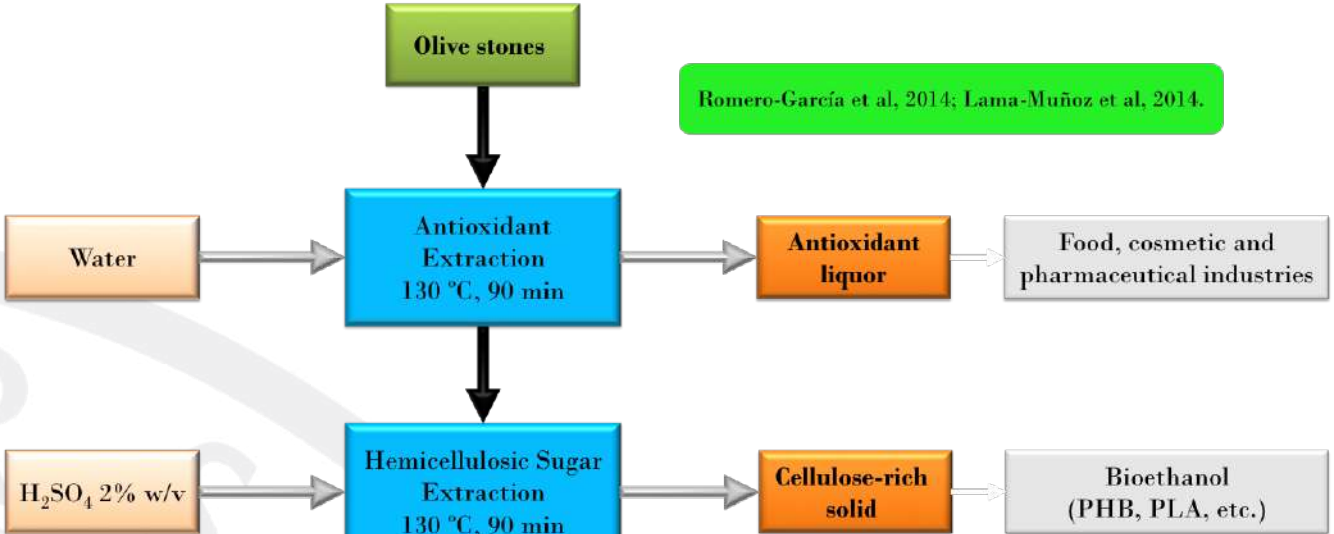
- 10/15% by weight of olive fruit
- Application as a biofuel for heat and electricity production

Components (% dw)	
Extractives:	8,9 ± 0,2
Glucose	0,4 ± 0,0
Xylose	0,4 ± 0,1
Galactose	0,5 ± 0,0
Arabinose	0,6 ± 0,1
Mannose	0,3 ± 0,0
Cellulose:	24,1 ± 0,7
Glucose	26,5 ± 0,7
Hemicelluloses:	**34,4 ± 0,6**
Xylose	28,8 ± 0,5
Galactose	5,2 ± 0,1
Arabinose	4,8 ± 0,1
Mannose	nd
Lignin acid insoluble (LAI)	30,9 ± 0,3
Lignin acid soluble (LAS)	1,6 ± 0,0
Ash	0,5 ± 0,0

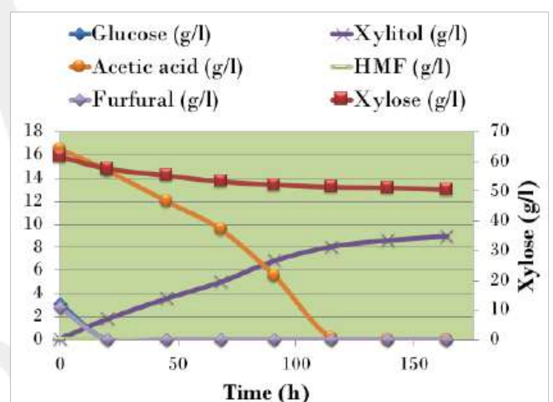
5. Pretreatment of olive derived biomass

OLIVE PITS

Romero-García et al, 2014; Lama-Muñoz et al, 2014.



Xylose-rich liquor	g/l
Glucose	3.11
Xylose	61.64
Acetic acid	16.51
Formic acid	1.64
HMF	0.04
Furfural	2.73



SUMMARIZING…

- 43% product yield
- 1300 kg bioproducts

3t olive-derived biomass

- 1t olive tree pruning biomass
- 1t olive leaves
- 1t de olive stones

→ OLIVE-BASED BIOREFINERY →

- 208 kg bioethanol
- 120 kg xylitol
- 124 kg oligosaccharides
- Natural Antioxidants
  - 79 kg phenols (GAE)
  - 16 kg oleuropein
  - 2.6 kg de hydroxtyrosol
  - 1.2 kg de flavonoids
- 676 kg lignin

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6. Conclusions

1. Pretreatment is a key step in the conversion process from lignocellulosic materials to bioproducts
2. Severity conditions must be carefully applied to get as much sugars and as low inhibitors as possible
3. The selection of the pretreatment depends usually on both the type of biomass and the main product targeted
4. The performance of the pretreatment can be assessed through surface modification, crystallinity, or other aspects, but especially on sugars released.

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ACKNOWLEDGEMENTS- FUNDING ORGANISATIONS

MARIE CURIE ACTIONS PEOPLE

BIOASSORT
Improvement of technologies and tools, e.g. biosystems and biocatalyst, for waste conversion to develop an assortment of high added value eco-friendly and cost-effective bio-products

Advances towards a flexible multi-feedstock, multi-product biorefinery in regions with high density of agroindustrial biomass: the olive case (2018-2020)

GOBIERNO DE ESPAÑA · MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD · UNIÓN EUROPEA FONDO EUROPEO DE DESARROLLO REGIONAL

Project ENE2017-85819

Design and optimization of a sustainable biorefinery based on biomass derived from olive tree and olive oil industry. Environmental and tecno-economic analysis (2015-2017)

GOBIERNO DE ESPAÑA · MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD · UNIÓN EUROPEA FONDO EUROPEO DE DESARROLLO REGIONAL

Project ENE2014-60090-C2)

Advanced processes for fractionation and biological conversion to obtain energy and chemicals from olive tree pruning (2012-2014)

GOBIERNO DE ESPAÑA · MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD · UNIÓN EUROPEA FONDO EUROPEO DE DESARROLLO REGIONAL

Project ENE2011-29112

Production of bioethanol from olive tree pruning biomass (2005-2008)

MINISTERIO DE EDUCACION Y CIENCIA · UNIÓN EUROPEA FONDO EUROPEO DE DESARROLLO REGIONAL

Project ENE2005-08822

Process integration for obtaining energy, liquid fuels, and value-added products from olive tree pruning biomass: an approximation to the biorefinery (2008-2011)

GOBIERNO DE ESPAÑA · MINISTERIO DE CIENCIA E INNOVACIÓN · UNIÓN EUROPEA FONDO EUROPEO DE DESARROLLO REGIONAL

Project ENE2008-06634

Olive tree biomass as a source of energy and chemicals. (2010-2016)

JUNTA DE ANDALUCIA

Proyectos de Excelencia. Plan Andaluz de I+D+i (Ref. AGR-6103

Universidad de Jaén

Chemical and Environmental Engineering Research Group
Universidad de Jaén, Spain

Thank you!

ecastro@ujaen.es
https://www.facebook.com/tep233/

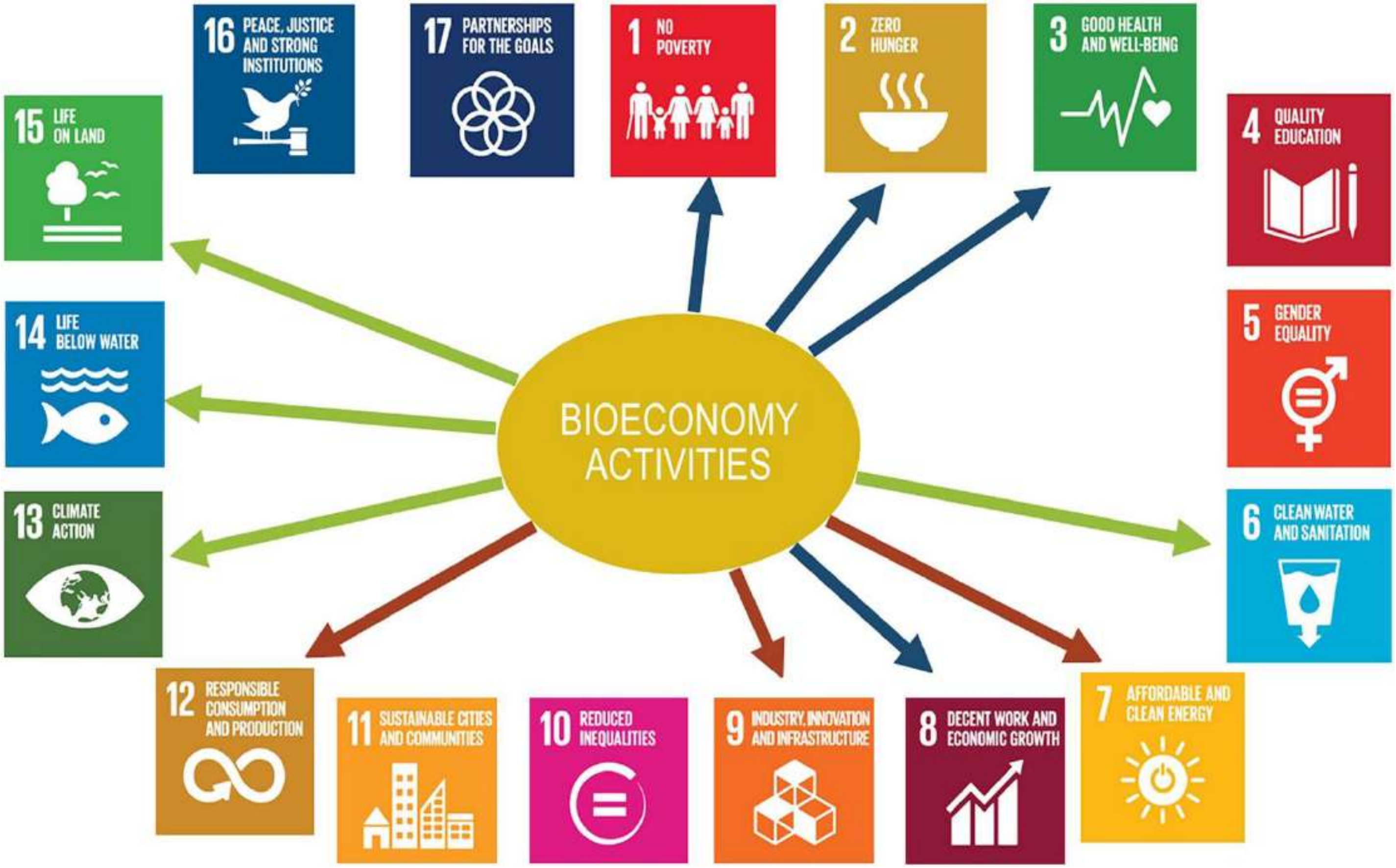
Lignocellulosic Biomass and Residues as Potential Substrates for the Industrial Biotechnology

Joachim Venus

Leibniz Institute for Agricultural Engineering and Bioeconomy

ATB Potsdam

Bioeconomy and SDGs: Does the Bioeconomy Support the Achievement of the SDGs?



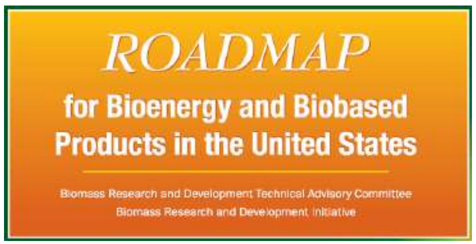
Sustainable Development Goals affected by bioeconomy activities

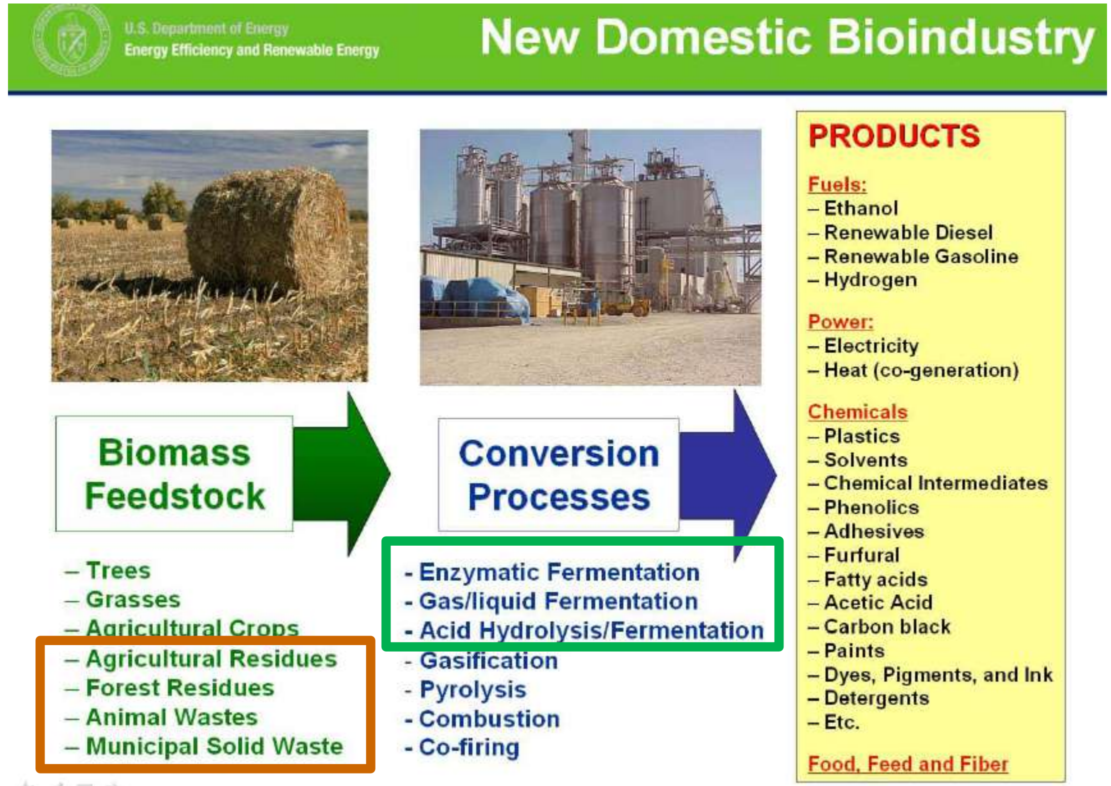
- **Blue arrow: socioeconomic targets**
- **Green arrow: ecological targets**
- **Red arrow: clean industry and economic targets**

Heimann, T. (2019). Bioeconomy and SDGs: Does the bioeconomy support the achievement of the SDGs? Earth's Future, 7,43–57. https://doi.org/10.1029/2018EF001014

Biotechnological

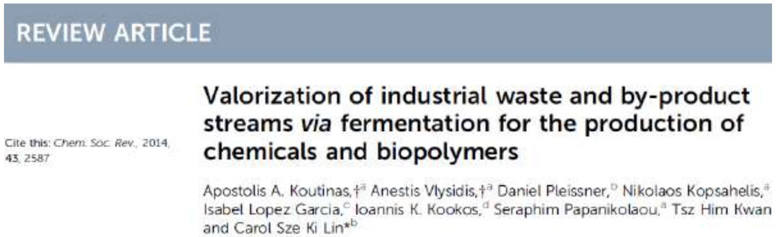
Biomass conversion into high-value chemical products and fuels





Biorefineries in theory would use multiple forms of biomass to produce a flexible mix of products, including fuels, power, heat, chemicals and materials. In a biorefinery, biomass would be converted into high-value chemical products and fuels (both gas and liquid). Byproducts and residues, as well as some portion of the fuels produced, would be used to fuel on-site power generation or cogeneration facilities producing heat and power.

Building blocks that could be produced via fermentation



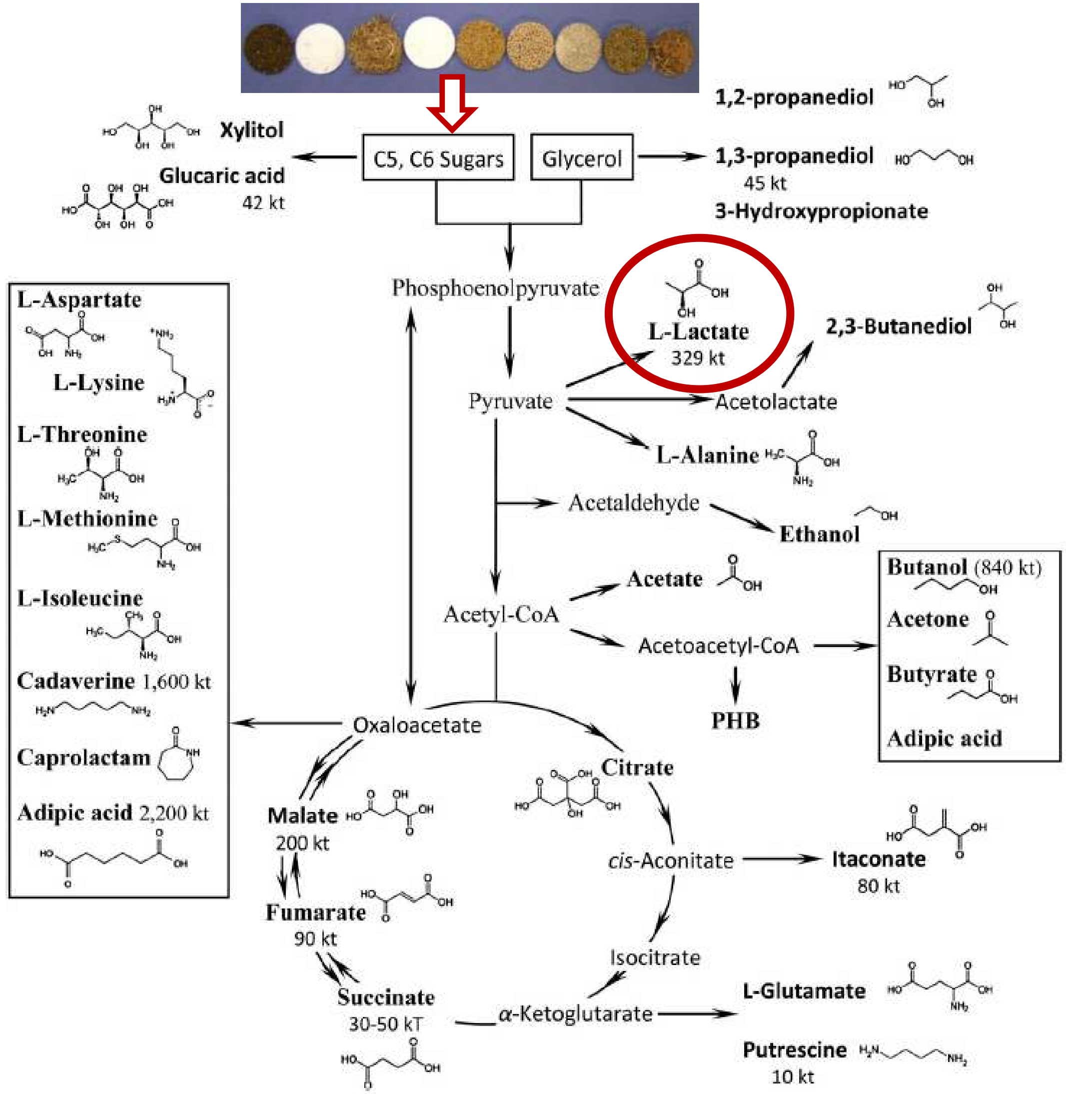
REVIEW ARTICLE

Cite this: *Chem. Soc. Rev.*, 2014, 43, 2587

Valorization of industrial waste and by-product streams *via* fermentation for the production of chemicals and biopolymers

Apostolis A. Koutinas,†[a] Anestis Vlysidis,†[a] Daniel Pleissner,[b] Nikolaos Kopsahelis,[a] Isabel Lopez Garcia,[c] Ioannis K. Kookos,[d] Seraphim Papanikolaou,[a] Tsz Him Kwan and Carol Sze Ki Lin*[b]

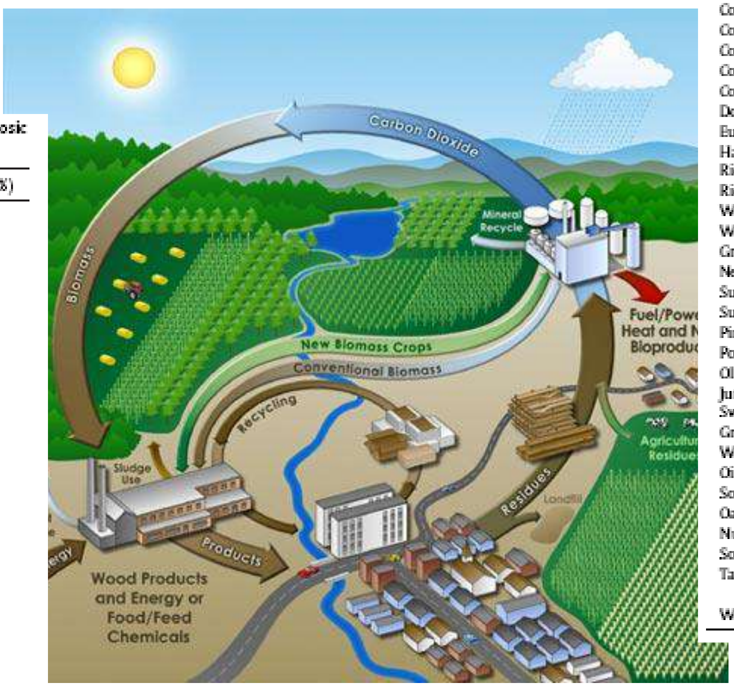
➔ Numbers next to biochemicals designate the total annual production in thousands of t



Berlin, 5 December 2018 – The results of the European Bioplastics' annual market data update, presented today at the 13thEuropean Bioplastics Conference in Berlin:

The global bioplastics production capacity is set to increase from around 2.1 Mio t in 2018 to 2.6 Mio t in 2023. Innovative biopolymers such as PLA and PHAs are driving this growth.

(Different) Composition & Behaviour of (lignocellulosic) Biomass



The contents of cellulose, hemicellulose, and lignin in various types of lignocellulosic biomass (% dry weight).[a]

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Algae (green)	20–40	20–50	NA[b]
Aspen hardwood	51	29	16
Birch Hardwood	40	39	21
Chemical pulps	60–80	20–30	2–10
Coastal Bermuda grass	25	35.7	6.4
Corn cobs	45	35	15
Cornstalks	39–47	26–31	3–5
Cotton seed hairs	80–95	5–20	0
Cotton, flax, etc.	80–95	5–20	NA[b]
Grasses	25–40	25–50	10–30
Hardwood	45 ± 2	30 ± 5	20 ± 4
Hardwood barks	22–40	20–38	30–55
Hardwood stems	40–55	24–40	18–25
Leaves	15–20	80–85	0
Newspaper	40–55	25–40	18–30
Nut shells	25–30	25–30	30–40
Paper	85–99	0	0–15
Pine softwood	44	26	29
Primary wastewater solids	8–15	NA[b]	24–29
Softwood	42 ± 2	27 ± 2	28 ± 3
Softwood barks	18–38	15–33	30–60
Softwood stems	45–50	25–35	25–35
Solid cattle manure	1.6–4.7	1.4–3.3	2.7–5.7
Sorted refuse	60	20	20
Spruce softwood	43	26	29
Swine waste	6.0	28	NA[b]
Switch grass	45	31.4	12.0
Waste papers from chemical pulps	60–70	10–20	5–10
Wheat straw	37–41	27–32	13–15
Willow Hardwood	37	23	21

M.A. Abdel-Rahman et al.
Journal of Biotechnology 156 (2011) 286– 301

Composition of representative lignocellulosic feedstocks.

Feedstocks	Carbohydrate composition (% dry wt)			References
	Cellulose	Hemicellulose	Lignin	
Barley hull	34	36	19	[12]
Barley straw	36–43	24–33	6.3–9.8	[13,14]
Bamboo	49–50	18–20	23	[15,16]
Banana waste	13	15	14	[17]
Corn cob	32.3–45.6	39.8	6.7–13.9	[18,19]
Corn stover	35.1–39.5	20.7–24.6	11.0–19.1	[20]
Cotton	85–95	5–15	0	[21]
Cotton stalk	31	11	30	[22]
Coffee pulp	33.7–36.9	44.2–47.5	15.6–19.1	[23]
Douglas fir	35–48	20–22	15–21	[24]
Eucalyptus	45–51	11–18	29	[16,25]
Hardwood stems	40–55	24–40	18–25	[26,27]
Rice straw	29.2–34.7	23–25.9	17–19	[28,29]
Rice husk	28.7–35.6	11.96–29.3	15.4–20	[30,31]
Wheat straw	35–39	22–30	12–16	[29,32]
Wheat bran	10.5–14.8	35.5–39.2	8.3–12.5	[33]
Grasses	25–40	25–50	10–30	[34,35]
Newspaper	40–55	24–39	18–30	[26]
Sugarcane bagasse	25–45	28–32	15–25	[16,36]
Sugarcane tops	35	32	14	[37]
Pine	42–49	13–25	23–29	[25]
Poplar wood	45–51	25–28	10–21	[38]
Olive tree biomass	25.2	15.8	19.1	[39]
Jute fibres	45–53	18–21	21–26	[40]
Switchgrass	35–40	25–30	15–20	[26]
Grasses	25–40	25–50	10–30	[26,27]
Winter rye	29–30	22–26	16.1	[41]
Oilseed rape	27.3	20.5	14.2	[41]
Softwood stem	45–50	24–40	18–25	[26,27]
Oat straw	31–35	20–26	10–15	[14]
Nut shells	25–30	22–28	30–40	[42]
Sorghum straw	32–35	24–27	15–21	[43,44]
Tamarind kernel powder	10–15	55–65	–	[45]
Water hyacinth	18.2–22.1	48.7–50.1	3.5–5.4	[46,47]

V. Menon, M. Rao
Progress in Energy and Combustion Science 38 (2012) 522-550

Percent dry weight composition of lignocellulosic feedstocks

Feedstock	Glucan (cellulose)	Xylan (hemicellulose)	Lignin
Corn stover[a]	37.5	22.4	17.6
Corn fiber[b,c]	14.28	16.8	8.4
Pine wood[d]	46.4	8.8	29.4
Popular[d]	49.9	17.4	18.1
Wheat straw[d]	38.2	21.2	23.4
Switch grass[d]	31.0	20.4	17.6
Office paper[d]	68.6	12.4	11.3

N. Mosier et al. / Bioresource Technology 96 (2005) 673–686

ATB

NREL
NATIONAL RENEWABLE ENERGY LABORATORY

Chemicals from Biomass: A Market Assessment of Bioproducts with Near-Term Potential

Mary J. Biddy, Christopher Scarlata, and Christopher Kinchin - *National Renewable Energy Laboratory*

Data Gaps

Scale-up of lactic acid production would require **clean, cheap sugars from lignocellulosic biomass** to compete with commodity sugar and starch substrates. There is a **lack of data about lactic acid production and purification from biomass hydrolysates, including issues of C5 sugar** utilization, although it appears work has started to address some of these issues.

This report is available at no cost from the National Renewable Energy Laboratory (NREL) at www.nrel.gov/publications
Technical Report NREL/TP-5100-65509 - March 2016/Contract No. DE-AC36-08GO28308

ATB

Renewable Resources

Angew. Chem. Int. Ed. 2011, 50, 10502 – 10509

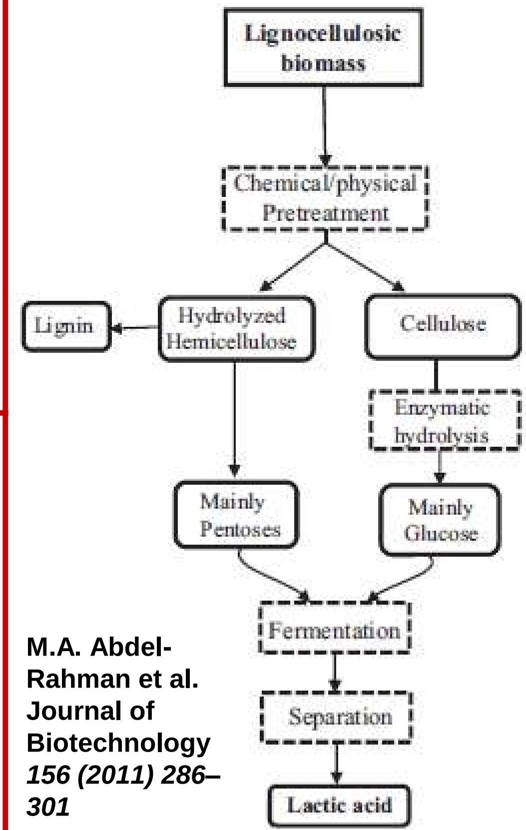
DOI: 10.1002/anie.201102117

Beyond Petrochemicals: The Renewable Chemicals Industry**

P. N. R. Vennestrøm, C. M. Osmundsen, C. H. Christensen, and Esben Taarning*

Chemical	Market type	Market size (Mt y^{-1})[a]	Major player(s)	Feedstock
acetic acid	existing	9.0	–	ethanol
acrylic acid	existing	4.2	Arkema, Cargill/Novozymes	glycerol or glucose
C_4 diacids	emerging	(0.1–0.5)	BASF/Purac/CSM, Myriant	glucose
epichlorohydrin	existing	1.0	Solvay, DOW	glycerol
ethanol	exisiting	60	Cosan, Abengoa Bioenergy, ADM	glucose
ethylene	existing	110	Braskem, DOW/Crystalsev, Borealis	ethanol
ethylene glycol	existing	20	India Glycols, Dacheng Industrial	glucose or xylitol
glycerol	existing	1.5	ADM, P&G, Cargill	vegetable oil
5-hydroxymethylfurfural	emerging	–	–	glucose/fructose
3-hydroxypropionic acid	emerging	(≥0.5)	Novozymes/Cargill	glucose
isoprene	existing/emerging	0.1 (0.1–0.5)	Danisco/Goodyear	glucose
lactic acid	existing/emerging	0.3 (0.3–0.5)	Cargill, Purac/Arkema, ADM, Galactic	glucose
levulinic acid	emerging	(≥0.5)	Segetis, Maine Bioproducts, Le Calorie	glucose
oleochemicals	existing	10–15	Emery, Croda, BASF, Vantage Oleochemicals	vegetable oil/fat
1,3-propanediol	emerging	(0.1–0.5)	Dupont/Tate & Lyle	glucose
propylene	existing	80	Braskem/Novozymes	glucose
propylene glycol	existing/emerging	1.4 (≥2.0)	ADM, Cargill/Ashland, Senergy, Dacheng Industrial	glycerol or sorbitol
polyhydroxyalkanoate	emerging	(0.1–0.5)	Metabolix/ADM	glucose

[a] Market size of an existing market is given as its current size including production from fossil resources; for emerging markets the expected market size is reported in parenthesis.



M.A. Abdel-Rahman et al. Journal of Biotechnology *156 (2011) 286–301*

Table 1: Overview of chemicals that are currently produced, or could be produced, from biomass together with their respective market type, size of the market, and potential biomass feedstock. Major players involved are also given.

The processes for producing lactic acid from biomass/residues include the following 4 main steps:

(1) **Pretreatment - breaking down the structure of the feedstock matrix**

(2) Enzymatic hydrolysis - depolymerizing biopolymers like starch, cellulose etc. to fermentative sugars, such as glucose (C6) and xylose (C5), by means of hydrolytic enzymes

(3) Fermentation - metabolizing the sugars to lactic acid, generally by LAB

(4) **Separation and purification of lactic acid - purification of lactic acid to meet the standards of commercial applications**

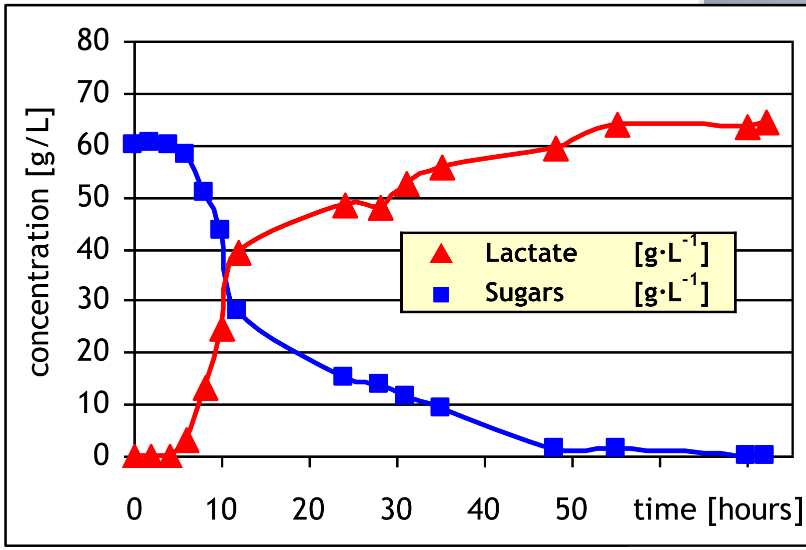
Pilot plant facility for lactic acid fermentation at Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB Potsdam)

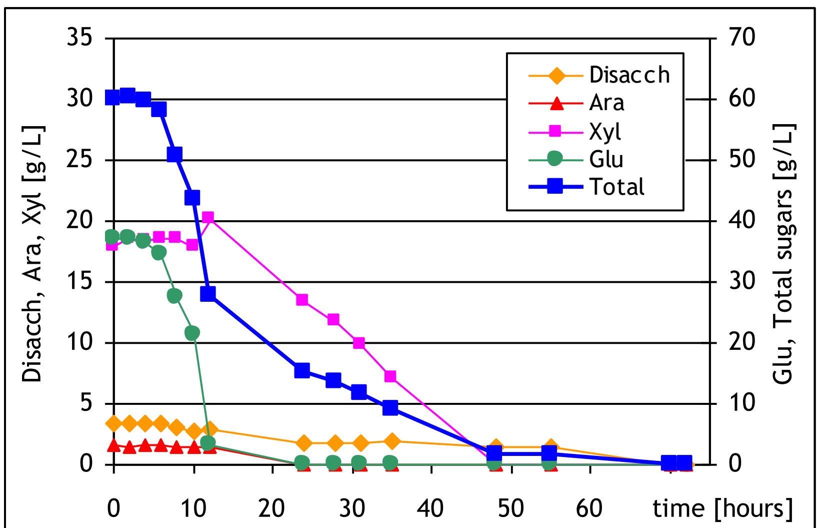
Fermentation feedstocks already tested:

Pleissner, D.; Venus, J.: Agricultural residues as feedstocks for lactic acid fermentation. - ACS Symposium Series, Vol. 1186 "Green Technologies for the Environment" (2014) pp 247–263

- *Starchy materials (cereals, industrial grade corn/potatoe starch, tapioca)*
- *Green biomass (alfalfa, grass juice, lupine, sweet sorghum, forage rye, silage, coco juice)*
- *Lignocellulosics (wood/straw hydrolysates, 2ndG sugars, bagasse, reed)*
- ***Residues & By-products (oilseed cake/meal, thick juice, molasses, whey, coffee residues, waste bread, waffle residues, algae biomass, fruit residues, rice bran, meat & bone meal, OMSW, AD digestates, corn stover…)***

Example wheat straw: Sugar uptake & product formation





- Fermentation ended after 50-60 hours with a yield of nearly 100% and 64 g/L (top left)
- (Total) Sugars (firstly Glucose followed by Arabinose/Xylose with residues of Disaccharides) have been used completely in the same time (bottom left)
- (Max) Lactate productivity (>5 $g \cdot L^{-1} \cdot h^{-1}$) is much higher than comparable published results
  [Li/Cui: Microbial Lactic Acid Production from Renewable Resources, pp. 211-228. In O.V. Singh and S.P. Harvey (Eds.), Sustainable Biotechnology - Sources of Renewable Energy. Springer, 2010]

WO 2013164423 A1; WO 2013164425 A1
Pleissner, D.; Venus, J.: Agricultural residues as feedstocks for lactic acid fermentation. - ACS Books "Green Technologies for the Environment" (2014) Chapter 13, pp 247–263

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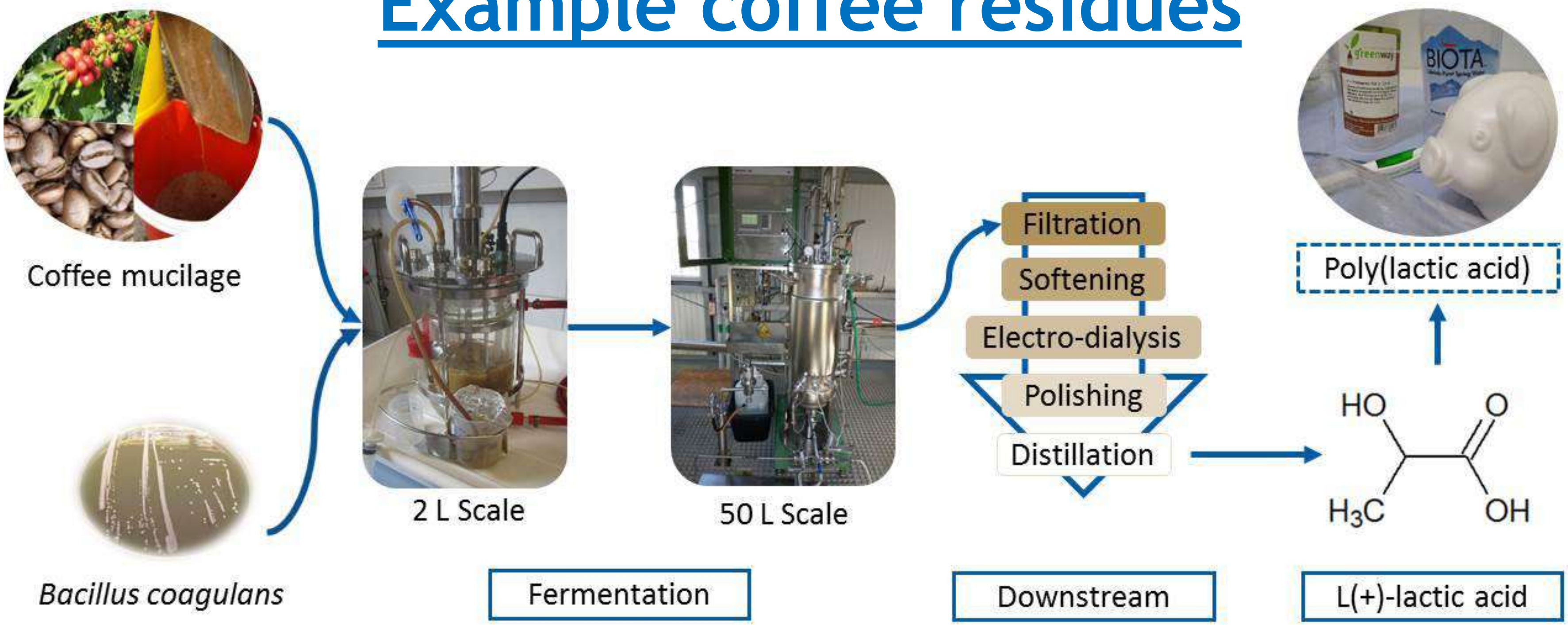
Bundesministerium
für Bildung
und Forschung

Example coffee residues

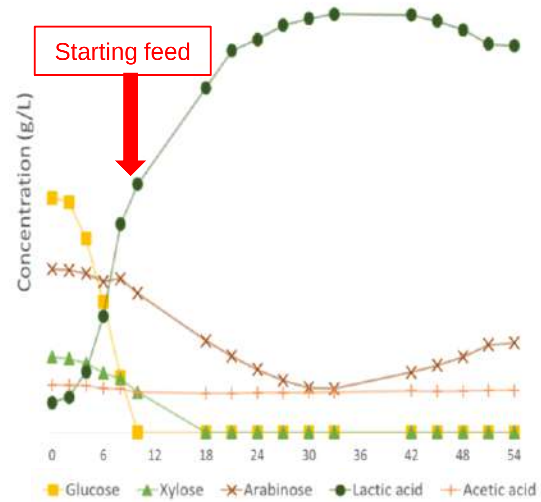


Pleissner, D.; Neu, A.-K.; Mehlmann, K.; Schneider, R.; Puerta-Quintero, G.I.; Venus, J.: Fermentative lactic acid production from coffee pulp hydrolysate using *Bacillus coagulans* at laboratory and pilot scales. Bioresource Technology 218 (2016) 167–173

Neu, A.-K.; Pleissner, D.; Mehlmann, K.; Schneider, R.; Puerta-Quintero, G.I.; Venus, J.: Fermenta-tive utilization of coffee mucilage using Bacillus coagulans and investigation of down-stream pro-cessing of fermentation broth for optically pure L(+)-lactic acid production. Bioresource Technology 211 (2016) 398–405, http://dx.doi.org/10.1016/j.biortech.2016.03.122

Lactic Acid Production from Lignocellulosic Hydrolysate using Cell Recycling Fermentation of *Bacillus coagulans*

- **Ph.D. Student:** Regiane Alves de Oliveira
- **Supervisor:** Dr. Rubens Maciel Filho
- **Co-Supervisor:** Dr. Carlos Eduardo Vaz Rossell



Partners:

FAPESP
FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PAULO

UNICAMP

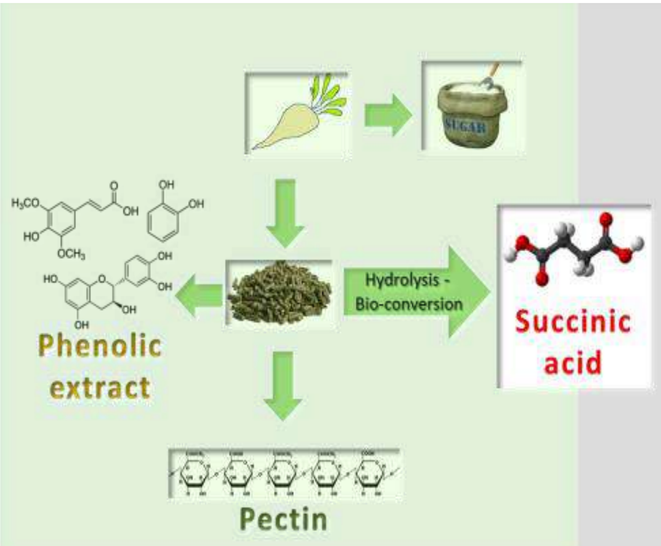
CTBE

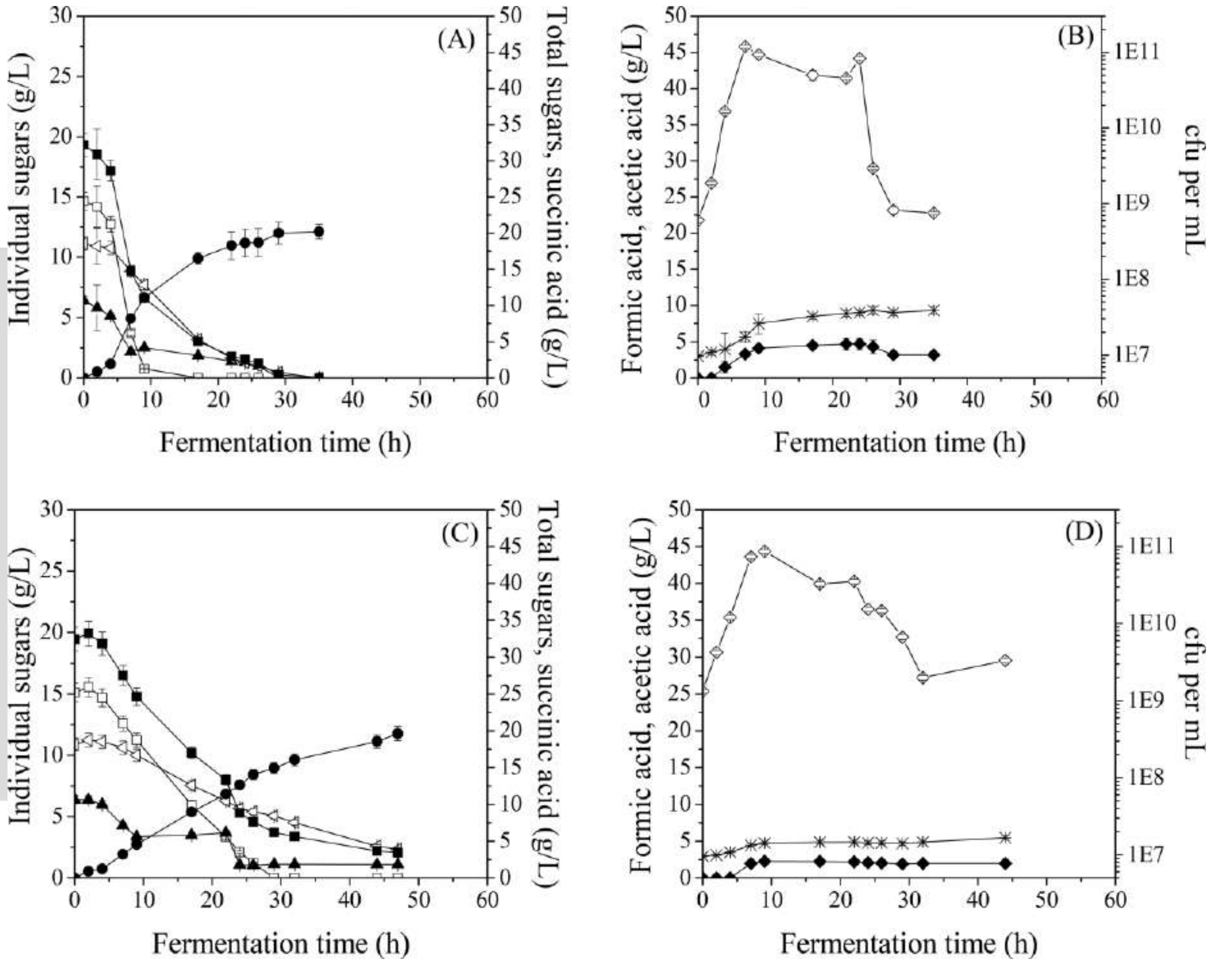
ATB

FAPESP Process No. 2016/14830-3, 01.02.2017-30.11.2017

Alves de Oliveira, R.; Schneider, R.; Vaz Rossell, C.E.; Maciel Filho, R.; Venus, J.: Polymer grade l-lactic acid production from sugarcane bagasse hemicellulosic hydrolysate using Bacillus coagulans. Bioresource Technology Reports 6 (June 2019) 26-31, https://doi.org/10.1016/j.biteb.2019.02.003

Example SBP: Biotechnological production of succinic acid





Batch fermentation of *A. succinogenes* using (A), (B) SBP hydrolysate supplemented with yeast extract and (C), (D) SBP hydrolysate alone. Glucose (□), xylose+fructose+galactose (▲), arabinose (◁) and total sugars (■), succinic acid (●), formic acid (♦) and acetic acid (✳), cfu per mL (◇)

Alexandri, M.; Schneider, R.; Papapostolou, H.; Ladakis, D.; Koutinas, A.; Venus, J.: Restructuring the conventional sugar beet industry into a novel biorefinery: Fractionation and bioconversion of sugar beet pulp into succinic acid and value-added co-products. ACS Sustainable Chem. Eng. 2019, 7, 7, 6569-6579

Metabolic Engineering 47 (2018) 279–293

Contents lists available at ScienceDirect

Metabolic Engineering

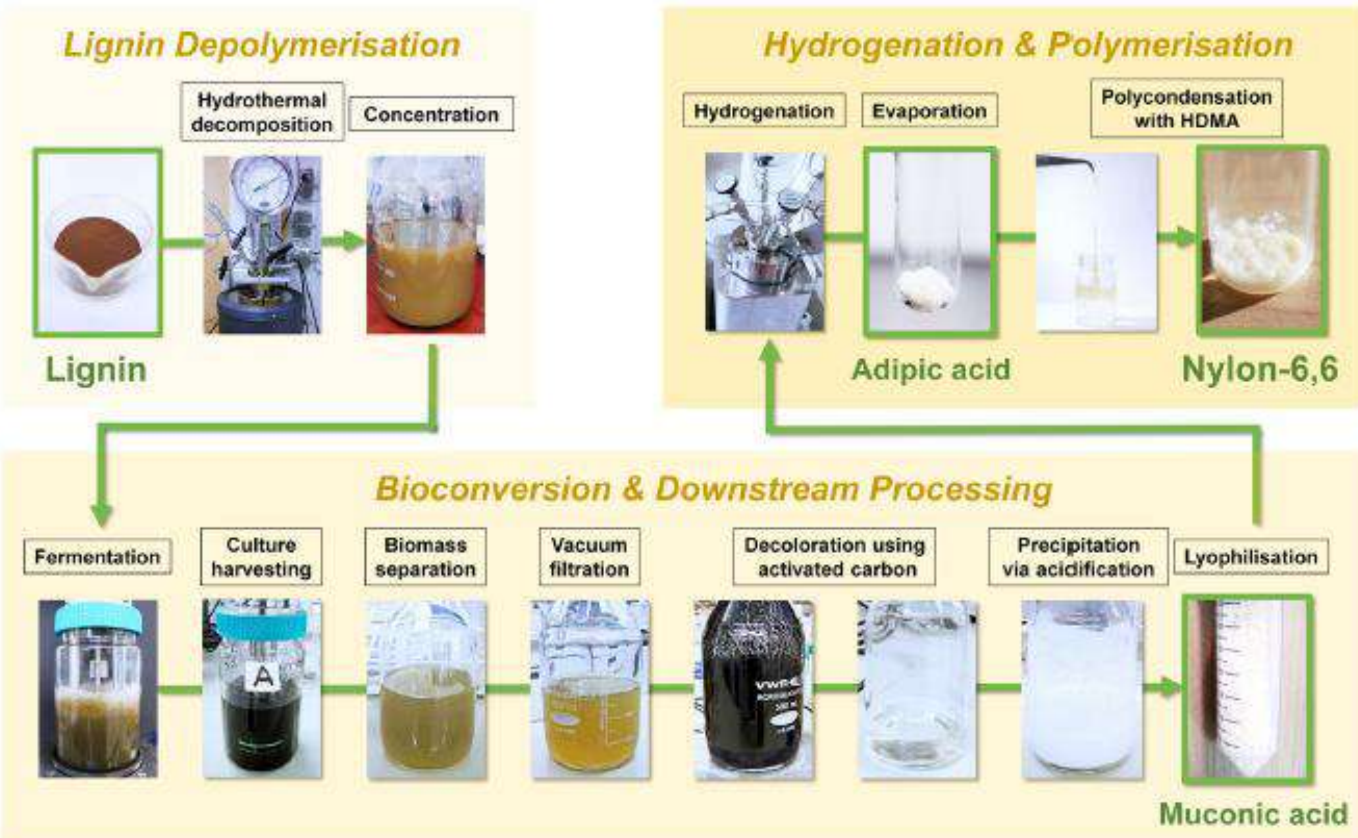
journal homepage: www.elsevier.com/locate/meteng

UNIVERSITÄT DES SAARLANDES

iSBio Institut für Systembiotechnologie

From lignin to nylon: Cascaded chemical and biochemical conversion using metabolically engineered *Pseudomonas putida*

Michael Kohlstedt[a], Sören Starck[a], Nadja Barton[a], Jessica Stolzenberger[a], Mirjam Selzer[a], Kerstin Mehlmann[c], Roland Schneider[c], Daniel Pleissner[c,d], Jan Rinkel[b], Jeroen S. Dickschat[b], Joachim Venus[c], Jozef B.J.H. van Duuren[a], Christoph Wittmann[a,*]



Demonstration of the value chain from lignin to nylon.

The cascaded process comprised hydrothermal depolymerization of lignin to a mixture of aromatics, containing mainly catechol, phenol and small amounts of cresols; biochemical conversion of the aromatics to cis,cis-muconic acid by the advanced producer Pseudomonas putida KT2440 MA-9; purification of cis,cis-muconic acid; hydrogenation to adipic acid; and final polymerization to nylon 6,6.

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Further process engineering challenges of producing bio-based products

"The most demanding efforts are to make the processes economical, with the production cost as low as possible"

Biofuels, Bioprod. Bioref. (2020); DOI: 10.1002/bbb.2104

- Improved DSP (incl. integrated/in-situ product recovery) for high-quality products
- Advanced strategies such as simultaneous saccharification and co-fermentation (SSF), develop continuous mode fermentation processes
- Scale-up to increase TRL

ATB

Downstream processing of raw lactates & lactic acid

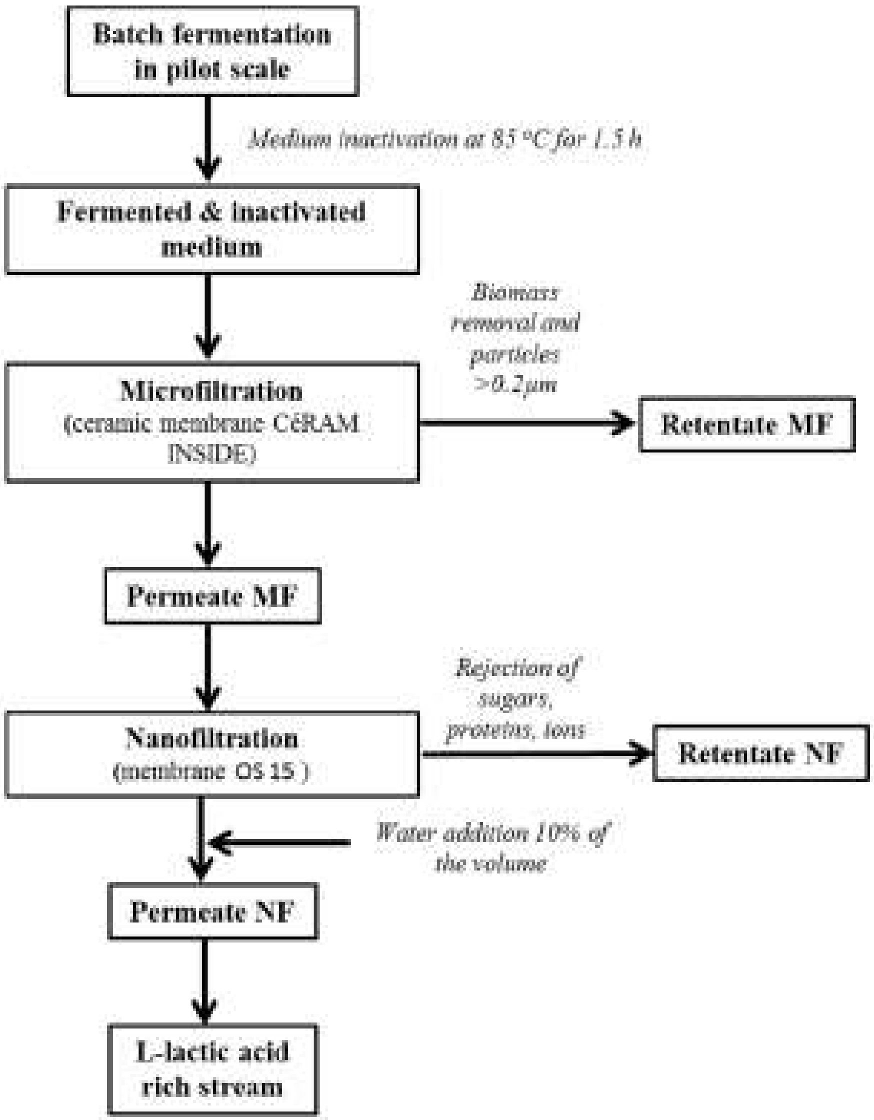
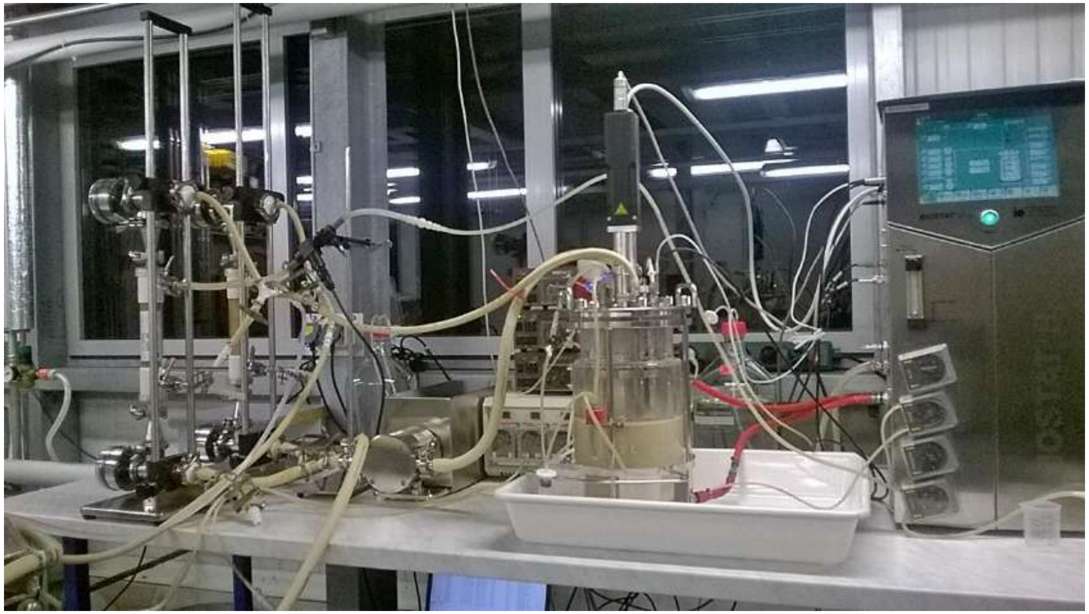
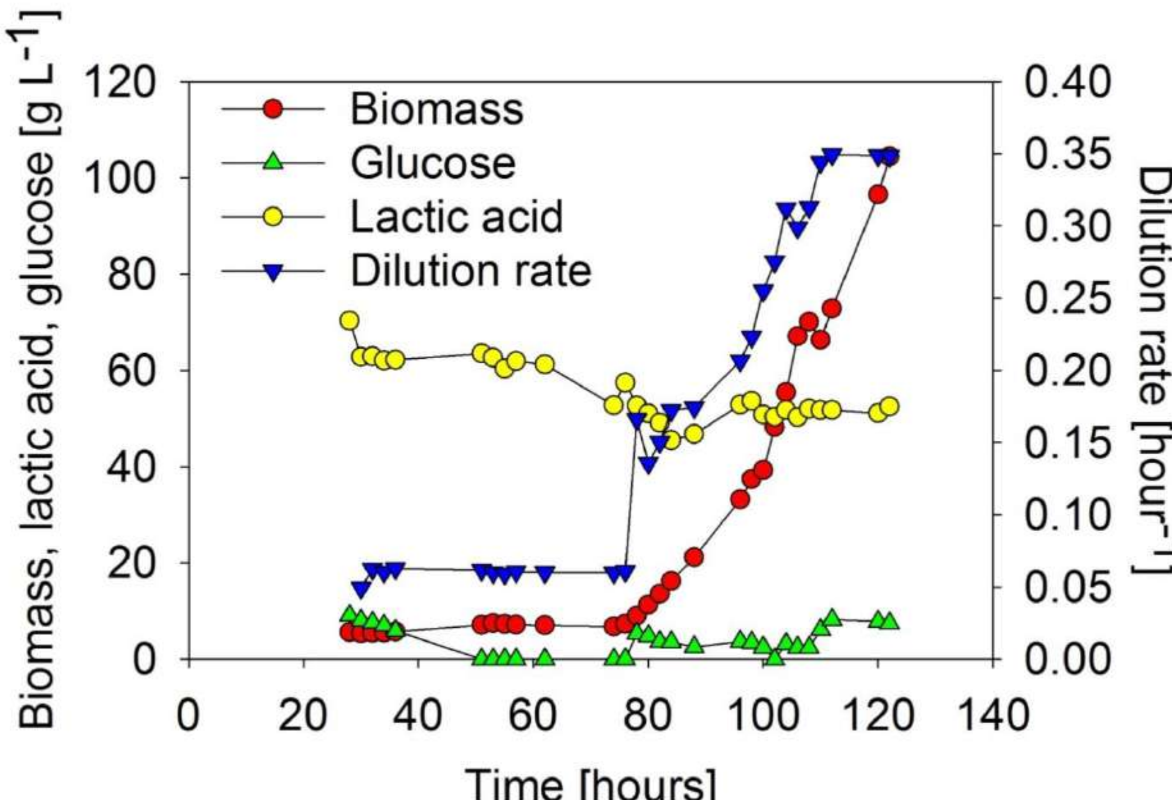


Figure 1. Schematic diagram of the studied process.

Alexandri, M.; Schneider, R.; Venus, J.: Membrane Technologies for Lactic Acid Separation from Fermentation Broths Derived from Renewable Resources. Membranes 2018, 8(4), 94, https://doi.org/10.3390/membranes8040094

Continuous mode fermentation with cell retention by hollow fibre membranes

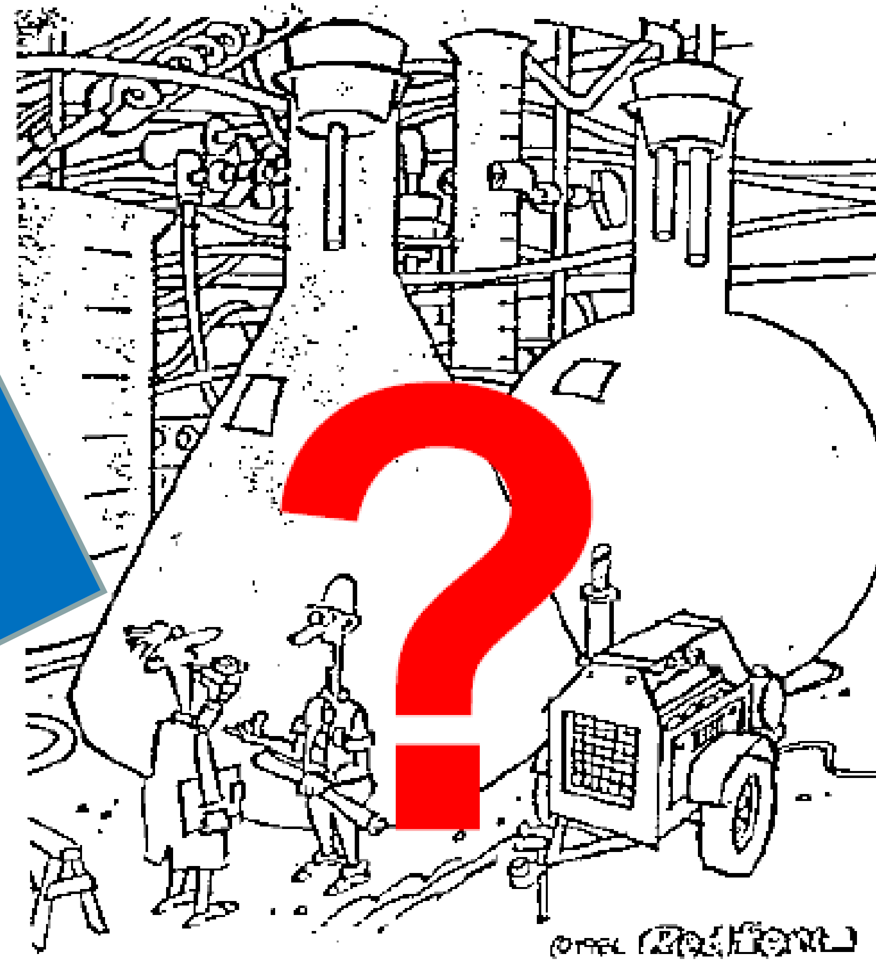




Pleissner, D.; Qi, Q.; Gao, C.; Perez Rivero, C.; Webb, C.; Lin, C.S.K.; Venus, J.: Valorization of organic residues for the production of added value chemicals: A contribution to the bio-based economy. Biochemical Engineering Journal 116 (2016) 3-16

Scale-up of bioprocesses

Process development up to xx m³ ??



"Got a few problems going from lab scale up to full-scale commercial."

Pilot plant facility

- **pilot facility for production of lactic acid** at the ATB consequently fills a gap in the various phases of bioprocess engineering
- **provision of product samples** is intended to open up the possibility of interesting **partners in industry with specific product requirements** in various applications

BIOSTAT® Bplus (Sartorius BBI Systems GmbH, Germany) equipped with a digital control unit DCU for the continuous fermentation with cell recycling

scale up

Pilot fermentor Type P, 450 L (Bioengineering AG)

Venus, J.; Richter, K.: Development of a Pilot Plant Facility for the Conversion of Renewables in Bio-technological Processes. Eng. Life Sci. 2007, 7, No. 4, 395-402
Pleissner, D.; Dietz, D.; van Duuren, J.B.J.H.; Wittmann, C.; Yang, X.; Lin, C.S.K.; Venus, J.: Biotechnological production of organic acids from renewable resources. Advances in Biochemical Engineer-ing/Biotechnology 166 (2019) pp. 373-410

ATB

Previous/Current EU-BBI/H2020 projects

- **PERCAL - Chemical building blocks from versatile MSW biorefinery 07/2017 - 12/2020**

Horizon 2020 European Union Funding for Research & Innovation

BIO-BASED INDUSTRIES Public-Private Partnership

- **BBI Project CAFIPLA** ”Combining carboxylic acid production and fibre recovery as an innovative, cost effective and sustainable pre-treatment process for heterogeneous bio-waste” (BBI grant agreement Nº 887115) – **06/2020–05/2023**, https://cafipla.eu/

CAFIPLA

- **BBI Project BeonNAT** ”Innovative value chains from tree & shrub species grown in marginal lands as a source of biomass for bio-based industries” (BBI grant agreement Nº 887917) – **07/2020–06/2025**, https://beonnat.eu/

BeonNAT

- **EU Project BIOMAC** “European Sustainable BIObased nanoMAterials Community” (H2020 grant agreement Nº 952941) – **01/2021–12/2024**, https://www.biomac-oitb.eu

BIOMAC

Thank you for your attention!

Contact:
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https://youtu.be/JnkB0WRIO-o

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AGRICULTURAL UNIVERSITY OF ATHENS

FOOD INNOVATION
Research Infrastructure

Life cycle analysis and life cycle costing for poly(butylene succinate) and poly(lactic acid) production using various renewable resources

Webinar *"Sustainable Production of Biobased Products in the Bioeconomy Era"*, 10 November 2021

Dimitrios Ladakis, Sofia Maria Ioannidou, Apostolis Koutinas

Agricultural University of Athens (AUA)

STAR ProBio
Sustainability Transition Assessment and Research of Bio-based Products
Grant Agreement Number 727740
www.STAR-ProBio.eu Funded by the EU H2020 Programme

European Union
European Regional Development Fund
ΕΣΠΑ 2014-2020
Partnership Agreement 2014 - 2020

FOOD INNOVATION Research Infrastructure

Agricultural University of Athens (Greece)

Department of Food Science and Human Nutrition

Group of Food Bioprocesses and Biorefineries

Main research interests

–Biorefinery development using agri-industrial waste and by-product streams

–Separation of value-added co-products

–Bioprocess development using entirely renewable resources for the production of platform chemicals, biopolymers and microbial lipids

–Biorefinery and bioprocess design including techno-economic evaluation and life cycle assessment

Chem Soc Rev 2014, **43**, 2587-2627

ROYAL SOCIETY OF CHEMISTRY

REVIEW ARTICLE

View Article Online

Cite this: DOI: 10.1039/c3cs60293a

Valorization of industrial waste and by-product streams *via* fermentation for the production of chemicals and biopolymers

Apostolis A. Koutinas,†[a] Anestis Vlysidis,†[a] Daniel Pleissner,[b] Nikolaos Kopsahelis,[a] Isabel Lopez Garcia,[c] Ioannis K. Kookos,[d] Seraphim Papanikolaou,[a] Tsz Him Kwan[b] and Carol Sze Ki Lin*[b]

Case studies

Selected bio-based products and uses

- **Poly(lactic acid) – PLA**
  Monomer: bio-based L-lactic acid
  Replacement for: biaxially oriented polypropylene (BOPP)

- **Poly(butylene succinate) – PBS**
  Monomers: bio-based succinic acid and 1,4-butanediol
  Replacement for: general purpose polystyrene (GPPS)

Selected feedstocks

- **Glucose syrup from corn**
  - ✓ Base case scenario

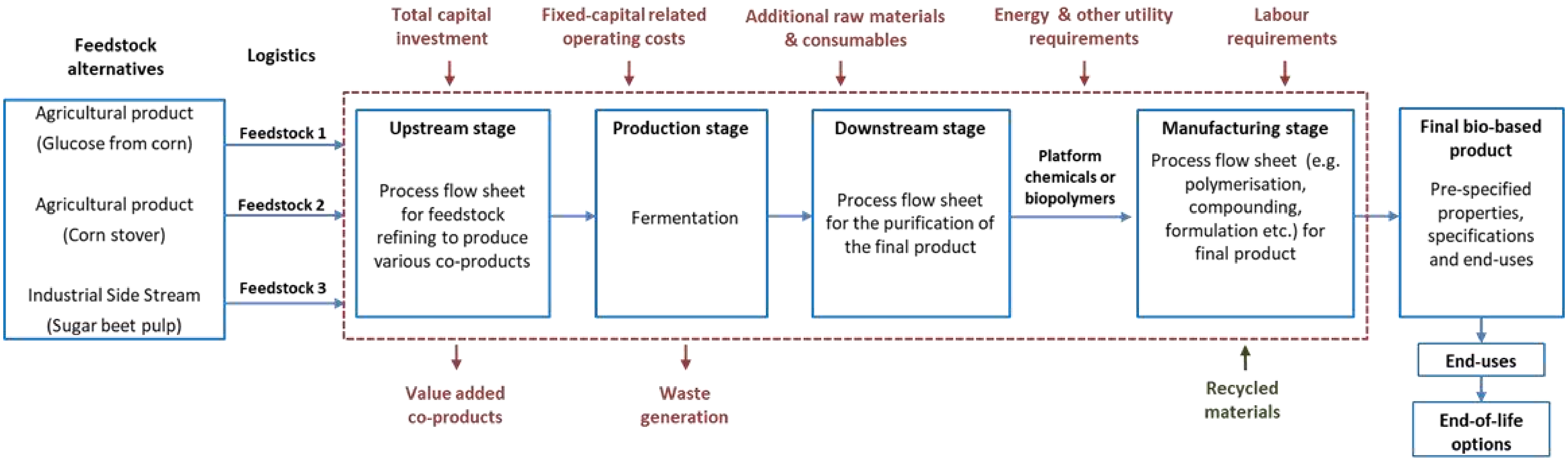
- **Corn stover (CS)***
  - ✓ Agricultural residue of corn cultivation
  - ✓ High carbohydrate content

- **Sugar beet pulp (SBP)****
  - ✓ Promising industrial side stream from the sugar production industry using sugar beet
  - ✓ Suitable for biorefinery development
  - ✓ Sufficient quantities are available in several EU-28 countries
  - ✓ High carbohydrate content
  - ✓ High pectin content

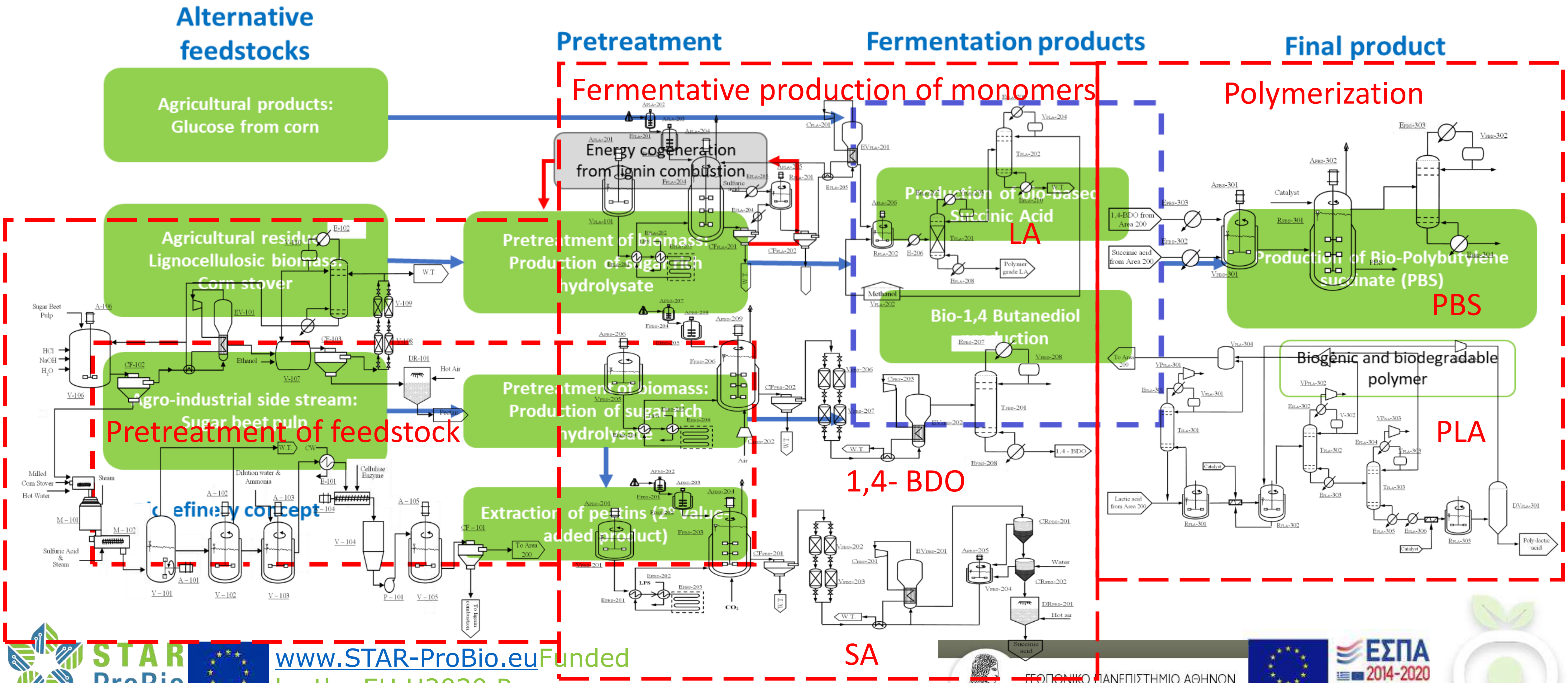
* considering that CS has 20% moisture content

** considering that SBP contains 70% water content; Drying and pelletisation have not been considered

System boundaries



Case studies process design



Sustainability analysis Principles, criteria & indicators

Principle	Criteria	Indicators
1.Sustainable techno-economical manufacturing	1.1 Process improvements	1.1.1 Techno-economic evaluation for producing the bio-based products in the current process 1.1.2 Techno-economic and externality cost evaluation presented as life cycle costs (LCC) 1.1.3 Risk Assessment to identify economic and technical risks including sensitivity analysis
	Case studies implementation 1.2 Alternative renewable feedstocks and biorefinery development	Indicators 1.1.1, 1.1.2 and 1.1.3 are evaluated for alternative renewable feedstocks and biorefinery concepts leading to the production of bio-based products
	1.3 Valorization of by-product and waste streams	Indicators 1.1.1, 1.1.2 and 1.1.3 are used to estimate the techno-economic sustainability of a process when side stream are valorized
	1.4 Recirculation of used bio-based products in the manufacturing stage	Indicators 1.1.1, 1.1.2 and 1.1.3 are used to estimate the techno-economic sustainability of a process in which recirculation of used bio-based products is applied

Selected economic metrics

Indicator Performance of TESA methodology for evaluating the production process of the selected case studies using alternative feedstocks

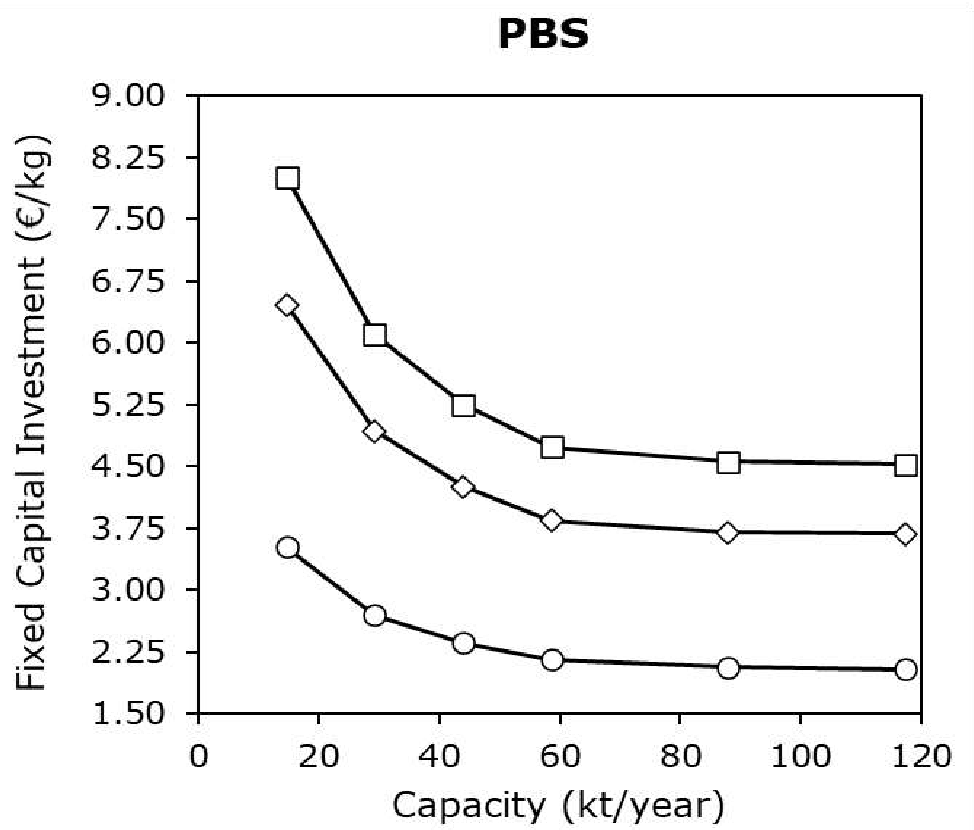
1. Variation of Fixed Capital Investment (FCI) at different plant capacities
2. Variation of Cost of Manufacture (COM) at different plant capacities
3. Estimation of Minimum Selling Price (MSP) via Discounted Cash Flow Analysis

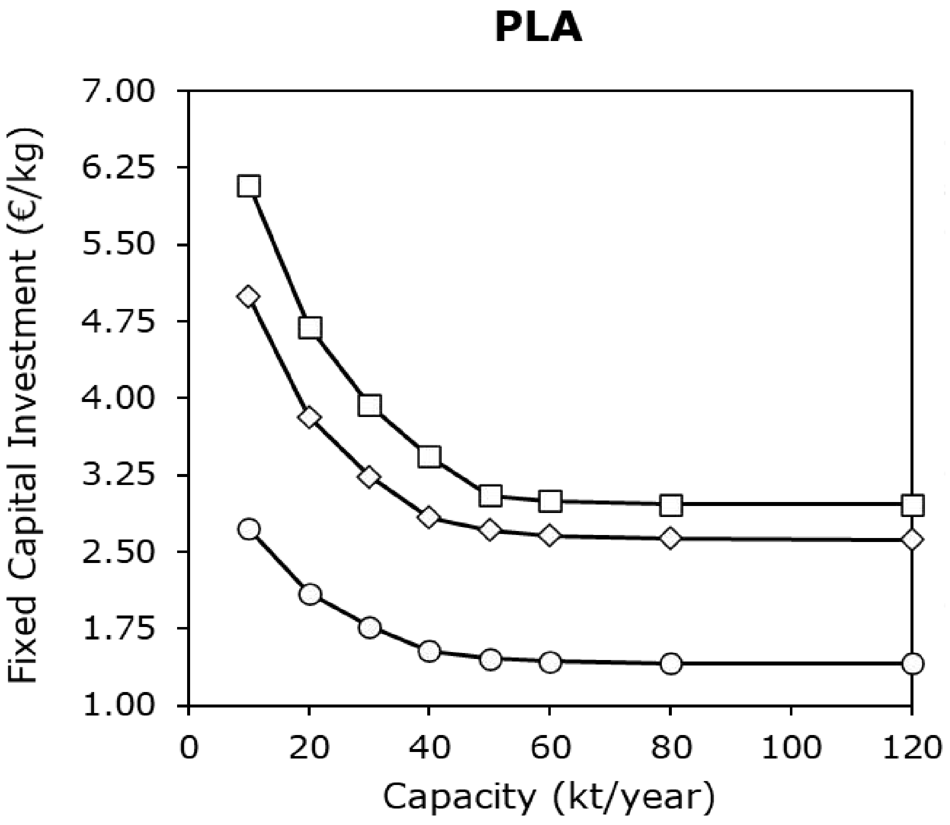
   (associated with zero Net Present Value (NPV) at the end of the useful life time of plant operation)
4. Optimum Plant Capacity (OPC) leading to minimum cost of manufacture
5. The Discounted Payback Period (DPP) is the time required, after the initiation of plant operation, to recover the capital investment
6. Minimum Feedstock Capacity Requirement (MFR) at the Optimum Plant Capacity
7. The ratio of Feedstock Capacity Requirement to Feedstock Availability in the region
8. Incorporate external environmental impact costs

Indicator Risk assessment focusing on application-specific technical aspects

1. Sensitivity analysis

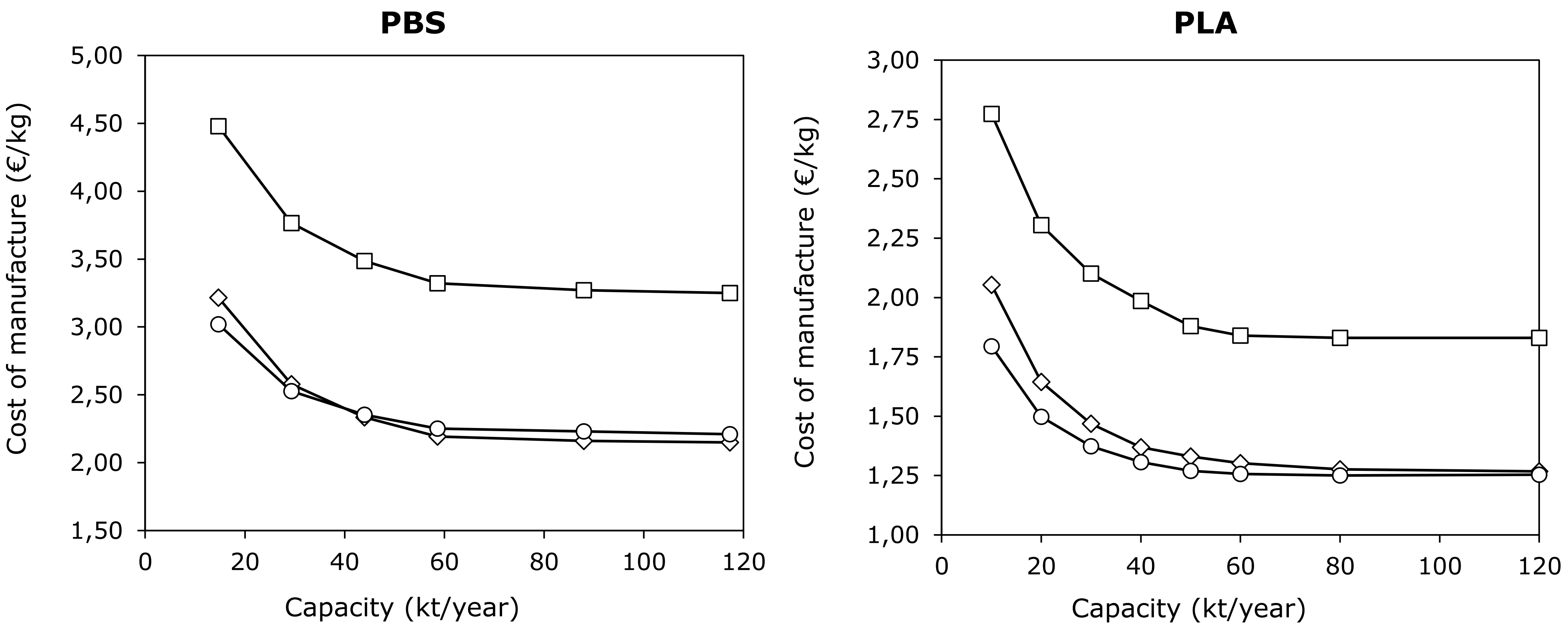
Fixed Capital Investment (FCI) at different plant capacities





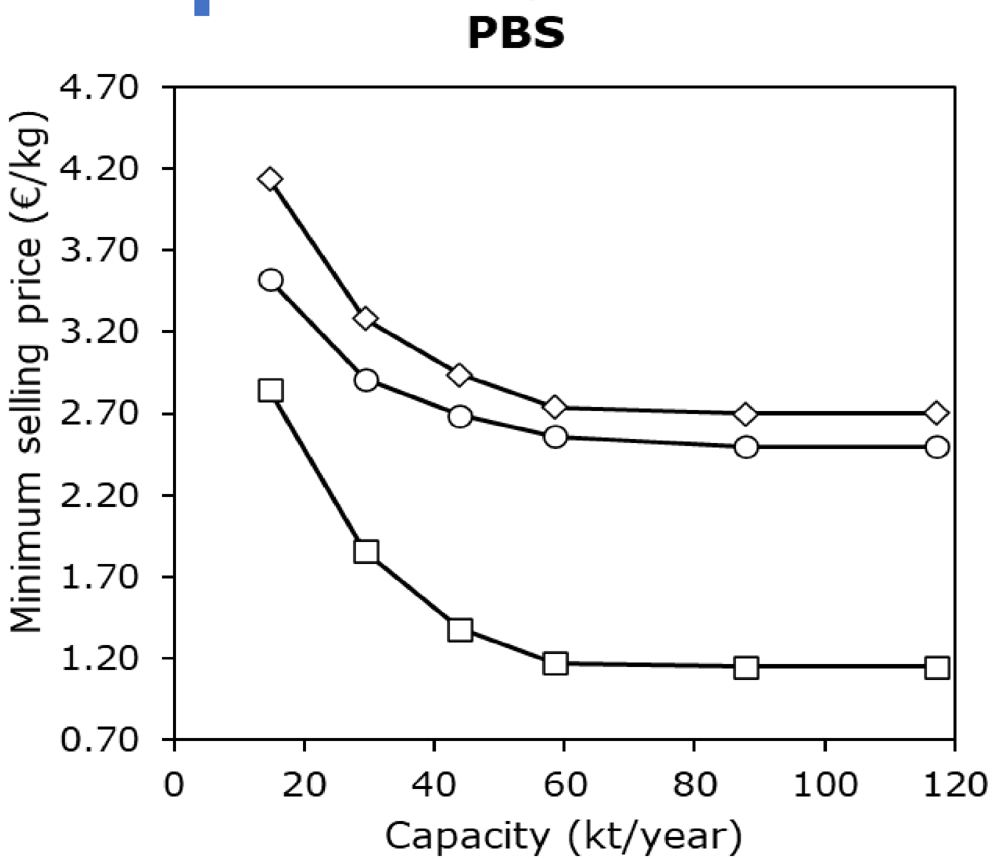
o glucose ◇ corn stover □ sugar beet pulp

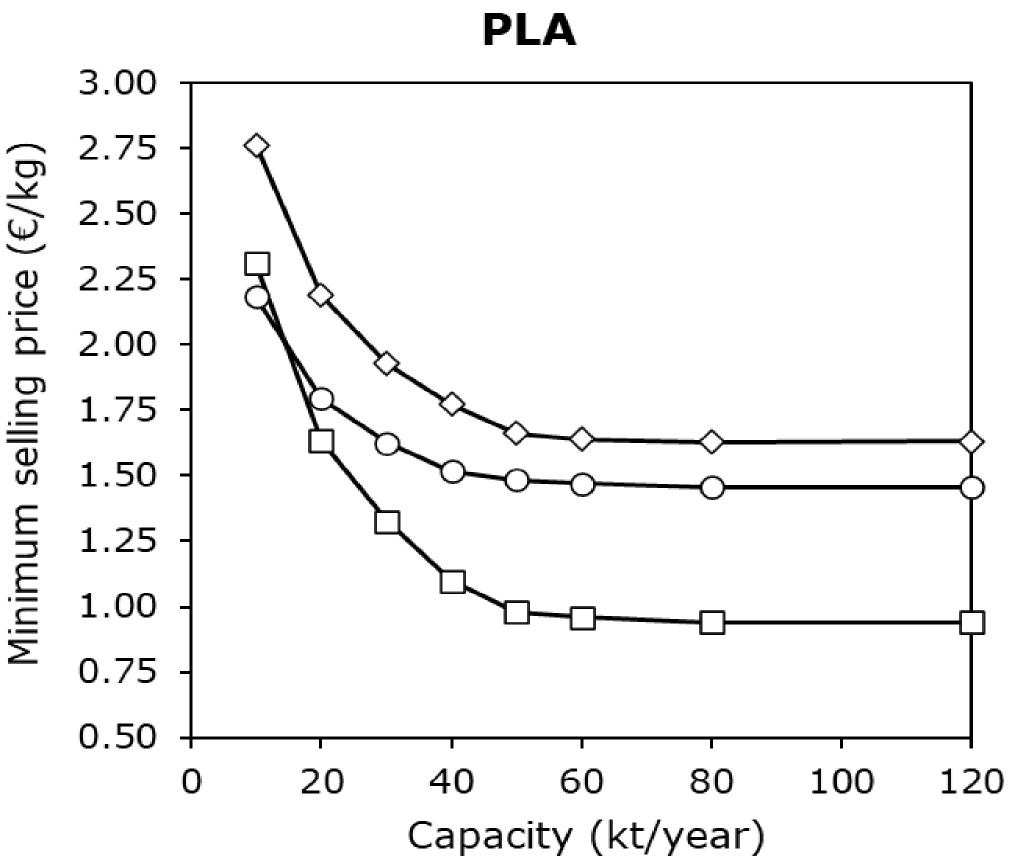
Cost of Manufacture (COM) at different plant capacities



o glucose ◇ corn stover □ sugar beet pulp

Minimum Selling Price (MSP) at different plant capacities





o glucose ◇ corn stover □ sugar beet pulp*

* considering that the sales price of pectin-rich extract is 4 €/kg in the case of PBS and mulch film production, while in the case of PLA production it is considered as 3 €/kg

Metrics in the optimum plant capacity

Poly(butylene succinate) – PBS

	OPC (kt/year)	COM (€/kg)	MSP (€/kg)	DPP (year)	MFR (kt/year)
Glucose	58.63	2.25	2.56	7	151.28
Corn stover	58.53	2.19	2.74	9	314.67
Sugar beet pulp	58.63	3.32	1.17 *	6	865.18 **

Poly(lactic acid) – PLA

	OPC (kt/year)	COM (€/kg)	MSP (€/kg)	DPP (year)	MFR (kt/year)
Glucose	50.00	1.27	1.48	7	64.84
Corn stover	50.00	1.33	1.66	12	134.88
Sugar beet pulp	50.00	1.88	0.98 *	6	370.85 **

OPC : Optimum Plant Capacity
COM : Cost of Manufacture
MSP : Minimum Selling Price
DPP : Discounted Payback Period
MFR : Minimum Feedstock Capacity Requirement

*considering that the sales price of pectin-rich extract is 4 €/kg for PBS and mulch film, and 3 €/kg for PLA

** considering that the SBP contains 70% water, which means that drying and pelletisation have not been carried out

STAR ProBio www.STAR-ProBio.euFunded by the EU H2020 Programme

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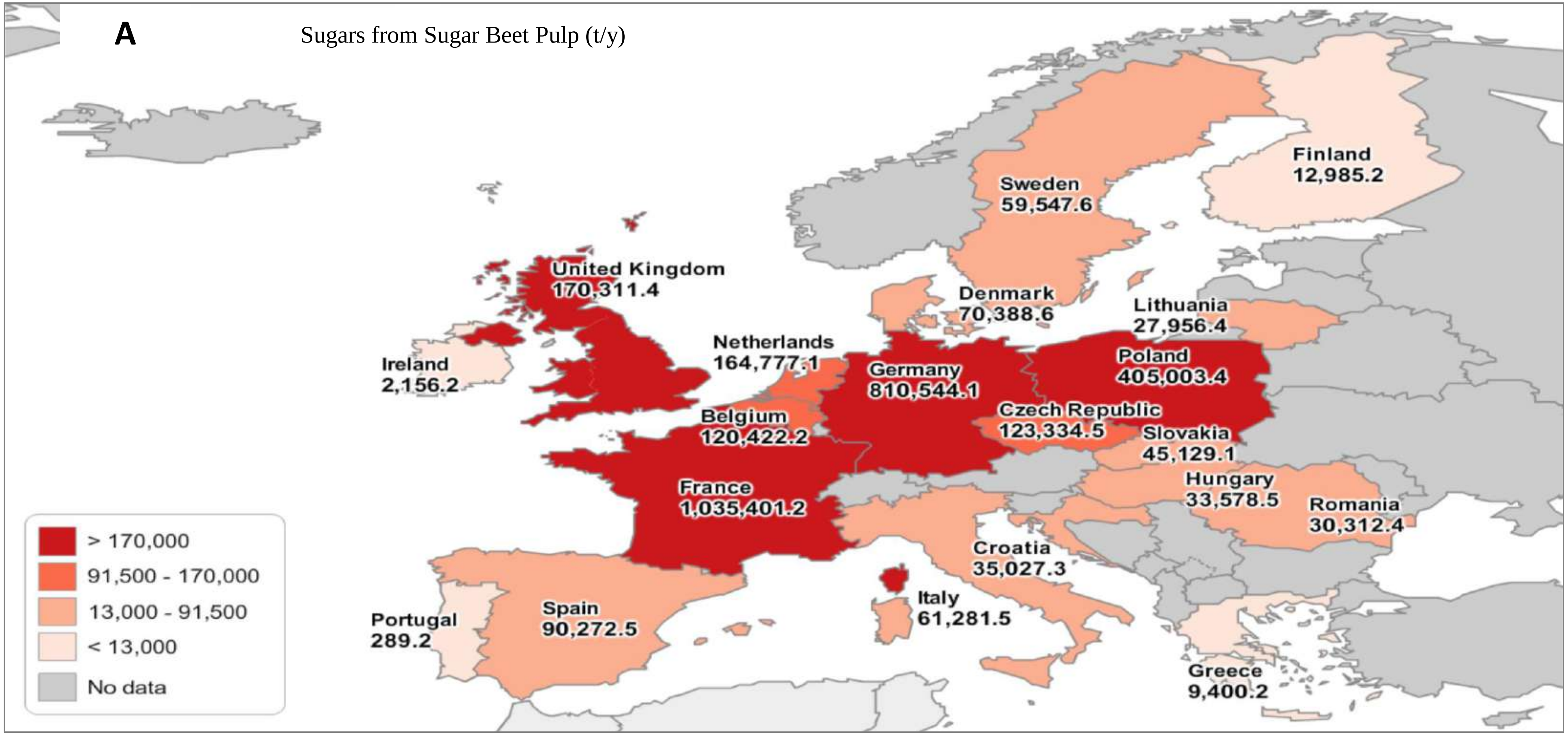
A
Sugars from Sugar Beet Pulp (t/y)
Finland
12,985.2
Sweden
59,547.6
United Kingdom
170,311.4
Denmark
70,388.6
Lithuania
27,956.4
Netherlands
164,777.1
Ireland
2,156.2
Germany
810,544.1
Poland
405,003.4
Czech Republic
123,334.5
Belgium
120,422.2
Slovakia
45,129.1
France
1,035,401.2
Hungary
33,578.5
Romania
30,312.4
Croatia
35,027.3
Italy
61,281.5
Portugal
289.2
Spain
90,272.5
Greece
9,400.2
> 170,000
91,500 - 170,000
13,000 - 91,500
< 13,000
No data

Ratio of feedstock capacity requirement to feedstock availability in the region

Corn stover

	Availability (million t for 2018)
France	3.80
Romania	5.60
Hungary	2.40

Ratio	France	Romania	Hungary
PBS	0.08	0.06	0.13
Mulch film	0.04	0.03	0.06
PLA	0.04	0.02	0.06

Sugar beet pulp

	Availability (million t for 2018)
France	2.34
Germany	1.55
Poland	0.85

Ratio	France	Germany	Poland
PBS	0.12	0.18	0.33
Mulch film	0.06	0.08	0.15
PLA	0.05	0.08	0.14

ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ AGRICULTURAL UNIVERSITY OF ATHENS
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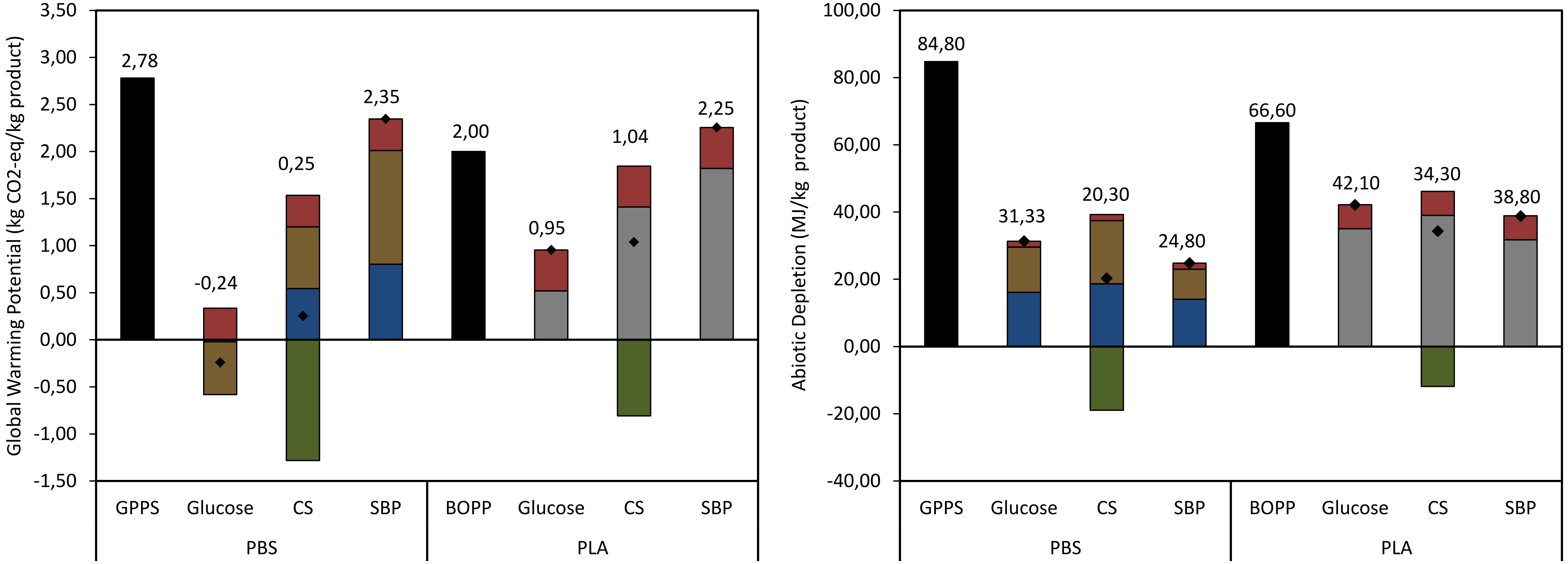
Environmental impact



Life cycle assessment of the main impact categories for PBS and PLA production from glucose, corn stover and sugar beet pulp. The environmental impacts of their fossil counterparts are also presented. Bars have been color-coded based on the contribution of each production stage: diamond – net total impact, black – fossil-based counterpart, blue – succinic acid, brown – BDO, grey – lactic acid, red – polymerization, green – savings from lignin combustion. Labels indicate the net total impact of each process.

STAR ProBio www.STAR-ProBio.euFunded by the EU H2020 Programme

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European Union European Regional Development Fund

ΕΣΠΑ 2014-2020 Partnership Agreement 2014 - 2020

Cost of externalities (methodology)

External costs
- physical parameter representing the unit of the impact
- economic parameter representing the accounting price per unit of impact

Principal stages for the implementation of externalities methodology

- Definition of the activity to be assessed
- Estimation of the impacts or effects of the activity (in physical units)

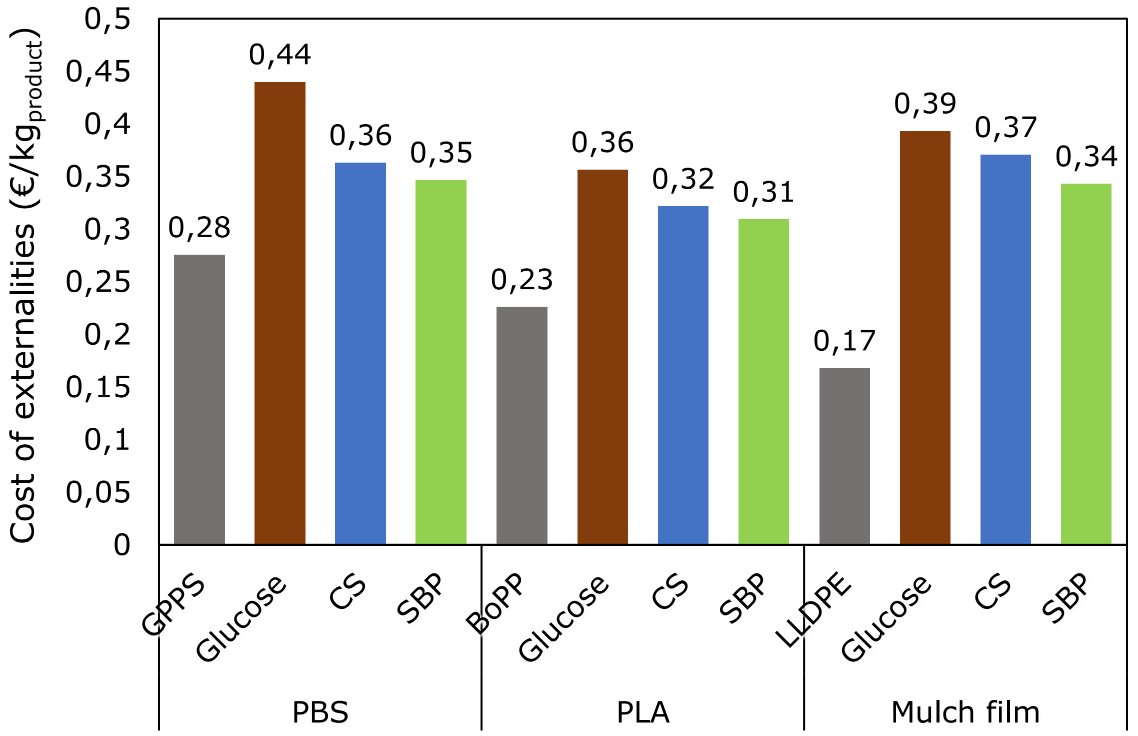
  → Gabi software, ReCiPe Mid/Endpoint methodology, version 1.08
- Monetisation of the impacts leading to external costs estimation.
- Assessment of uncertainties and sensitivity analysis.
- Analysis of the results and conclusions

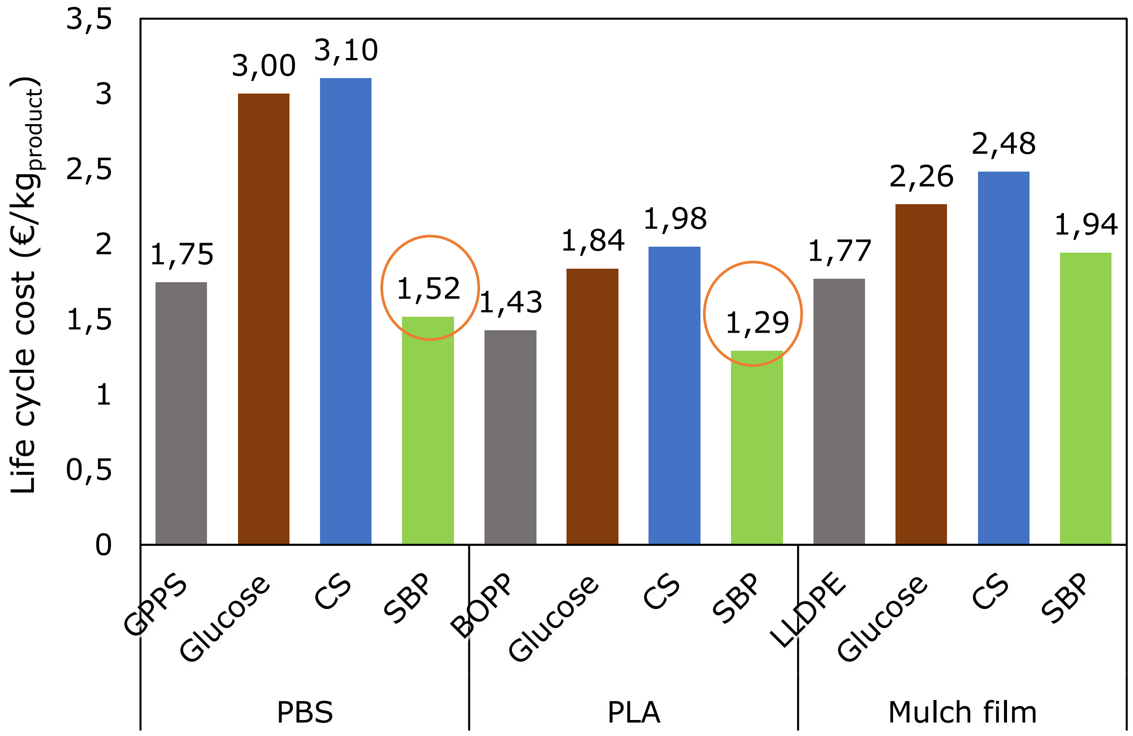
Impact Category	Unit	Monetary Value[1]
Climate Change	€/kg $CO_{2\text{-eq}}$	0.0566
Stratospheric Ozone Depletion	€/kg CFC-$_{11\text{-eq}}$	30.4000
Human Toxicity	€/kg 1,4 $DCB_{\text{-eq}}$	0.0991
Photochemical oxidant formation	€/kg $NMVOC_{\text{-eq}}$	1.1500
Fine Particulate Matter Formation	€/kg $PM_{10\text{-eq}}$	39.2000
Ionizing Radiation	€/kg kBq $U_{235\text{-eq}}$	0.0461
Acidification	€/kg $SO_{2\text{-eq}}$	4.9700
Freshwater eutrophication	€/kg $P_{\text{-eq}}$	1.8600
Marine eutrophication	€/kg $N_{\text{-eq}}$	3.1100
Terrestrial ecotoxicity	€/kg 1,4-$DB_{\text{-eq}}$	8.6900
Freshwater ecotoxicity	€/kg 1,4-$DB_{\text{-eq}}$	0.0361
Marine ecotoxicity	€/kg 1,4-$DB_{\text{-eq}}$	0.0074

[1]Bijleveld et al. Environmental Prices Handbook EU28 version - Methods and numbers for valuation of environmental impacts. CE Delft.

Externalities & Life Cycle Cost (LCC) – Comparison to fossil counterparts

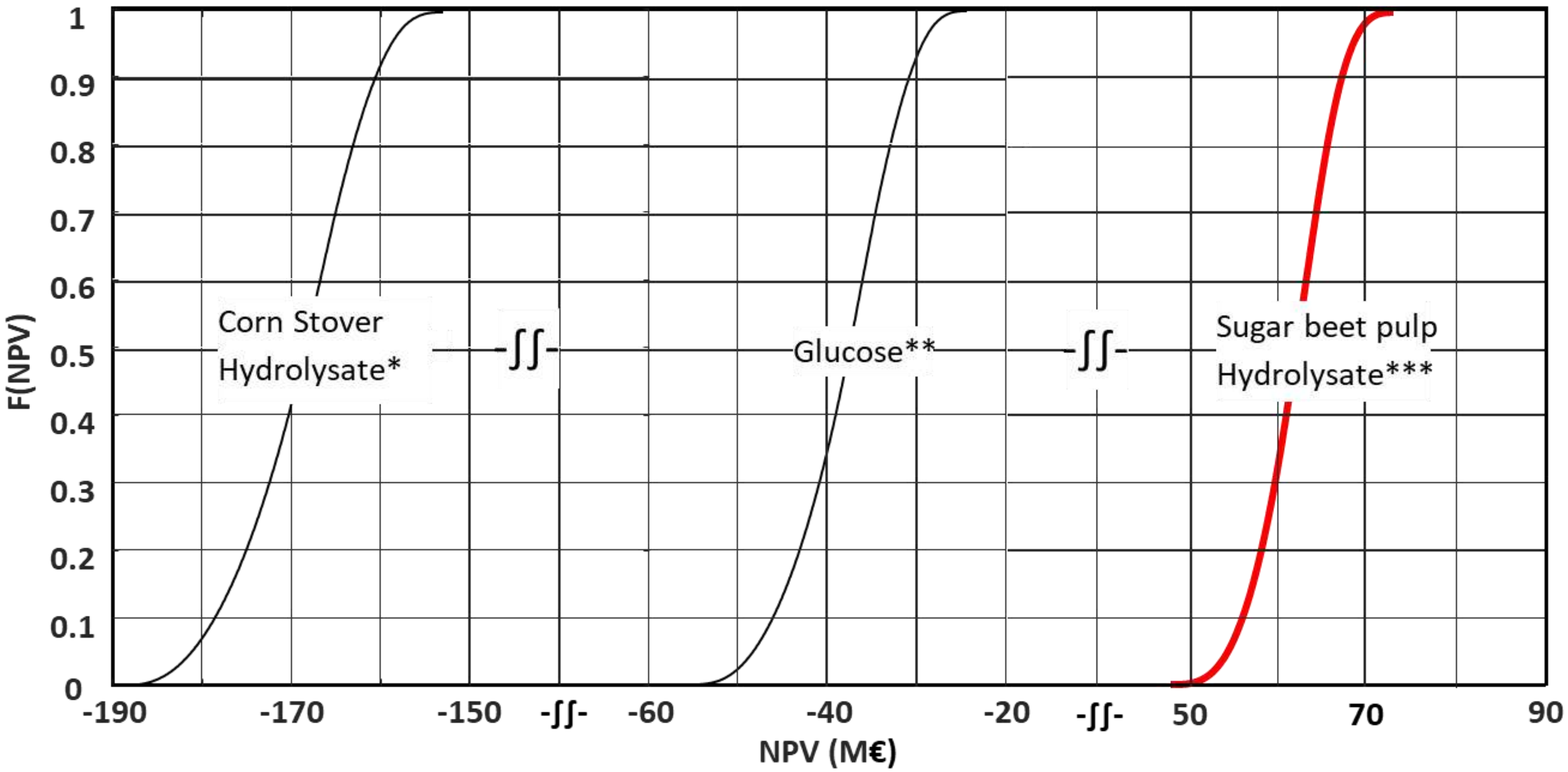
Life cycle cost = Minimum Selling Price or Current Price + Cost of externalities





Risk assessment - PLA

Probability of profitable (net present value-NPV) production of PLA when the biopolymer is sold at the current price of fossil counterpart (1.20 €/kg BOPP)



Process variables	Range	Design parameters	Value
Fermentation duration for lactic acid production (h)	50-75	Optimum PLA annual production capacity (kt/y)	50
Cost of steam (€/t)	8.09-8.23	Lactic acid concentration at the end of fermentation (kg/m^3)	182
Electricity cost (€/kWh)	0.0577-0.0685	Lactic acid to PLA polymerization yield (kg_{LA}/kg_{LA})	0.8
Total sugar to lactic acid conversion yield (kg_{LA}/kg_{TS})	0.85, 0.90, 0.97		
Glucose price (€/kg)	0.15, 0.21, 0.26		
Assumed PLA market price (€/kg_{PLA})		1.20	

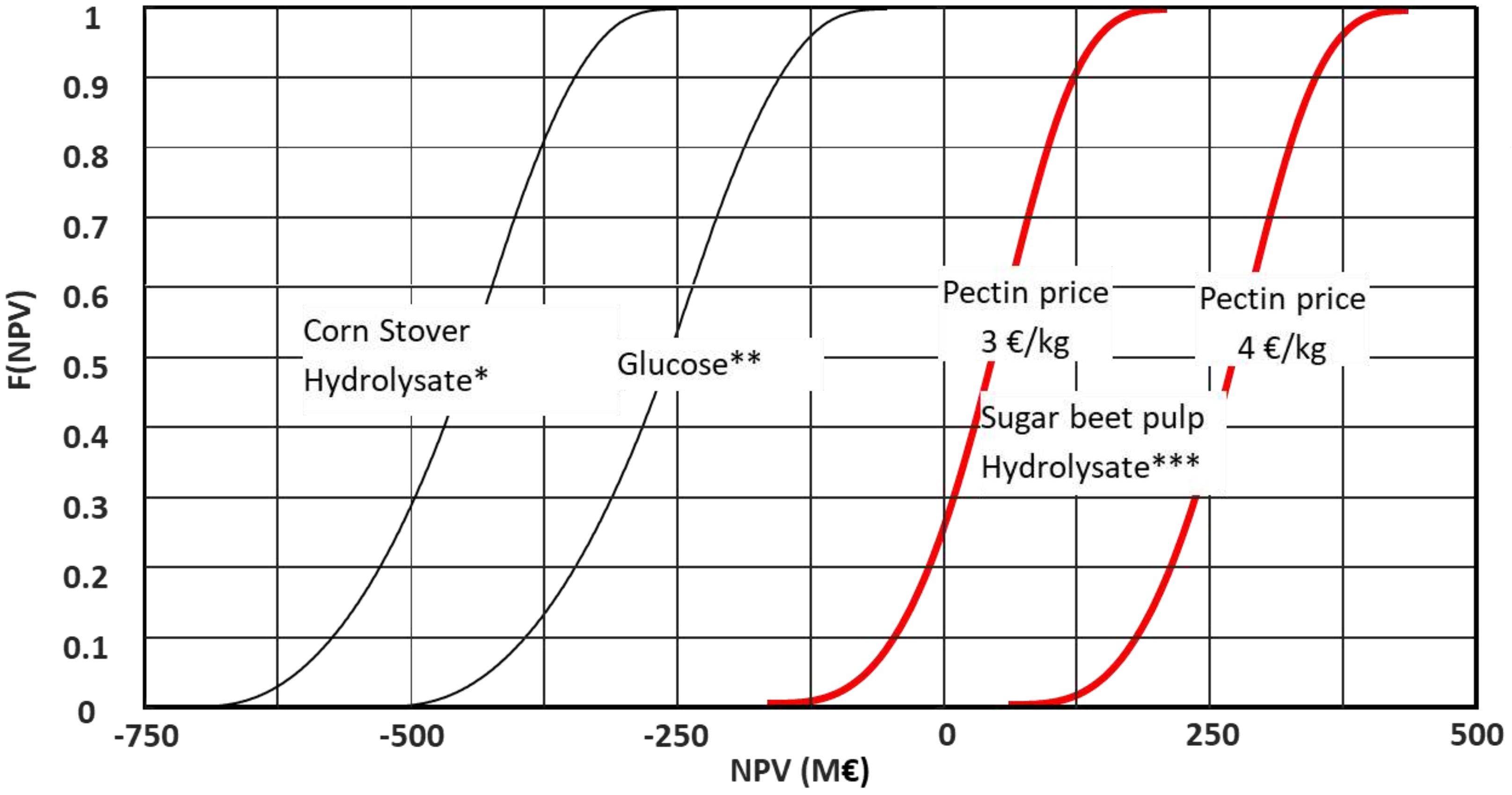
*PLA production from corn stover hydrolysate, with sugar to LA yield of 0.97 (kg_{LA}/kg_{TS})

**PLA production from glucose, with sugar to LA yield of 0.97 (kg_{LA}/kg_{TS}) and glucose price of 0.15 €/kg

***PLA production from sugar beet pulp hydrolysate, with sugar to LA yield of 0.85 (kg_{LA}/kg_{TS}) and pectin price of 3 €/kg

Risk assessment-PBS

Probability of profitable production of PBS when the biopolymer is sold at the current price of fossil counterpart (1.47 €/kg GPSS)



Process variables	Range	Design parameters	Value
Fermentation duration of 1,4-butanediol (BDO) production (h)	20-50	Optimum PBS annual production capacity (kt/year)	58.6
Cost of steam (€/t)	8.09-8.23	BDO annual production capacity (kt/year)	40
Electricity cost (€/kWh)	0.0577 - 0.0685	Succinic acid annual production capacity (kt/year)	40
Succinic acid market price (€/kg)	0.93-1.87	BDO concentration at the end of fermentation (kg/m^3)	125
Total sugar to BDO conversion yield (kg_{BDO}/kg_{TS})	0.32, 0.40, 0.48		
Glucose price (€/kg)	0.15, 0.21, 0.26		
Assumed PBS market price (€/kg_{PBS})		1.47	

* PBS production from corn stover hydrolysate, with sugar to BDO conversion yield of 0.48 (kg_{BDO}/kg_{TS})

** PBS production from glucose, with sugar to BDO conversion yield of 0.48 (kg_{BDO}/kg_{TS}) and glucose price of 0.15 €/kg

*** PBS production from sugar beet pulp hydrolysate, with sugar to BDO conversion yield of 0.32 (kg_{BDO}/kg_{TSS}) and pectin prices of 3 or 4 €/kg

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Work Programme BB-01-2016: Sustainability schemes for the bio-based economy

Online Workshop “Sustainable Production of Biobased Products in the Bioeconomy Era” 10/11/21

Symbeeosis

love your self love your planet

Pavlos Voulgaris – Agronomist Biotechnologist
Agriculture Project Manager at Symbeeosis SA

“Regenerative Economics as part of an Ethic and Sustainable Food Production Business”

Circular - Regenerative Economics as taught to us by nature

Complete recycling of all elements and their return to the food chain and consequently to the life chains (water, nitrogen, carbon, nutrients, etc.)

Organisms that have evolved based on the utilization of "locked elements" making them available to the rest (decomposers).

Utilization of energy from the sun and the available resources, collaborations between the plant kingdom and fungi to meet their energy needs and create an energy reservoir for other species.

Evolution of social intelligence with the aim of excellent communication, full utilization of available resources and energy (bee society).

The power of business to do good

REGENERATE

ACHIEVING NET ZERO GREENHOUSE GAS EMISSIONS BY 2050

Challenges

+0.9°c temperature rise

The average global temperature has risen by 0.9 degrees Celsius, between 1993 and 2016.

8 inches in 100 years

Sea levels have risen by about 8 inches in the past 100 years.

90% of CO2 emission

Fossil fuels and industry created nearly nine in ten of all CO_2 emissions (2018) and they are currently catering for over 85% of our energy consumption.

134 million tonnes CO2 reduced

The UK has reduced the amount of carbon dioxide it emits by 134 million tonnes since 2010.

364 million tonnes CO2 produced

Yet it still produced 364 million tonnes of carbon dioxide, in 2018.

75 - 175 million tonnes

In order to achieve Net Zero it is likely that between 75 and 175 million tonnes of greenhouse gases will have to be removed by carbon capture and storage technology alone. For comparison, the UK's biggest carbon capture plant is set to capture just 16 million tonnes by the mid 2020s.

0% coal in the UK energy mix

By June 2020, the UK achieved a new record of 67 days without coal power.

CREATING A CIRCULAR ECONOMY

Challenges

45% of emissions from everyday products

45% of all global emissions come from the production and disposal of cars, clothes, food, and other everyday products.

1.5 million tonnes of plastic littering

In the UK an estimated five million tonnes of plastic are used every year, just under a third (30 percent) of which is littering the soil, rivers, and oceans.

9.5 million tonnes food waste

The UK wastes around 9.5 million tonnes of food a year (2018), causing the emission of as much as 25 million tonnes of greenhouse gases since rotting food produces methane, a powerful greenhouse gas, as it decomposes.

16% of food is thrown away

The average household throws away 16% of all food.

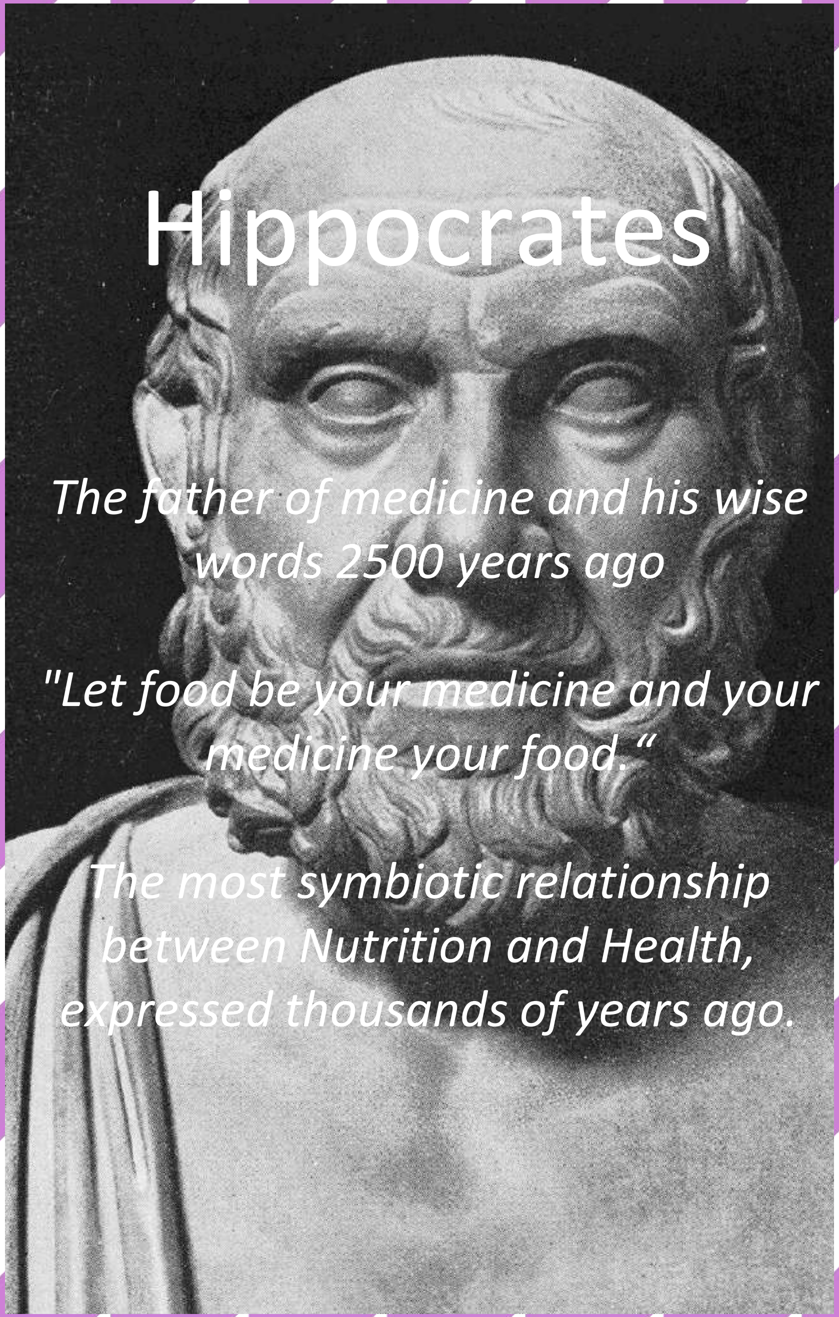
52.3 million tonnes of waste to landfill

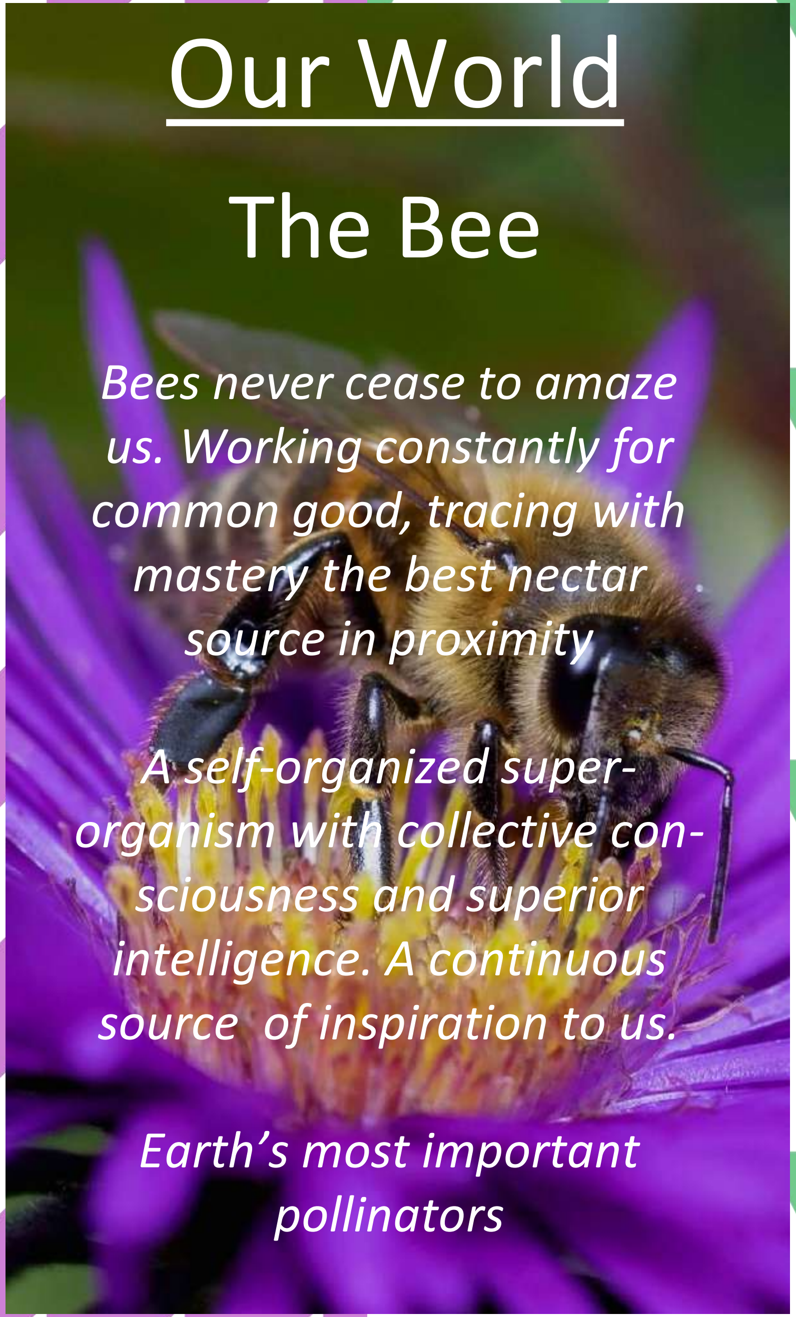
In the UK, 52.3 million tonnes of waste was sent to landfill (2016), up 8.5 percent from 2014.

Symbeeosis

from the Greek word Symbiosis
< Syn + biosis

and the bee, the most important pollinator of nature and model-inspirer of harmonious coexistence.


Hippocrates
The father of medicine and his wise words 2500 years ago
"Let food be your medicine and your medicine your food."
The most symbiotic relationship between Nutrition and Health, expressed thousands of years ago.


Our World
The Bee
Bees never cease to amaze us. Working constantly for common good, tracing with mastery the best nectar source in proximity
A self-organized super-organism with collective con-sciousness and superior intelligence. A continuous source of inspiration to us.
Earth's most important pollinators


Greek Biodiversity
Also known as the biological heaven of the Mediterranean, Greece has a very high number of indigenous species due to its geographical position, climatic and geomorphological variance and geological history.
Its flora consists of 6750 different plants, 1490 of them being indigenous.

Our Vision

We dream of restarting the planet through radical change: sustainable cultivation of the Earth, high quality food, ethical business. We believe in the symbiosis between people and the planet.

Our Purpose

«Our food is our medicine »

We are a company that bees would build: we offer symbiotic products and services that are inspired by the Hippocratic principles and the rich Greek biodiversity, with sustainable operations and respect to the planet and people.

Our Philosophy

love your self

love your planet

Symbeeosis

"Reframing economics"

Regenerative Business Model

→ Planet

All businesses depend directly or indirectly on the use of land, air, water, fire, minerals and / or green space. Our relationship with nature must be balanced.

→ People

Implementing change comes from people and teams.

→ Products

A profound transformation is taking place in the development of the products that will feed us in the future.

→ Production

Smaller, more flexible production units will become the way of escalation in the future. This will require new flexible models of machinesproduction to energy dependence and packaging.

→ Platforms

Business adaptability will be determined by data management and knowledge interconnection.

Holistic Green Marketing

In a business environment that speaks of three P (profit profit profit) we apply different P (people planet purpose platforms) through philosophy, values, corporate social responsibility and sustainable development.

Product **Price** **Place** **Promotion**	→	**Purpose** **Pollination** **People** **Passion** **Platforms** **Personal Responsibility** **Processes** **Packaging** **Product** **Price** **Place** **Promotion**

EU FARM TO FORK & BIODIVERSITY STRATEGY

Moving towards a more healthy and sustainable EU food system, a corner stone of the European Green Deal

- Make sure Europeans get healthy, affordable and sustainable food
- Tackle climate change
- Protect the environment and preserve biodiversity
- Fair economic return in the food chain
- Increase organic farming

Stepping up the fight against food waste

Halving per capita food waste at retail and consumer levels by 2030.

The Commission will propose legally binding **targets** to reduce food waste across the EU by 2023.

Research and innovation

EUR 10 billion under Horizon Europe to be invested in R&I related to food, bioeconomy, natural resources, agriculture, fisheries, aquaculture and environment. Knowledge transfer will be essential. The CAP's Farm advisory services and Farm sustainabilty data network will be instrumental in assisting farmers in the transition.

Promoting the Global transition

Making European food famous for its sustainability can add a competitive advantage and open new business opportunities for European farmers.

The EU will collaborate with third countries and international actors to support a global move towards sustainable food systems. **A sustainability food labelling framework will facilitate consumer choice.**

Our uniqueness

innovation + sustainability

Use of active dry extracts:
modern green methods for obtaining extracts concentrated in active ingredients

Development of genomic protocols:
Specific origin of the genetic material, from populations with the best desired characteristics to ensure quality

Collaboration with leading research institutes
and the Greek and international academic community

Minimum footprint
From farm to cup

Organic and regenerative cultivations

Ethical Business
Transparency throughout the Supply Chain / Everyone is a stakeholder

Goal is B-corp certification

ORGANIC

NON-SYNTHETIC INGREDIENTS

SUSTAINABLY SOURCED

BIO ACTIVE

RESPONSIBLE PACKAGING

CAFFEINE FREE

1. Regenerative Agriculture

- Regenerative Agriculture is a holistic practice of land management, which restores health to the soil. This increases its organic matter and therefore the resilience of crops and the availability of nutrients, resulting in an increase in biodiversity, the continuous development and development of individuals, farms and communities for the realization of their innate potential.
- Regenerative agriculture is directly related to organic farming, aiming at strengthening the ecosystem.

5 Principles of Regenerative Agriculture

Soil Health
- Builds Soil Organic Matter
- Conservation Tillage
- Cover Crops
- Crop Rotations
- No GMOs or Gene Editing
- No Soilless Systems
- No Synthetic Inputs
- Promotes Biodiversity
- Rotational Grazing

Social Fairness
- Fair Payments for Farmers
- Freedom of Association
- Good Working Conditions
- Living Wages
- Long Term Commitments
- Transparency and Accountability

Animal Welfare

Protection of pollinators
Regenerative Beekeeping

Our Cultivations

- → *We have our own fields in Mount Olympus and Arcadia*
- → *We utilize, disseminate and teach the best practices of modern agriculture*
- → *We select types of herbs that grow in the cultivation areas, ensuring the genetic material and maintaining intact their best quality characteristics*
- → *We apply at a research and pilot level new Regenerative Practices of Earth Cultivation, combining technological development with sustainability and data platforms.*
- → *We carry out quality analyses from the field to the production of the final product*

Precision Agriculture + Platforms

- ✓ *Mapping of all measurable phases on the parcel*
- ✓ *Customization depending on the type of production*
- ✓ *Saving resources and energy*
- ✓ *Adaptation to climate change*
- ✓ *Provision of market data, trends, market needs and scientific research.*
- ✓ *Risk and risk assessment .*

gaiasense

Agroknow

2.Regenerative Beekeeping

- Regenerative Beekeeping : A holistic approach to the production of beekeeping products. Going beyond organic, to organic + practices & nomadic beekeeping. Emphasis is placed on the genetic material of the queens, in order to ensure that their origin and characteristics are appropriate for the area where the production takes place.
- A new model of beekeeping, as it aims to minimize the input of artificial feeds for bees as well as the environmental footprint of beekeepers. At the same time contributing to the protection of local breeds and the increase of financial benefits for beekeepers, leading to the development of unique local products.

Doing business through the eyes of bees

- *We utilize and disseminate the best practices of organic beekeeping, creating content and using educational platforms*
- *Our Beehives reside in areas with native herbaceous vegetation and / or organic crops for honey from naturally grown plants*
- *Nomadic Organic + Practices*
- *Use of technology to minimize our impact on the planet*
- *Recycling and using only eco friendly materials.*

Our beehives

We have our own hives - 1,500 bee flocks

Just by cooperating with so many bees we contribute to local ecosystems and farms, as our bees visit at least 30 billion flowers per day, enhancing local plant biodiversity.

A natural example of circular-regenerative economy.

We are also contributing to the local economy as we expand our beekeeping capital, giving hives to young passionate beekeepers. We buy their products and provide processing services in our factory.

Precision Beekeeping + Platforms

miBeez → an integrated toolbox that could be used during all beekeeping activities to help beekeepers manage production, monitor colonies and get advice for optimal decision making, in order to improve quantity and quality of apiculture products, as well as reducing time, cost and environmental impact.

Data collected, consist of : environmental parameters, weight and internal conditions of the cell environment, as well as the concentration of microparticles in the air.
Also, we can follow the history of the apiculture of apiaries (different locations, types of vision and environmental conditions, under which our product was developed).

miBeez

Our Products

We offer Greek, certified organic functional foods & drinks in 4 categories:
Tea • Herbs • Honey • Nutritional Supplements

Our products, based only on medicinal plants and beekeeping ingredients, are equally beneficial to human health and well-being, and the planet

Our target:

To highlight the value of proper nutrition and Greek organic functional food

To pollinate the principles of harmonious coexistence with the planet and the importance of healthy eating

Our packaging

Metal plastic free packaging
for tea & herbs

Made of tinplate, certified Plastic Free & Metal Recycles Forever™
It can be reused or recycled continuously

PLASTIC FREE

METAL recycles forever

Compostable pyramids
for tea & herbs

From PLA (Polylactic acid), a fiber derived from maize starch
Biodegradable naturally in soil or water, without emitting pollutants and leaving zero footprint

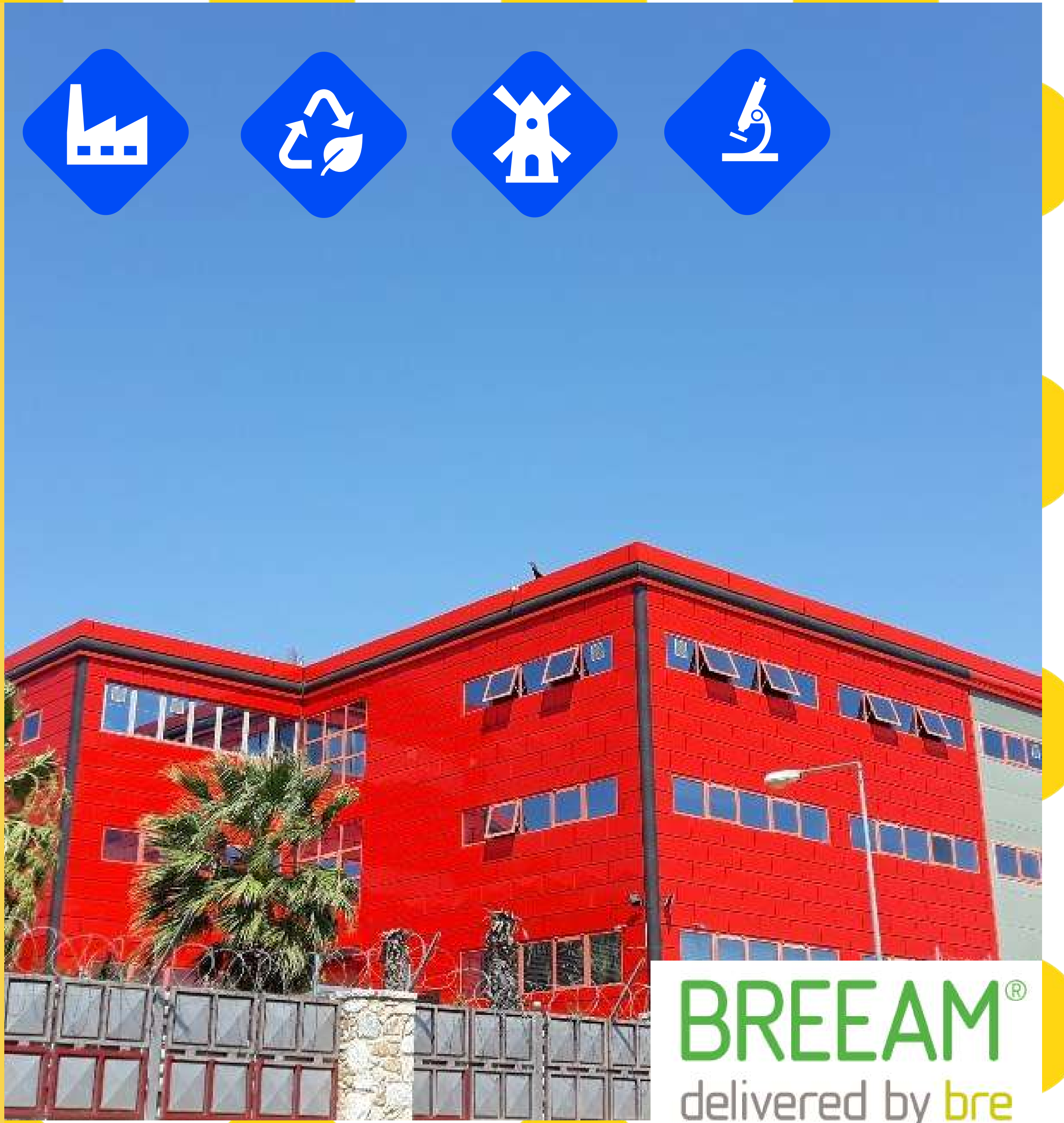
Glass jars for honey

With a metal lid
Can be reused or recycled.

Cardboard box for honey

FSC certified paper, which ensures that it comes from responsibly managed forests, controlled sources, recycled materials or a combination of these. Recyclable.

OUR INOVATIVE PRODUCTION FACTORY



BREEAM®
delivered by bre

Our production unit

We illuminate our spaces with low energy lamps and use motion sensors to lower electricity consumption.

We have an installed ventilation network with the use of heat exchangers and enthalpy to save the energy needs of the air conditioning units.

We utilize solar energy through solar panels to supply hot water to the heating circuits of our tanks and water.
We also plan the installation of photovoltaic panels and the use of other forms of renewable energy sources for the energy autonomy of the installation.

Energy and water consumption measurements are made at the process level to monitor our environmental KPIs.

Recycling bins for plastic, aluminum, glass & paper

Waste Management Plan

- Innovation: Zero production of non-reusable waste
- Production of products with low environmental footprint
- Honey not used in production is returned to feed the hives in August and January.
- Unsuitable plant material or pulp from production is sent for composting.
- From "dust" resulting from the cutting and sieving of plants (plant material with a diameter of less than 1mm), a part is currently used together with the plant for extraction. The extract is incorporated into the chopped plant material to enhance the action and properties of the final product. At the same time, studies are carried out on the utilization of the powder for the preparation of granules in combination with the plant extract.

"We can not solve our problems by thinking in the same way we used to create them."
Albert Einstein

THANK YOU FOR YOUR ATTENTION

Symbeeosis

Bioeconomy in Transition Research Group

School of Sustainability Studies and Circular Economy
UnitelmaSapienza University of Rome

Policy strategies, green finance and the transition to a circular bioeconomy

Piergiuseppe Morone, UnitelmaSapienza University of Rome

Athens, 10.11.2021

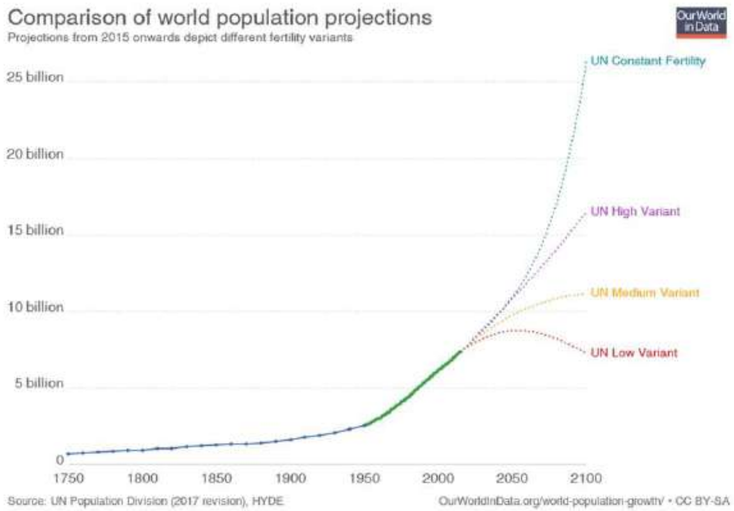
RESEARCH INFRASTRUCTURE ON FOOD BIORPOCESSING DEVELOPMENT & INNOVATION EXPLOITATION – Food Innovation RI

Online Workshop

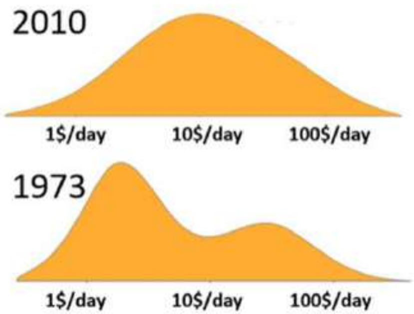
"Sustainable Production of Biobased Products in the Bioeconomy Era"

Economic growth and demography

Economic growth and demography

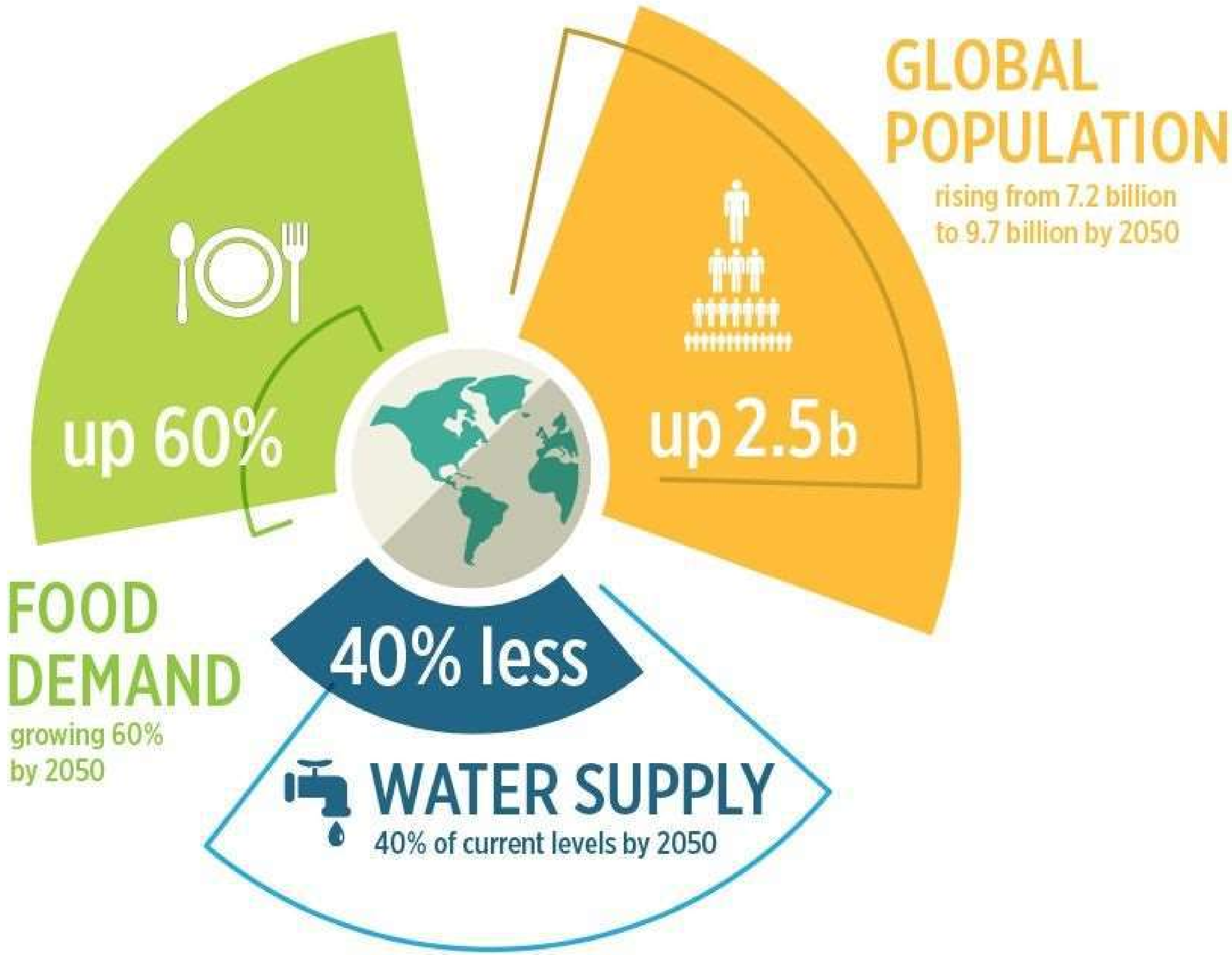


UN projection of population growth under high-medium-low fertility assumption



The explosion of the middle-class

The rise in global food demand



Population growth and mass consumption society

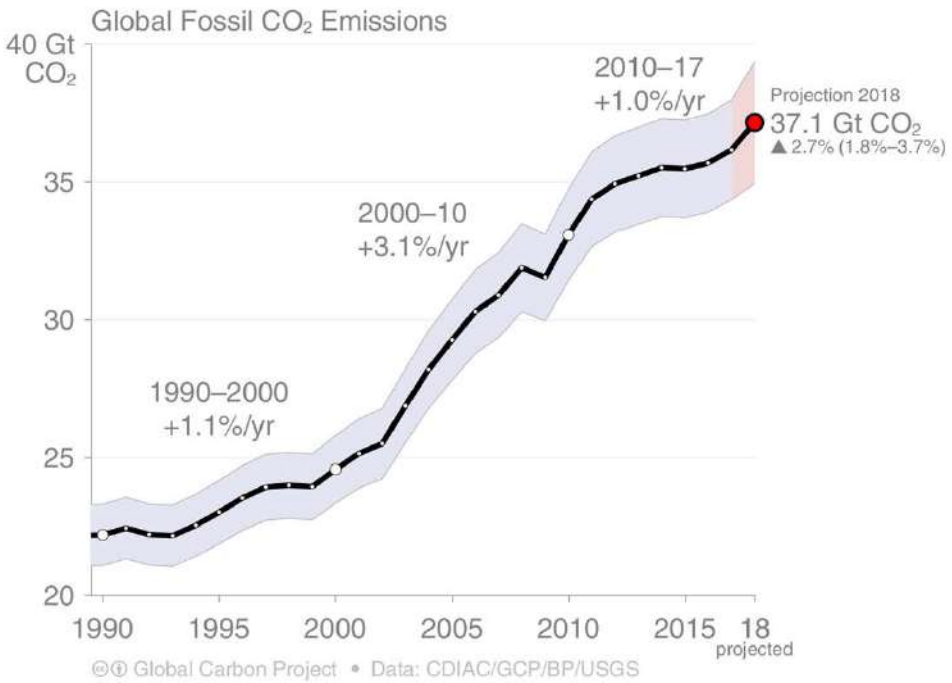
CORONAVIRUS

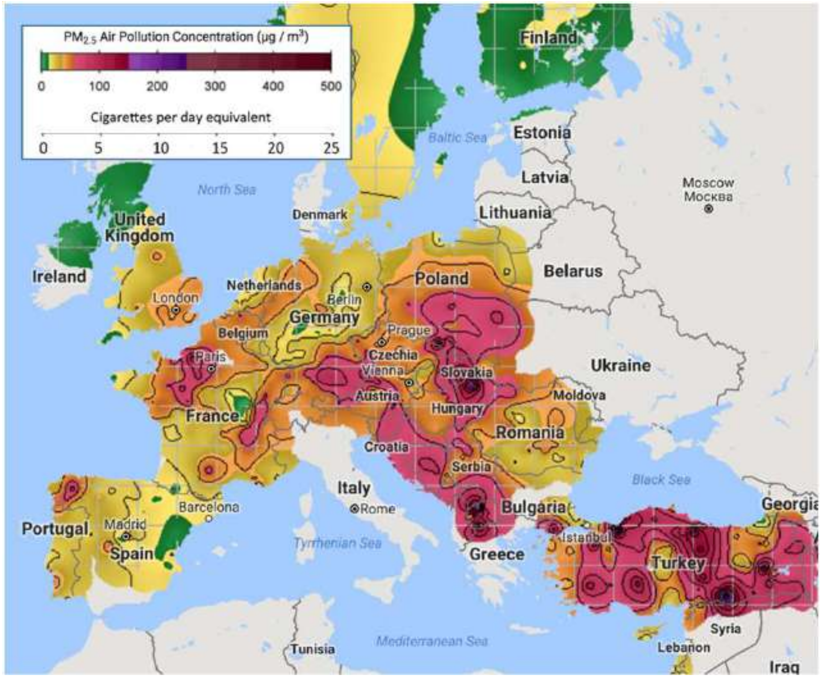
In volo da Hong Kong a Hong Kong: l'idea per chi non sa rinunciare all'aereo

The rise in waste production

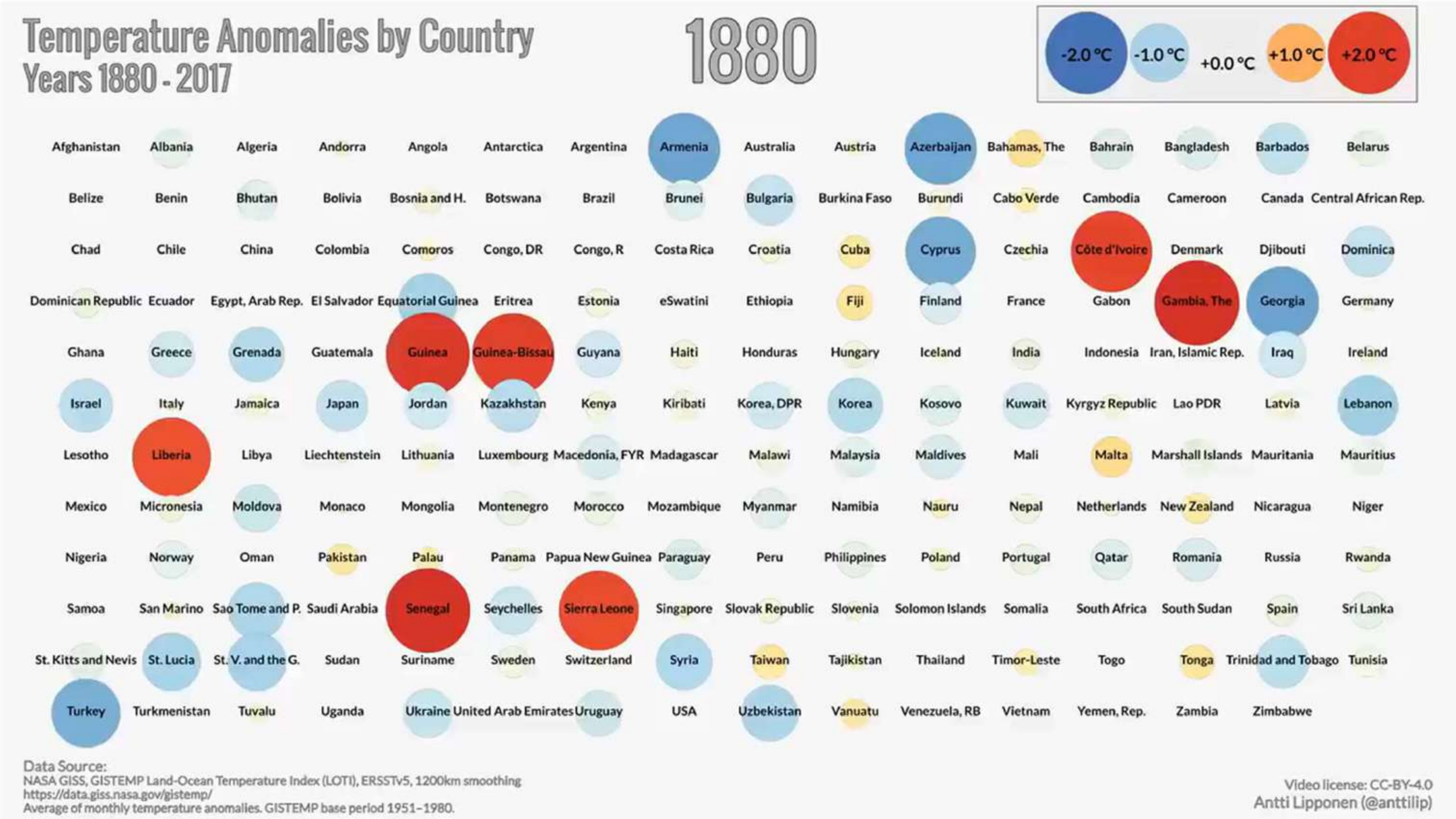
The rise in per-capita waste production determines an explosion in the global waste production if associated with population growth and middle class expansion

The rise in global and local pollutants

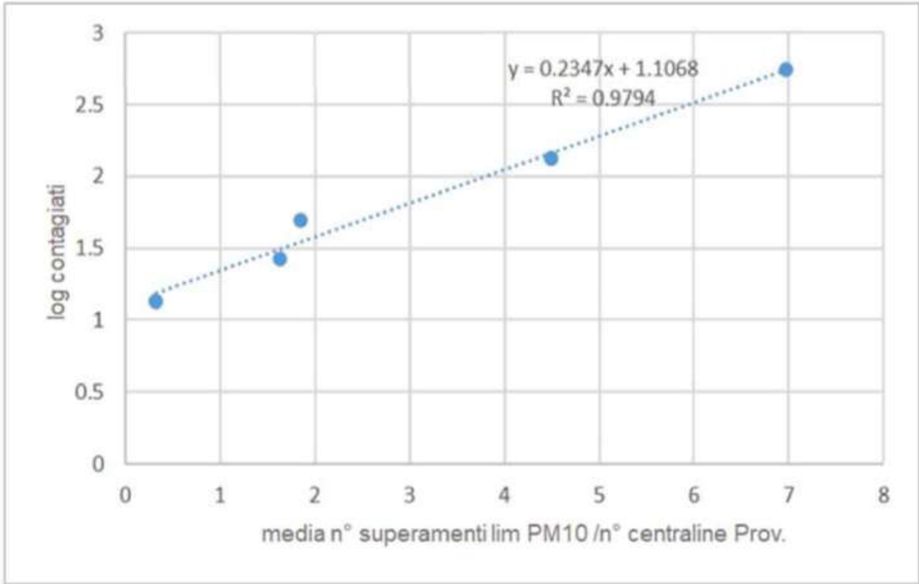




Global pollutants and climate change



Local pollutants and human health

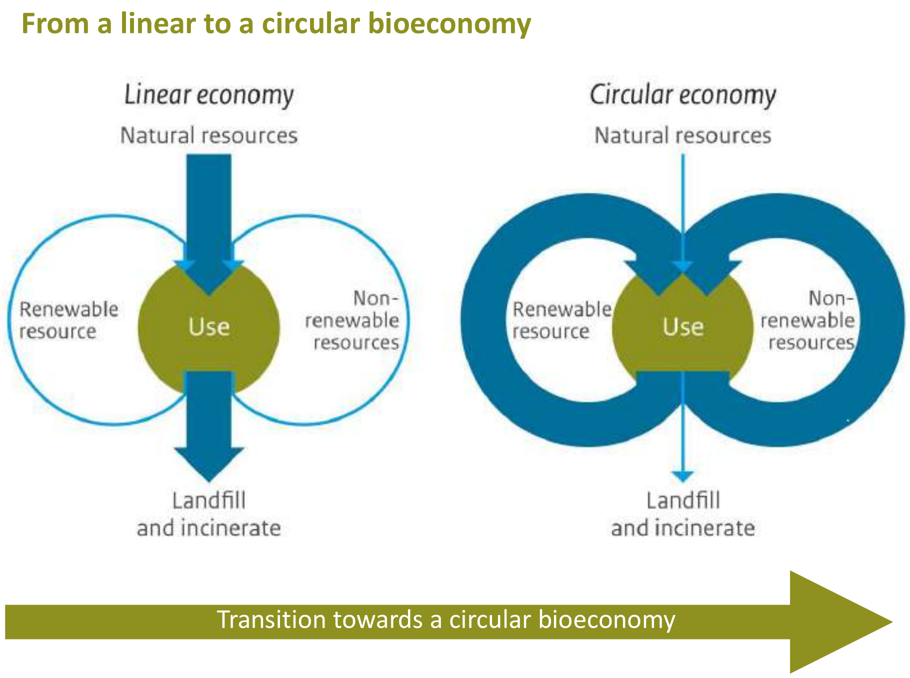


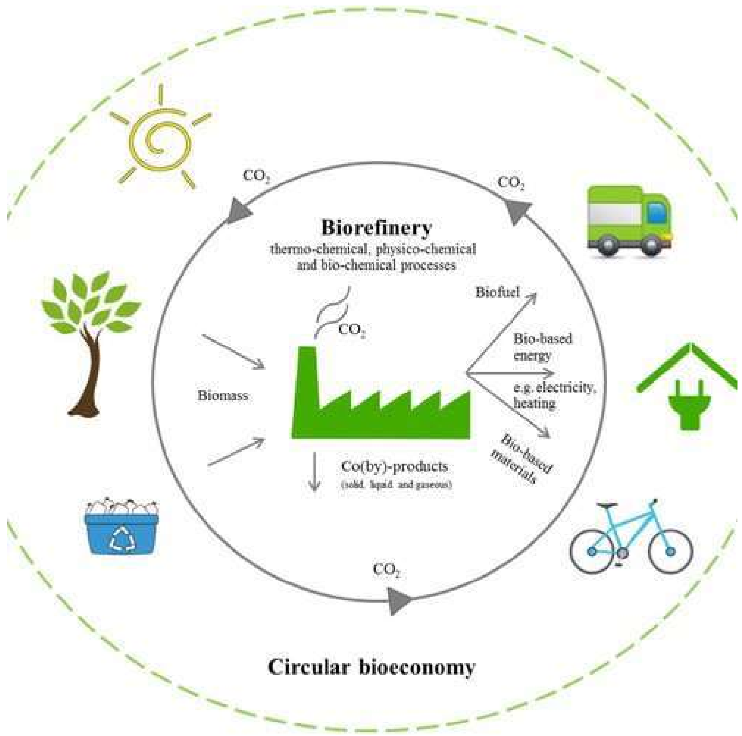
How to revert these trends?

The transition towards sustainability

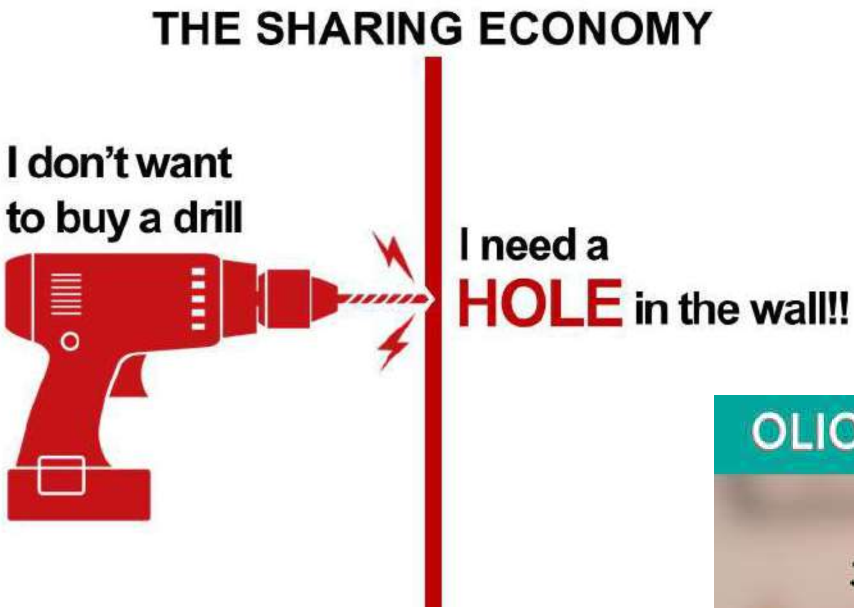
New production and consumption models

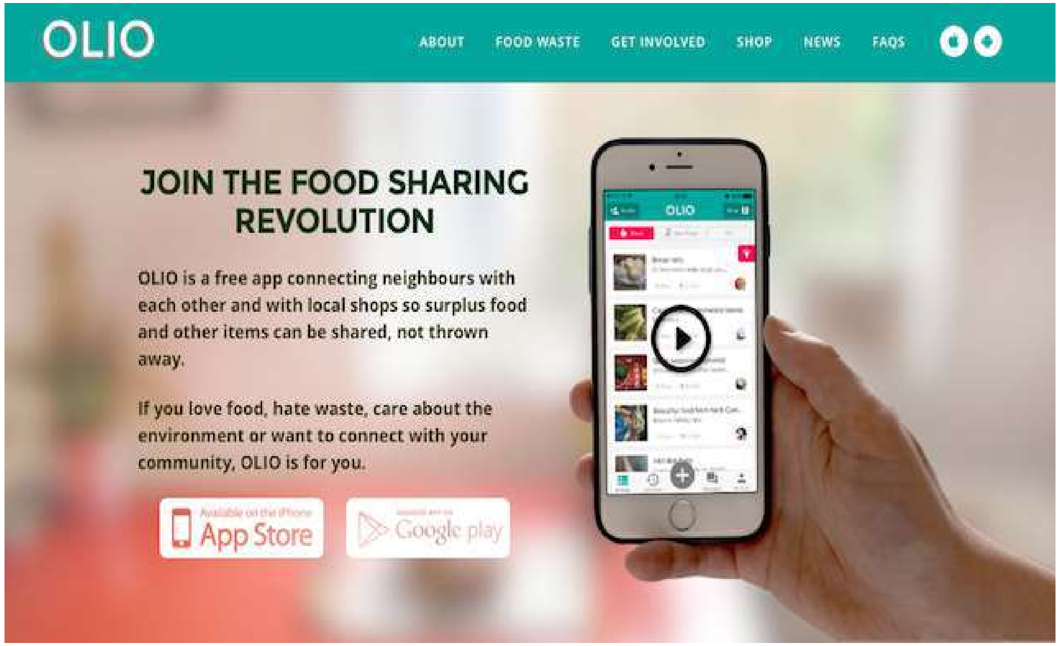
From linear and fossil-based to circular and bio-based



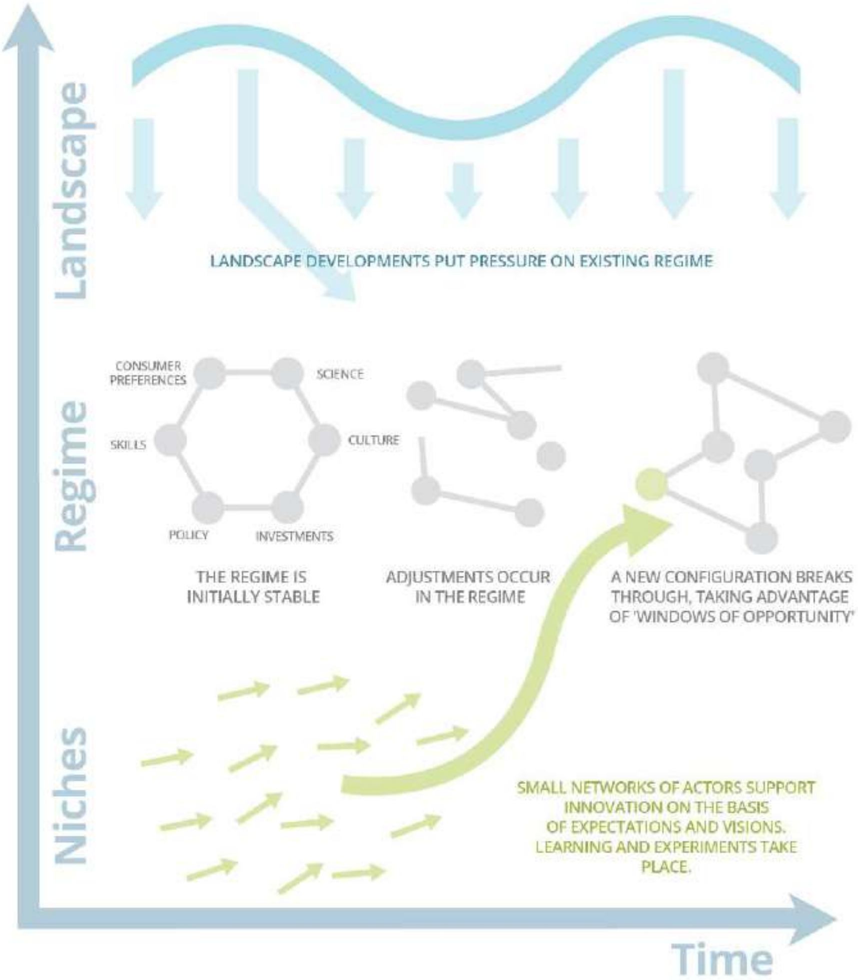


From ownership to access





The multi-level perspective on sustainability transitions



The role of green finance

The purpose of green finance (or sustainable finance) is to direct public and private financial resources towards **sectors, projects and initiatives** functional to the ***transition*** of the economy towards more sustainable models, that is, more inclusive and with a reduced impact on the environment and able to integrates the **SDGs** in their activities

Green finance aims to generate positive impacts for society, along with returns for investors

The role of green finance

- It is a sector that more than others is based on **<u>trust and information asymmetries</u>** that characterize its relationships

- In this regard, the **<u>European institutions</u>** are carrying out an **important regulatory activity** (both legislative and policy oriented) to introduce coherent criteria and definitions in the field of sustainable finance

Timeline

December 2015	December 2016	March 2018	June 2018
After **Paris Agreement on Climate** the European Union placed environmental and social sustainability at the centre of its policies	The European Commission set up a group of experts, the **High-Level Expert Group on Sustainable Finance** (HLEG), with the task to develop recommendations for the development of sustainable finance	The European Commission published the **Action Plan to finance sustainable** growth with 10 initiatives	June 2018, the European Commission appointed a **Technical Expert Group** (TEG) on Sustainable Finance with the task of providing technical advice on some issues of the Action Plan

The legislative and policy initiatives of the EU – Instruments: Taxonomy Regulation

The TEG focused on developing the taxonomy of eco-friendly economic activities, European standards on Green Bonds and eco-labels for financial products

Taxonomy Regulation: a classification of eco-compatible economic activities which represent a tool to guide the choices of investors and businesses

It directly applies to Member States, which can not introduce national rules that would compromise the integrity of the taxonomy regime

The legislative and policy initiatives of the EU – Instruments: Taxonomy Regulation

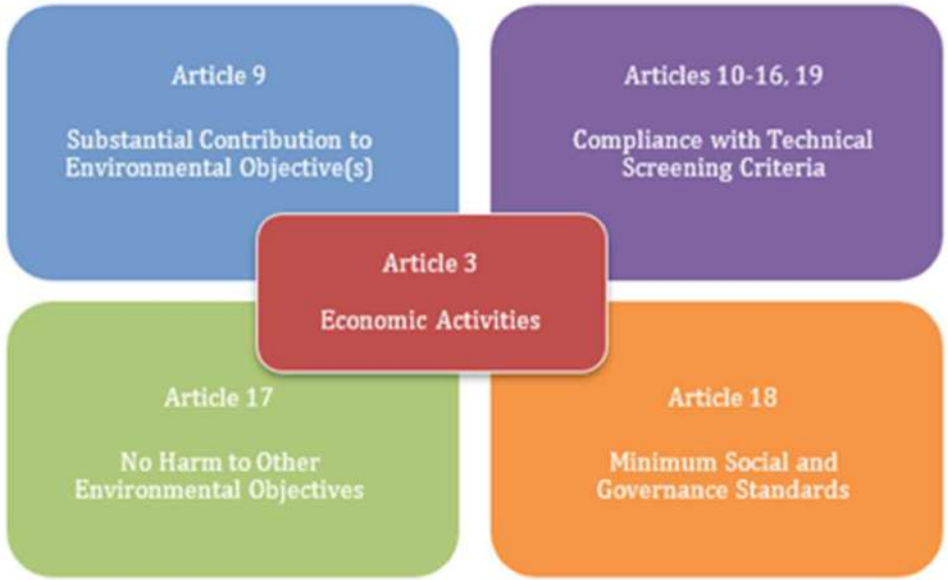
The Taxonomy is a classification of eco-compatible economic activities which represent a tool to guide the choices of investors and businesses
To be eco-compatible, an activity must:

1. positively contribute to at least one of the six environmental objectives

2. do not produce negative impacts on any other objective ("do no significant harm")

3. be carried out in compliance with minimum social guarantees (for example, the OECD guidelines for multinational enterprises and the United Nations Guiding Principles on Business and Human Rights)

4. comply with the technical screening criteria



The legislative and policy initiatives of the EU – Instruments: Green Bonds

The Commission asked the TEG to develop a proposal for a European standard on green bonds (or EU Green Bond Standard - GBS), or a system of criteria shared at European level for issuing green bonds

With the introduction of this standard, it will be possible to attribute the "EU Green Bond" certification to any type of bond or debt instrument, listed or unlisted, issued by a European or international operator, which proves to be GBS compliant

Consumers' behaviours towards green bonds - A field experiment

The rationale for the emergence of a green premium in bond markets: the role of certifications, framing and cooperation

Annarita Colasante[1], Andrea Morone[2], Piergiuseppe Morone[1]

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[2]Department of Economics, Managment and Law, Unieversità degli studi di Bari

Consumers' behaviours towards green bonds - A field experiment

Research question:

What is the role played by certifications in shaping the willingness to invest in green bonds?

Consumers’ behaviours towards green bonds - A field experiment

To address this RQ we design a lab experiment

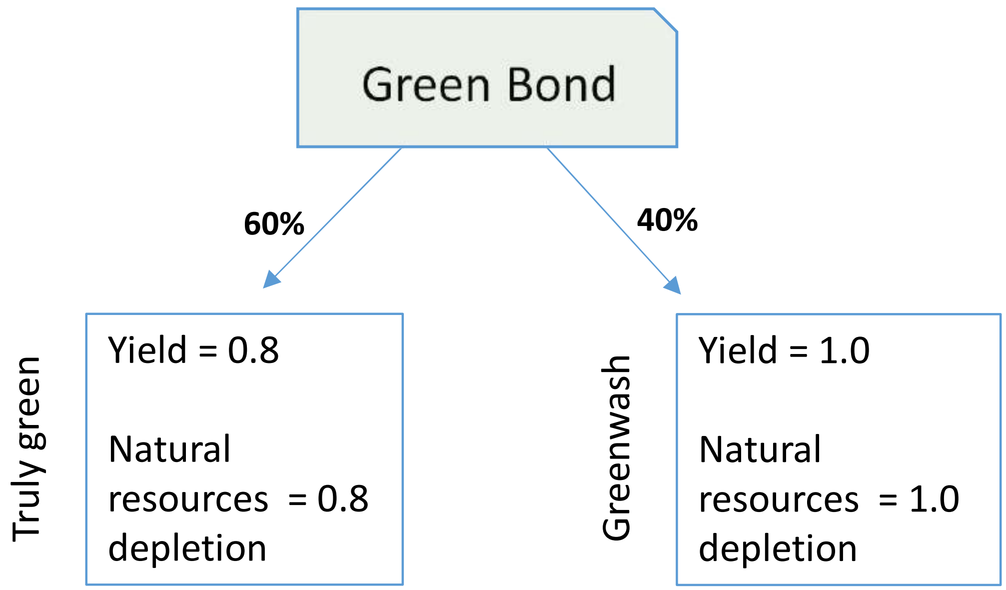
- A total of 165 participants took part to an **online experiment**
- The sample includes Spanish undergraduate students (heterogeneous background)
- Participants were recruited by means of ORSEE (Greiner, 2015)
- The experiment was computerized and used z-tree (Fischbacher,2007)
- All participants were connected during the whole experiment in a **Google Meet** (individual) room
- Subjects read the instructions on their own screen and the experimenter answered to clarifying questions
- Subjects were paid using either **Bizum** or a bank transfer

Consumers’ behaviours towards green bonds - A field experiment

- Participants have a single task to perform: after receiving the initial endowment, they are asked to **allocate this amount between two investment options**;
- at the beginning of the experiment, 35 participants are split in groups of 5;
- each group receives an amount of natural resources and an amount of financial resources such that **1 unit of resources = 1 ECU**; both kind of resources are evenly split among same group members;
- participants may invest the share of financial resources they own in two options (**option B** and **option G**) that will be used to subsidise the production activity of two firms;

Consumers’ behaviours towards green bonds - A field experiment

The experimental design



Treatment 1 (High quality signal)	Treatment 2 (Low quality signal)
90% right signal 10% wrong signal	30% right signal 70% wrong signal

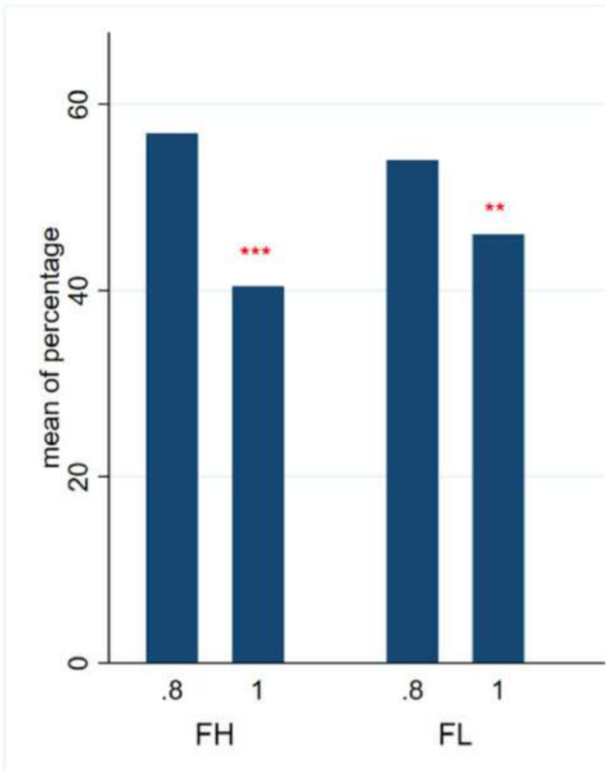
Consumers' behaviours towards green bonds - A field experiment

Similar to a Public Good Game, we may identify two solutions:

- At the individual level, the best option (**Nash equilibrium**) is invest the entire endowment in option B to get the maximum (current) payoff.
- At the aggregate level, the best option (**Pareto optimum**) is to invest in every period in option G.

Subjects investing in option B the entire amount is playing the (rational) **Nash strategy**.

Consumers' behaviours towards green bonds - A field experiment



Experimental results:

- **Greenium effect**: in both treatments subjects tend to prefer Green bonds over Brown bonds
- **Treatment effect**: in the low quality signal the investments in green bonds are significantly lower with respect to the high quality treatment
- **Green wash effect**: if subjects receive the wrong signal (green instead of brown) several times in a row, they revert their choices from G to B

Consumers’ behaviours towards green bonds - A field experiment

Policy implications

- Provide low quality information confuses people rather than help them in making a decision, whereas high quality information increases investment in the green option
- Disclose high quality information helps to reduce the uncertainty produce by the ***greenwashing*** practice
- In order to drag investments in green bond, a unified certification scheme provided by a **public and trustworthy** institution (e.g. national government) is needed.

Thank you!

Pretreatments – the Holy Grail of Biorefineries

Mikhail Iakovlev

November 10th, 2021

Outline

- Pretreatments: Industrial State of the Art and the Shortcomings
- SO_2-Ethanol-Water (SEW) Pretreatment = Fractionation

American Process (API)

1995
API LLC, USA
API Europe, Greece
Process Integration Studies

2005
Biorefinery Technologies
R&D Center

2011
GP+ ® Technology
Alpena Biorefinery

2013
SEW (AVAP®) Technology
Thomaston Demonstration
Biorefinery

2019
New generation SEW
technologies

1995
Application of Pinch Technology, Process Integration and Energy Efficiency.

IT Products and Services including apiMAX™ – proprietary simulation program modelling biomass-based process industries.

2005
API built a R&D center in Thomaston, GA and developed technologies for the commercial production of sugars, biofuels and nanocellulose from non-food based biomass.

2011
Alpena Demonstration Biorefinery:
- Alpena, MI
- GP+® pretreatment technology
- 2,700 t/y.

The production of advanced ethanol from hardwood C5 and C6 sugars was demonstrated first time ever. The product was sold to USA fuels market.

2013
Thomaston Demonstration Biorefinery:
- Thomaston, GA
- SEW (AVAP®) pretreatment technology
- up to 1 t/d lignocellulosic sugars and 0.5 t/d of nanocellulose

2019
New generation SEW technologies

SEW = SO_2-ethanol-water

Economic considerations for a viable biorefinery (roughly in the order of importance)

- Effective pretreatment:
  - High yield of products – sugars and lignin
  - High availability – being able to run without interruptions
- Price of feedstock
- Price and amount of enzymes
- Simple pretreatment, good chemicals recovery, low energy/water usage
- Feedstock quality / Versatile pretreatment - ability to diversify to ever increasing value products
- Production capacity

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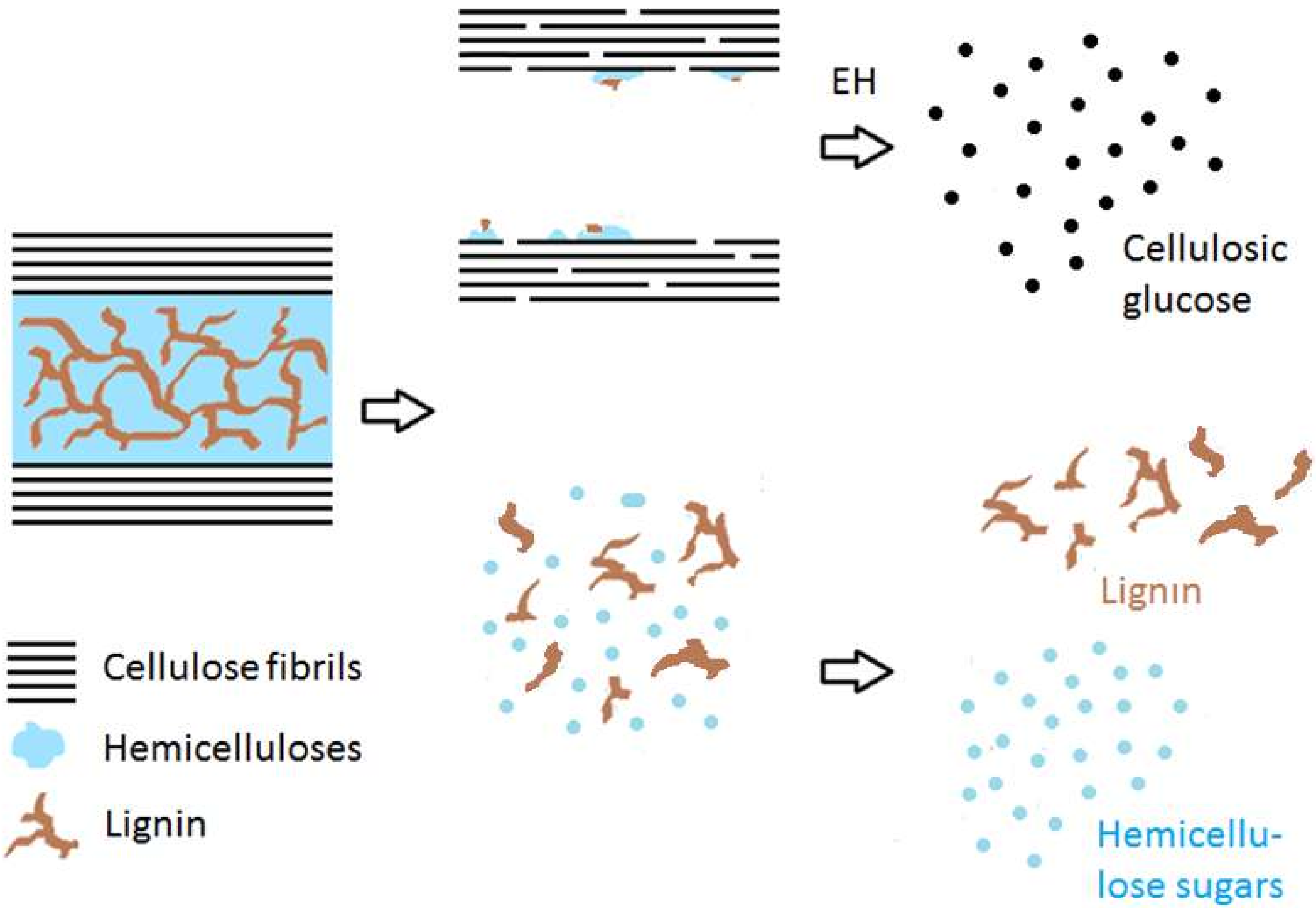
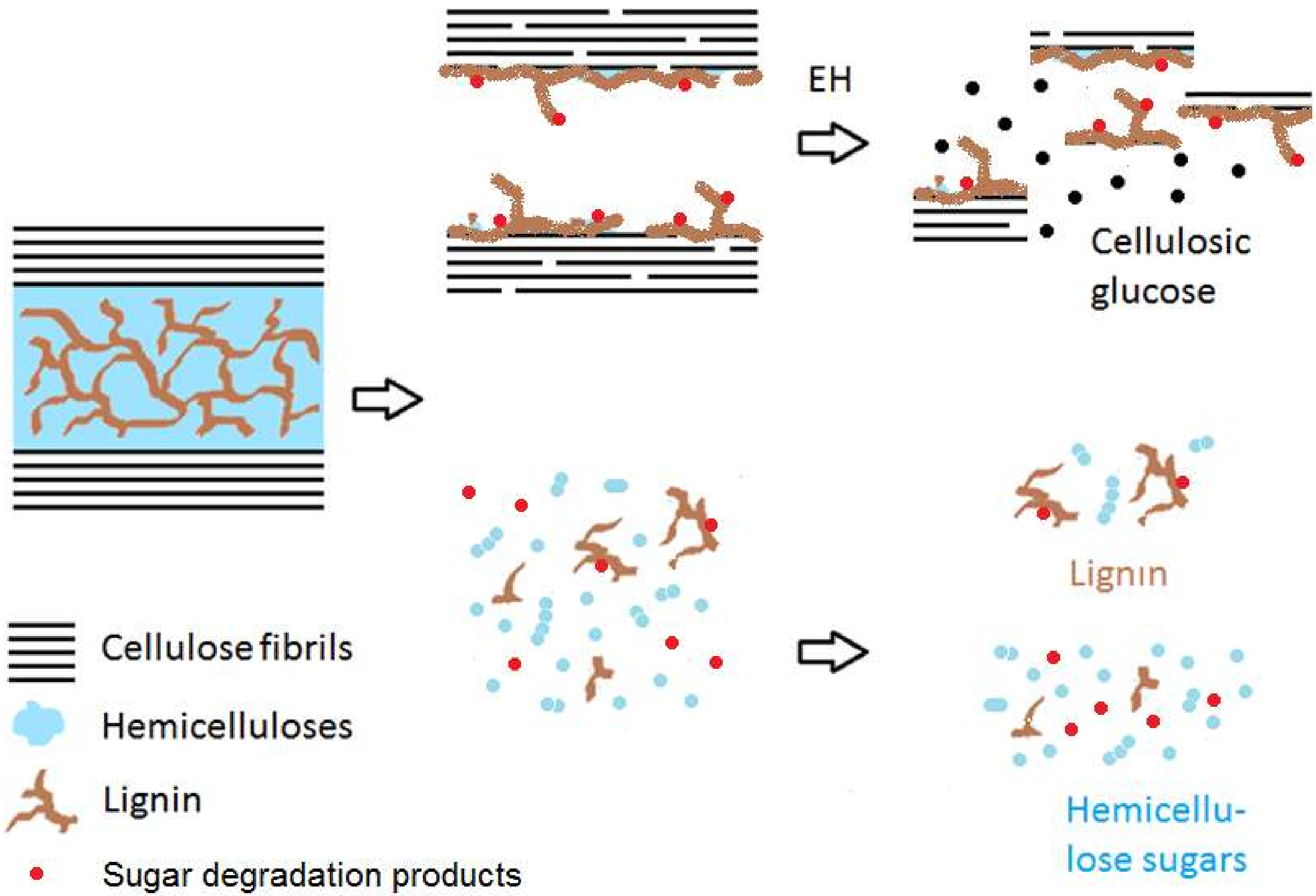
Ideal Pretreatment = Fractionation



EH – enzyme hydrolysis

Classical Mild Acid/Hot Water Pretreatment



EH – enzyme hydrolysis

Mild Acid/Hot Water Pretreatment

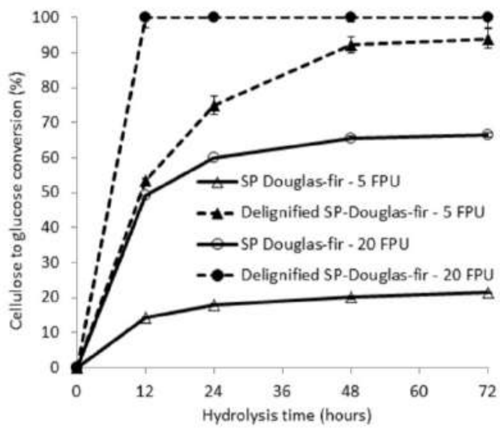


Figure 12 Enzymatic hydrolysis of steam pretreated Douglas-fir and the corresponding delignified substrates at both high and low enzyme loadings. Steam pretreatment conditions were 200°C, 4% SO_2 for 5 minutes. (Log Ro=3.64). The complete delignification was done at room temperature using acidified sodium chlorite solution. Error bars represent deviations from the mean. (n=2 for enzymatic hydrolysis with subsequent HPLC analysis of the sugars in two replicates for each sample).

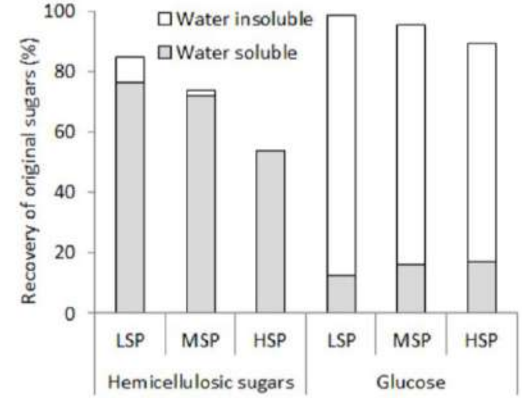


Figure 22 Influence of steam pretreatment severity on the recovery of sugars in the water soluble and insoluble fractions (% of original sugars present in the raw material). LSP, MSP and HSP refer to low, medium and high severity steam pretreatment respectively. Hemicellulose refers to the sum of arabinan, galactan, xylan and mannan.

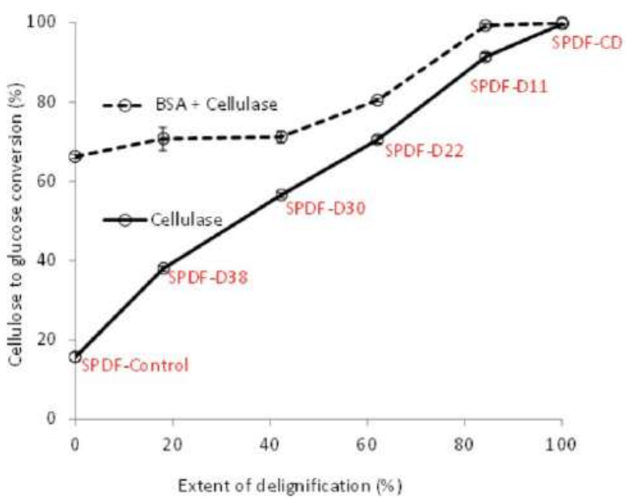


Figure 16 Influence of substrate delignification on 72 h enzymatic hydrolysis of steam pretreated Douglas-fir (SPDF) when low enzyme loadings (5 FPU/g glucan) are used with and without pre-incubation of BSA. Error bars represent deviations from the mean (n=2 for

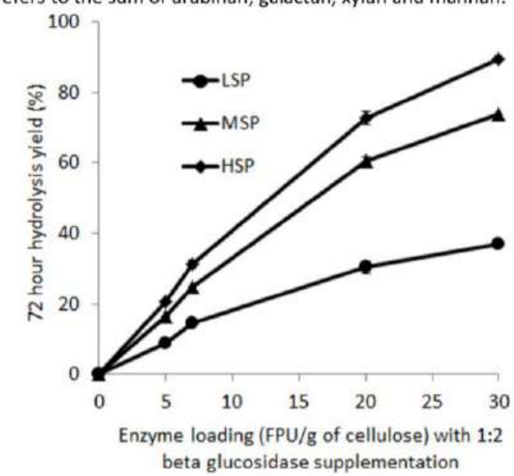


Figure 23 Influence of enzyme loadings on the 72 hour hydrolysis yields of the water insoluble cellulosic component of Douglas-fir after steam pretreatment at different severities. LSP, MSP and HSP refer to low, medium and high severity steam pretreatment respectively. Error bars represent standard deviations from the mean (n=2 for enzymatic hydrolysis followed by a single HPLC analysis for each sample).

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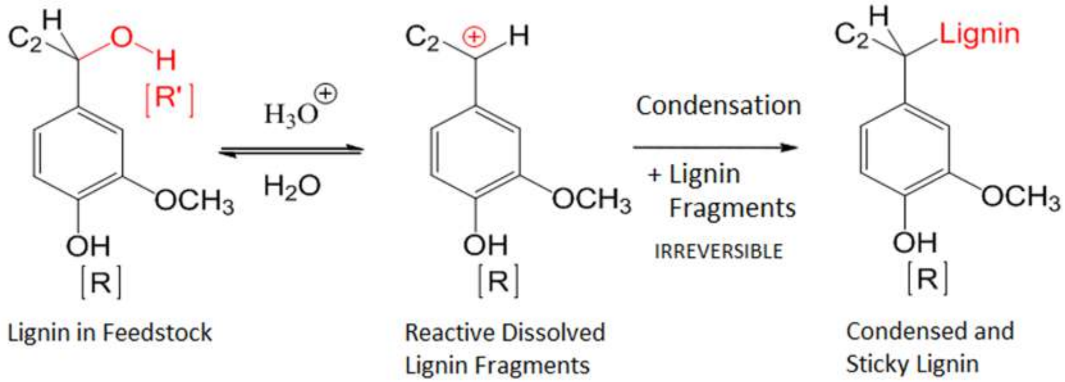
Kunchikannan, PhD Thesis, 2013

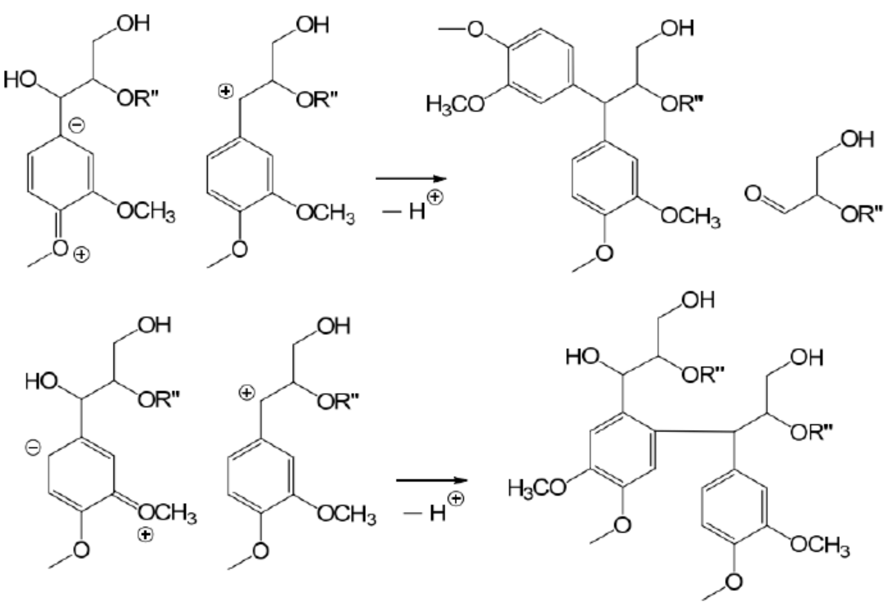
Lignin Secondary Condensation and Sticky Precipitates

Lignin condenses with itself and with sugar degradation products (furanics, humins):



Sticky lignin precipitates:

- Often not evident in lab experiments
- Deposit and plugs equipment in continuous operations
- Deposit on cellulose decreasing enzyme digestibility



Condensation of lignin in acid: Formation of α-1 and α-6 bonds (adopted from Sixta et al. 2006)

Stanciu and Ciurea, 2008
Leschinsky, 2008, 2009
Gutsch and Sixta, 2011
Gutsch, 2012
van Heiningen et al., 2017, 2018

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Industrial State of the Art Pretreatments

Species	Pre-treatment	Total Monosugars Yield kg/dry tonne feedstock	Technology, Company	Location
Scots Pine	Mild H_2SO_4	?	Cellunolix®, St1	Kajaani, Finland
Spruce	Acid sulfite	(only hemicellulose sugars)	Bali™, Borregaard	Sarpsborg, Norway
Sugarcane straw	Hot water	456 [1]	Bioflex®, GranBio	São José Alagoas, Brazil
Agricultural waste	Hot water	507 [2]	Versalis (orig. Beta Renewables)	Crescentino, Italy
Bagasse	Mild H_2SO_4	363 [3]	Raizen	Piracicaba SP, Brazil
Corn stover	Mild H_2SO_4	456 [4]	Liberty, POET-DSM	Emmetsburg, Iowa
Wheat straw	Steam	440-550 [5]	Sunliquid®, Clariant	Romania
Softwood	SO_2-Ethanol-Water (SEW)	640-680	To be announced	

[1] Based on public technology disclosures of expected 70 USG/t of biomass

[2] Based on published target of 4.3 t biomass / t ethanol http://task39.sites.olt.ubc.ca/files/2013/05/IEA-2012_Chemtex-Italia_PROESA_Vienna-2.pdf

[3] Based on 211 L/BDT to 289 L/BDt target, https://economia.estadao.com.br/noticias/geral,raizen-avanca-no-etanol-de-2-geracao,10000089302

[4] Based on public technology disclosures of expected 70 USG/t of biomass

[5] Based on the ethanol yield of 1 tonne per 4-5 tonne biomass, https://www.clariant.com/en/Business-Units/New-Businesses/Biotech-and-Biobased-Chemicals/Sunliquid

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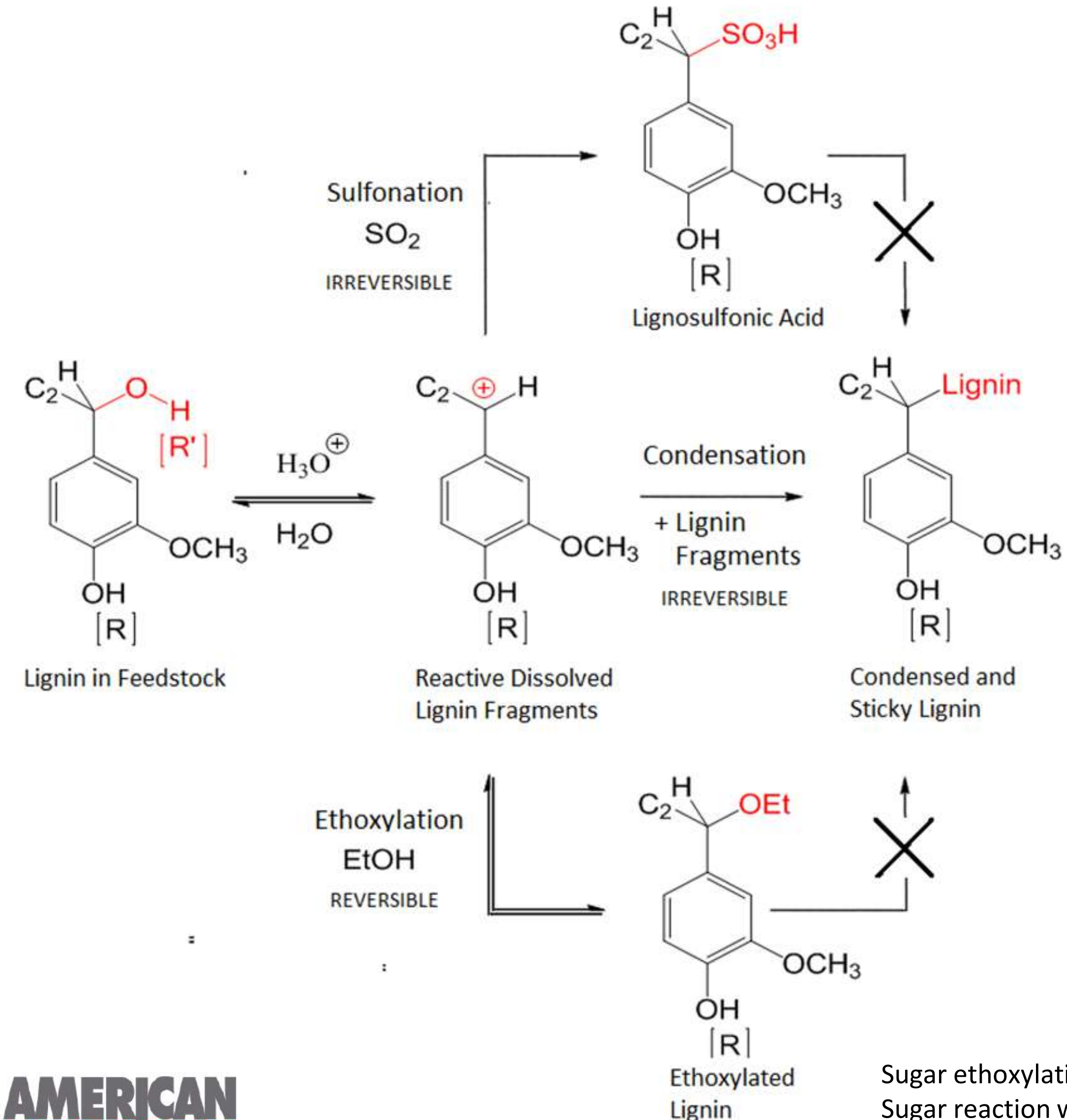
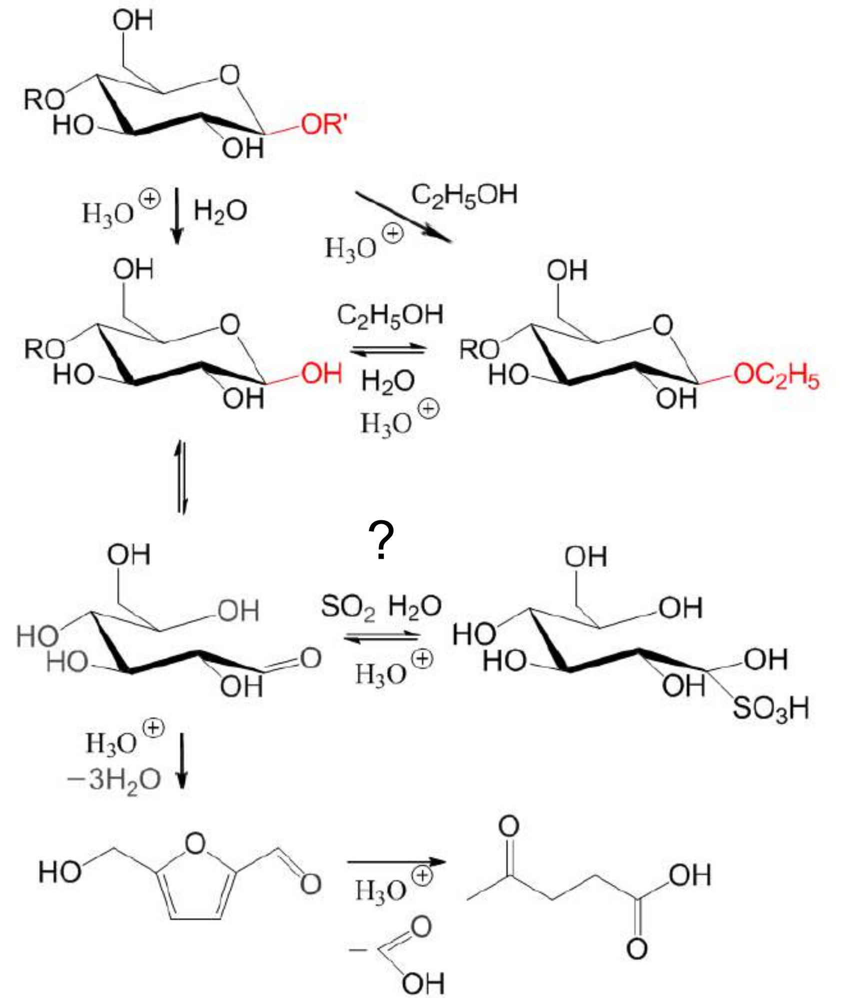
Lignin and Sugar Protection in SEW Process





Sugar ethoxylation: Sharazi and van Heiningen, 2017; Sharazi et al., 2017, Bouxin et al., 2014
Sugar reaction with SO_2/HSO_3^-: Shi et al., 2012
Lignin ethoxylation: Sharazi et al., 2018; Balakshin, Capanema, 2015
Lignin sulfonation: general pulping knowledge

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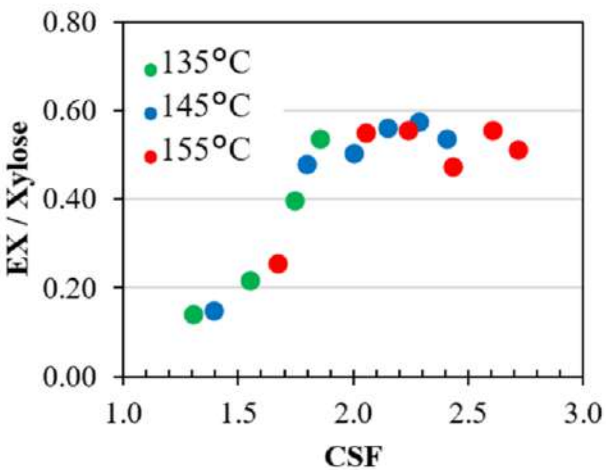
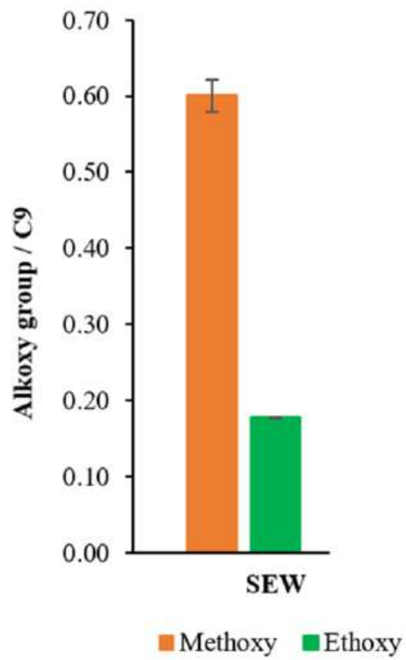
SEW = SO_2-ethanol-water

Reversible Ethoxylation of Sugars and Lignin in SEW Fractionation of Sugarcane Straw

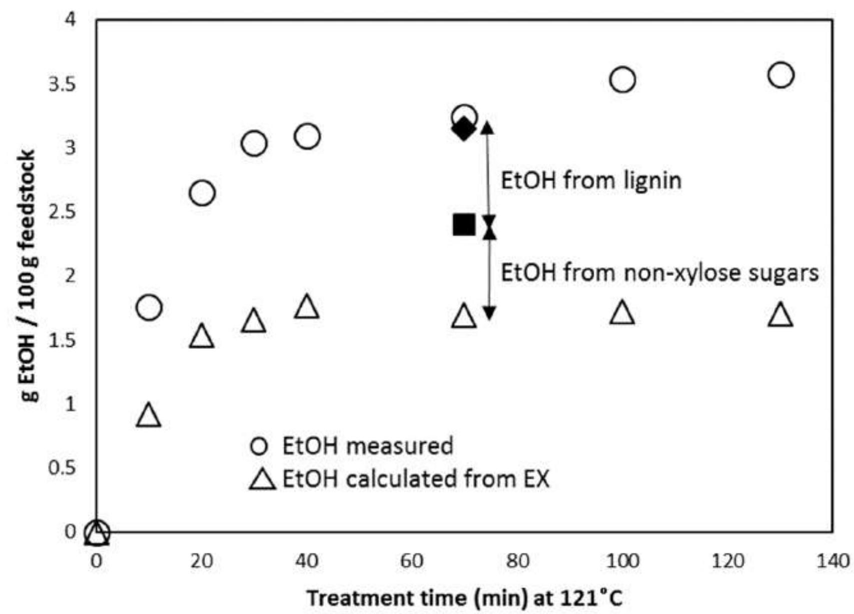
Ethoxylated xylose/xylose ratio vs. pretreatment severity



Lignin alkoxyl content



De-ethoxylation in the subsequent heat treatment (in the absence of ethanol):



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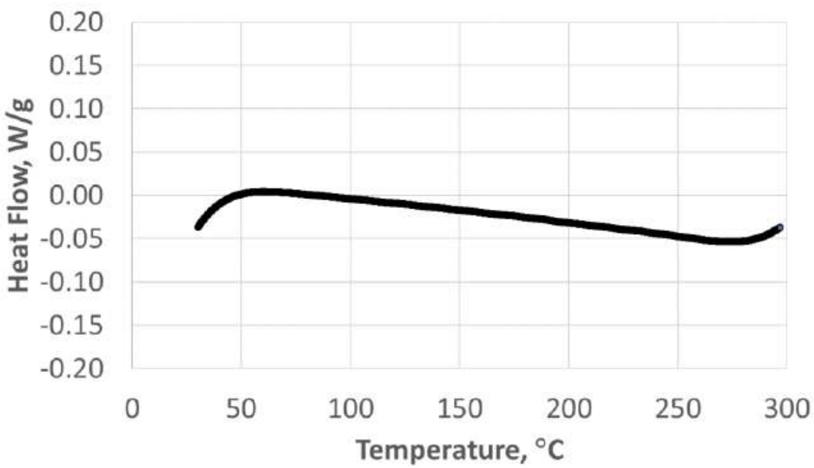
Pretreatment conditions: SO_2:EtOH:Water = 12:44.5:44.5 (w/w)

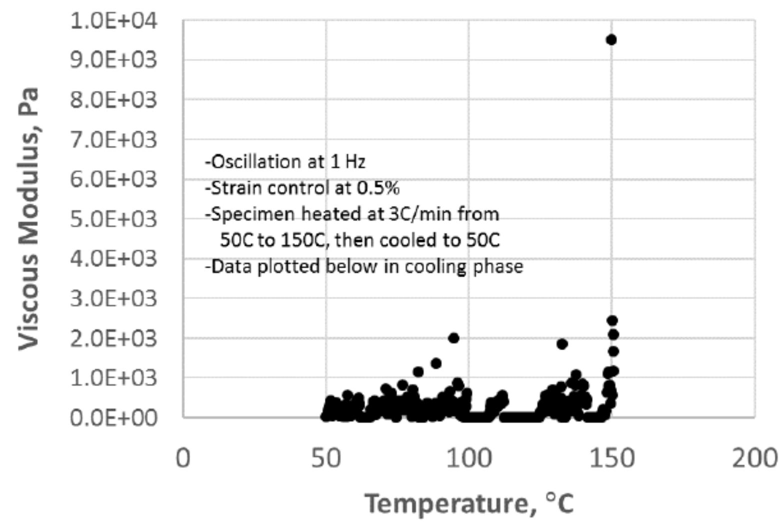
Van Heiningen et al. 2018 (EWLP, Aveiro, Portugal)

SEW Lignin Properties



Differential Scanning Calorimeter Curves (heat/cool/heat method)

No evidence of melting up to 300°C.



Viscous(loss) modulus vs. Temperature

No change in viscous modulus with temperature. When the sample was taken out after the test, it was still in the same dry powder form, showing that this lignin does not melt.

Thus, SEW Lignin is not sticky and does not become sticky when heated up to 150°C.

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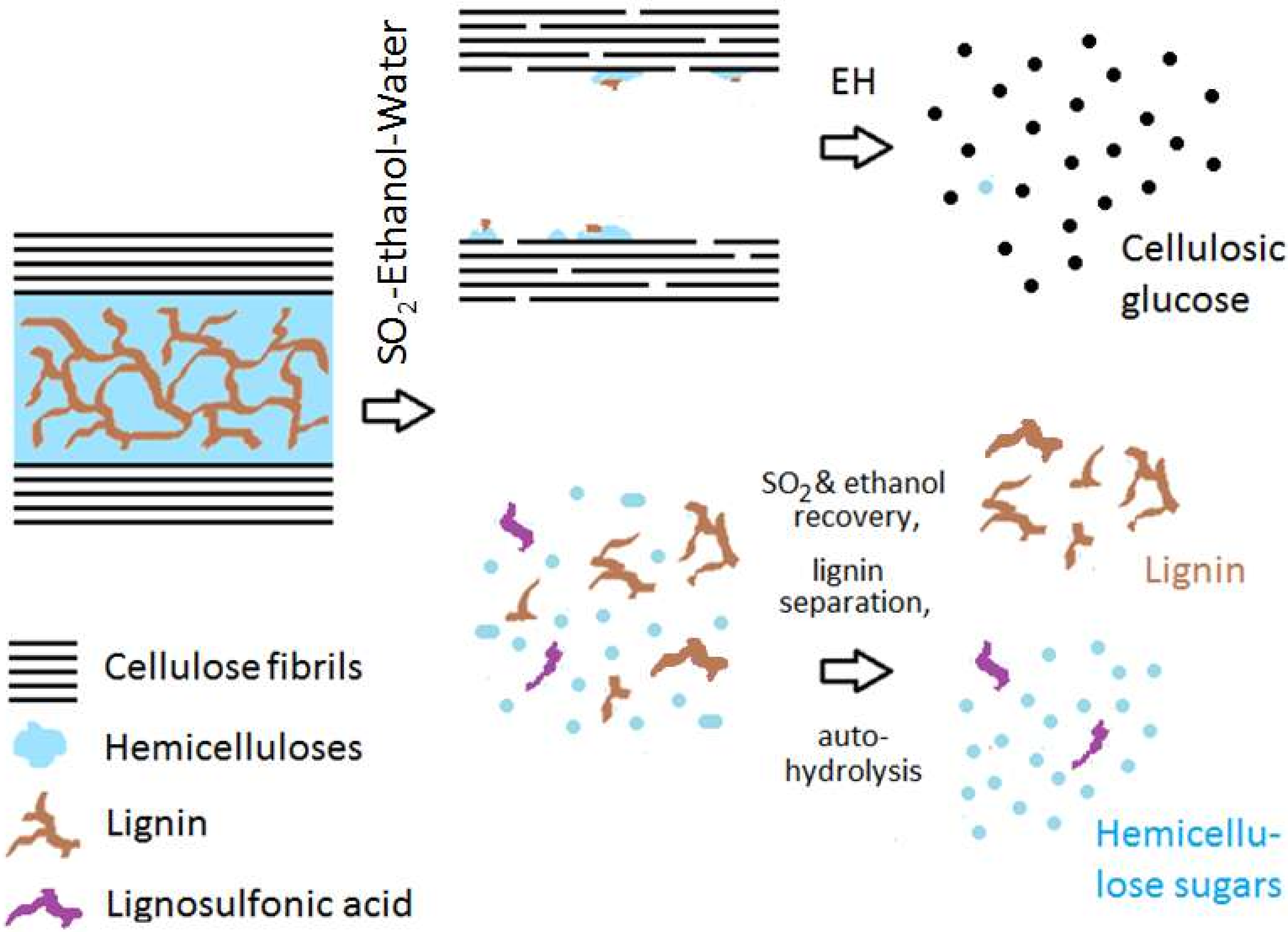
US patent 11,118,017 B2 (2021)

SO_2-Ethanol-Water Process = Biomass Fractionation

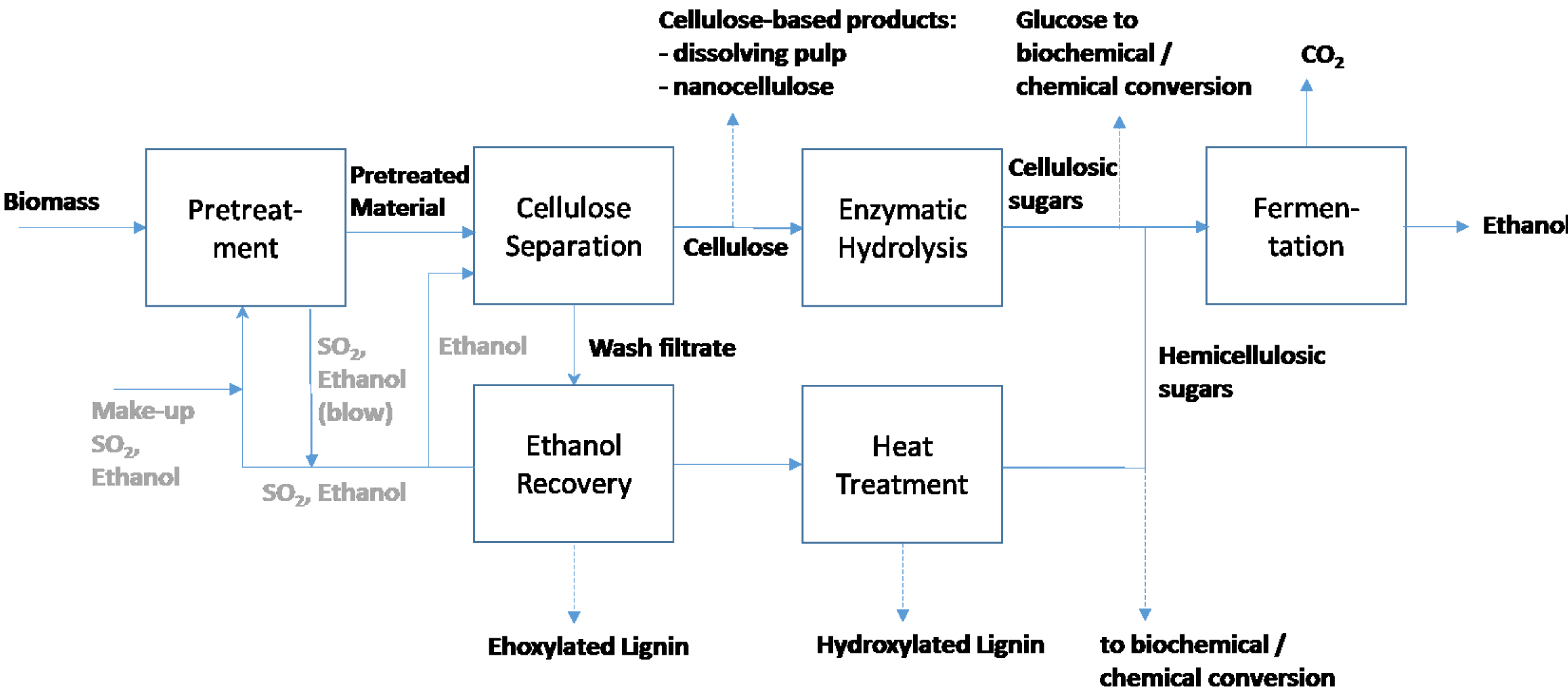


Cellulose

Lignin

EH – enzyme hydrolysis

SO_2-Ethanol-Water Process



- Over 90-95% carbohydrates are recovered as monomeric sugars.
  - Sugar degradation <1% on dry biomass
  - >90-95% cellulose-to-glucose with <4-9 FPU per g cellulose.
- Recent development: Over 65-70% of lignin is recovered in the non-condensed form (ethoxylated or non-ethoxylated).

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US patent 11,118,017 B2 (2021)

SO_2-Ethanol-Water (SEW) Process: Versatility in Raw Materials

Succesfully Tested SEW Feedstocks:

Angiosperms

- Trees (hardwoods):
  - Aspen
  - Beech
  - Birch
  - Black ash
  - Eucalyptus
  - Maple
  - Poplar
  - Mixed hardwood biomass
- Herbs (agricultural residues):
  - Corn (stover and fiber)
  - Oil palm (empty fruit bunch)
  - Sugarcane and energy cane (straw and bagasse)
  - Tobacco (stalks)
  - Wheat (straw)

Gymnosperms

- Trees (softwoods):
  - Spruce
  - Larch
  - Red pine
  - Jack pine
  - Loblolly pine
  - Balsam fir
  - Douglas fir
  - Mixed softwood biomass
  - Recycle wood

Mixed angiosperms and gymnosperms

The process is not sensitive to the particle size (from sawdust to relatively large biomass pieces) - due to penetration ability of ethanol.

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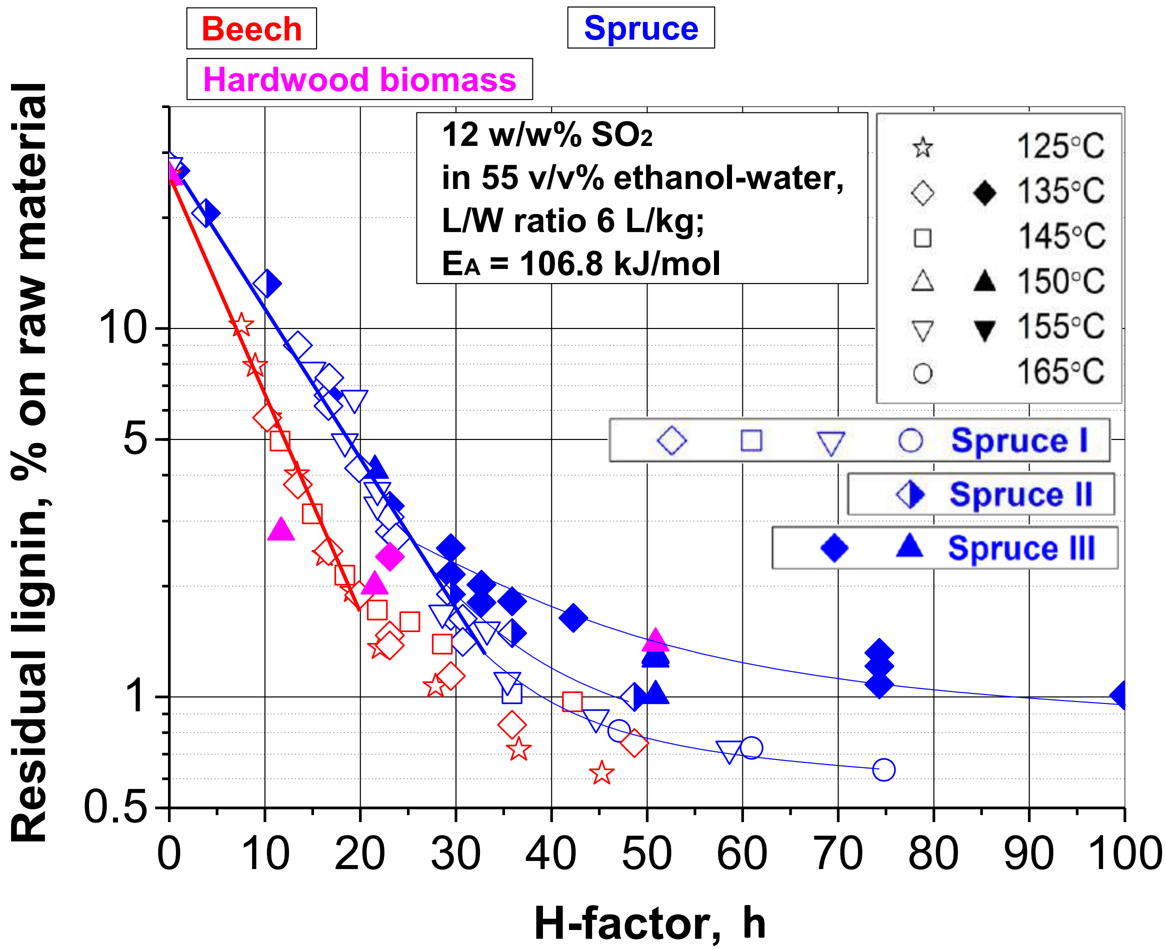
SEW Delignification of Different Lignocellulosics



Softwoods, hardwoods and annual plants are successfully delignified by SEW process.

Iakovlev et al. 2011, 2012, 2014
Yamamoto et al. 2014

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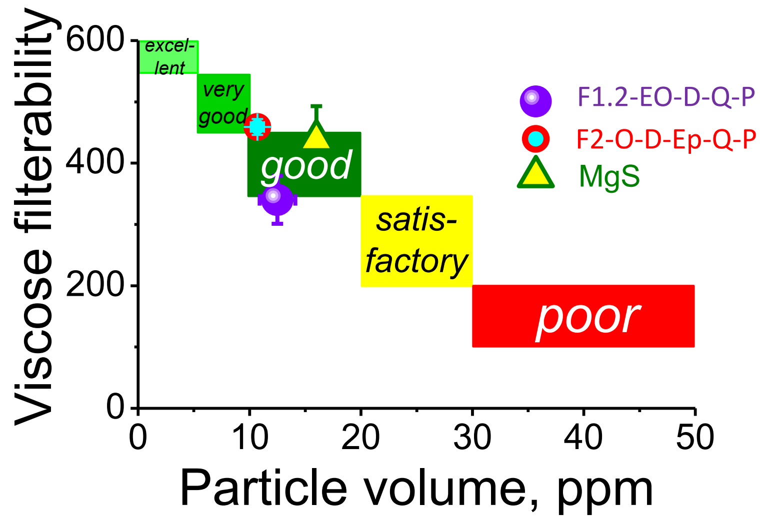
SO_2-Ethanol-Water (SEW) Process: Versatility in Products

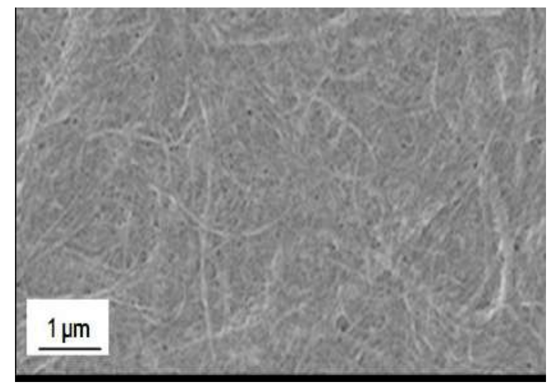
Typical SEW Glucose Syrup Composition (Pilot Runs):

Component	g/L
Monomeric glucose	650
Other monosaccharides	20-40
Oligosaccharides	10-20
Lignin	7
Acetic acid	0-2
Formic, levulinic acids	Non-detectable
Furfural, HMF	Non-detectable

Very high-purity glucose stream can be used as a direct replacement for corn sugar for conversion to biochemicals.



The quality of viscose produced from SEW cellulose is good to very good.



SEW Cellulose Nanofibrils

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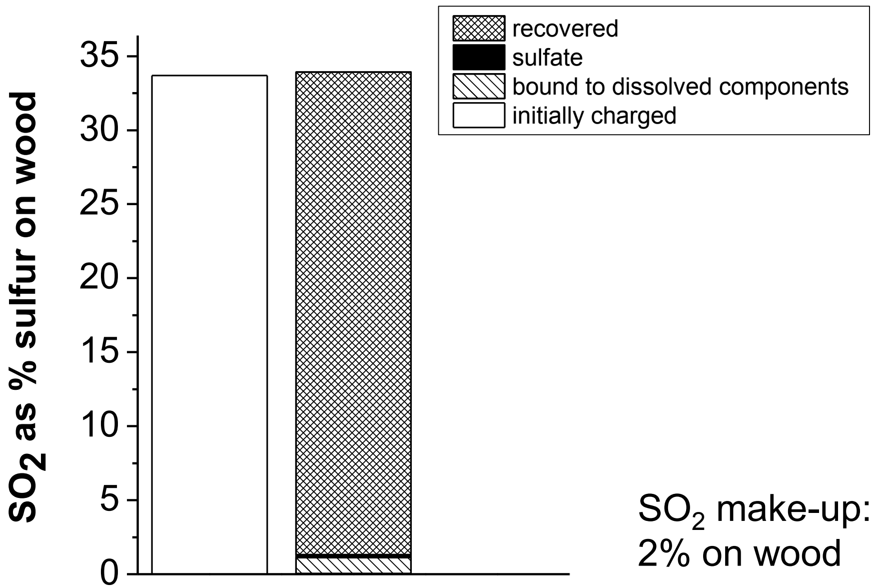
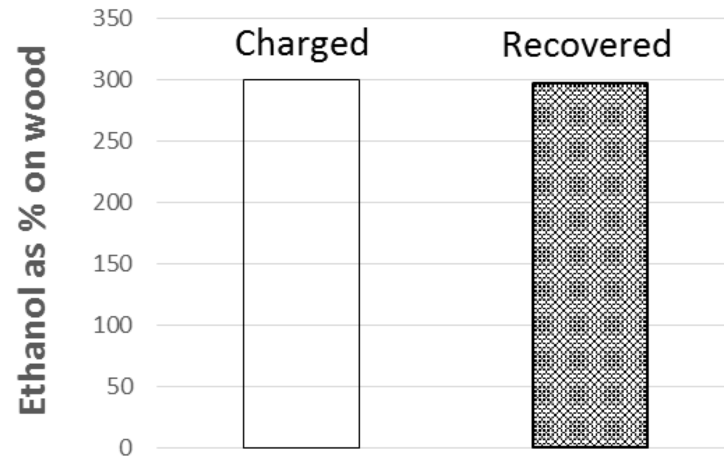
Iakovlev et al., 2014
Nelson et al., 2016

SO_2 and Ethanol Recovery (Softwood)



SO_2 make-up:
2% on wood



99.2% of total ethanol recovered by distillation from spent fractionation system.

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Iakovlev and van Heiningen 2012
Primakov 1961

Conclusions

- Pretreatment is the key element of lignocellulosic Biorefinery determining its ultimate economical performance.
  - High sugar and lignin yields
  - Lignin in usable form
  - High availability – no plugging
  - High enzymatic digestibility
  - Versatility in raw materials and products
  - Efficient chemical recovery
- Conventional hot water/acid and other pretreatments struggle to satisfy these requirements.
- SO_2-Ethanol-Water (SEW) process makes a viable Biorefinery possible
  - the Holy Grail?