

Biotechnology of Polysaccharides for Biofuels and Bio-based Products

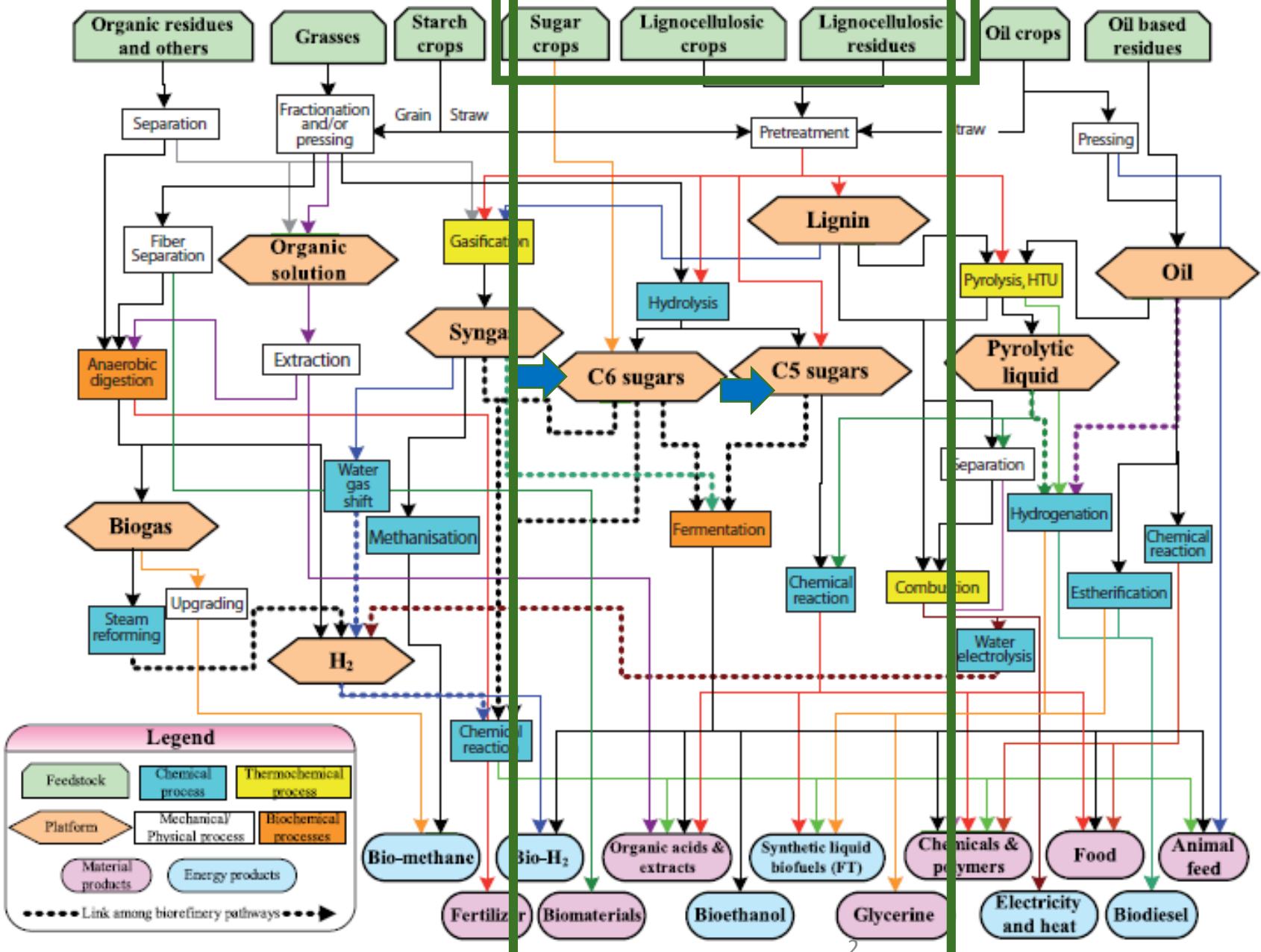
Francisco Gírio

Head of Bioenergy Unit

LNEG - Laboratório Nacional de Energia e Geologia

Lisboa, Portugal

Several Platforms and Multi-Products



Source: IEA Bioenergy: Task 42- Biorefineries

LC BIOMASS & OTHERS

Forest residues



Eucalyptus

Agriculture residues



Rice straw, corn stover

Bio-wastes



Grasses, MSW

Agro-industrial



Food wastes, Sewage

Perennial non-food crops



Miscanthus, etc

By-products of biodiesel



Glycerol, ...

Fisheries and aquaculture residues



Seaweed, fish bones...

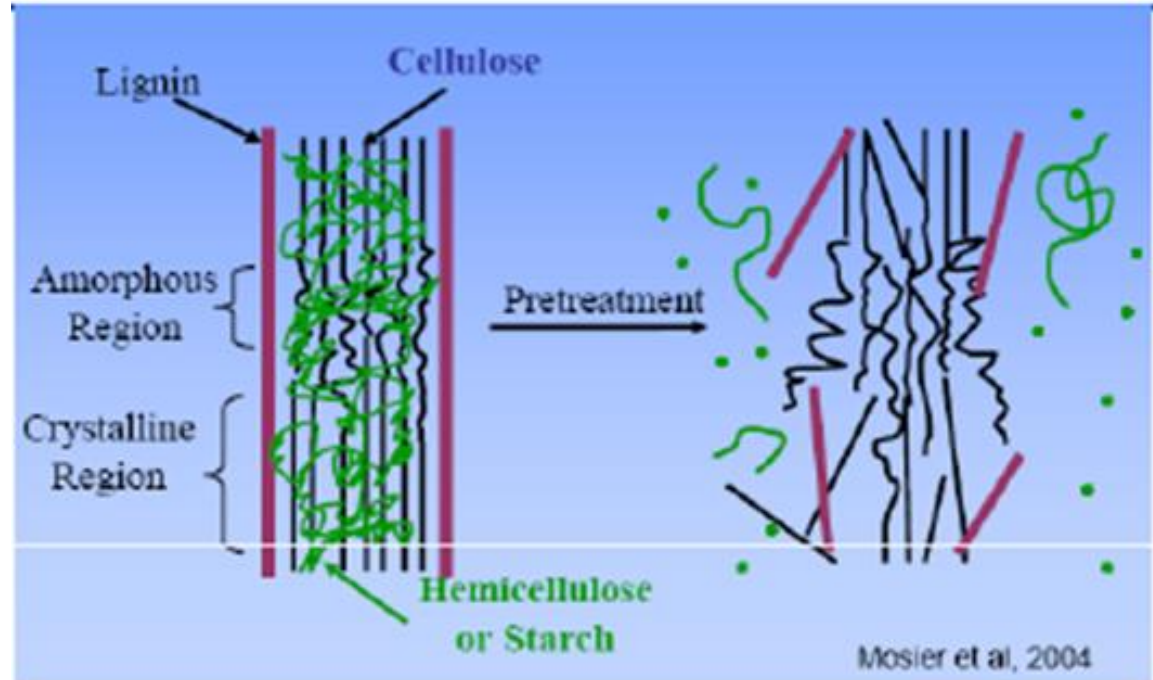
Algae & CO₂



CO₂ uptake and algae

LC Biomass (heterogeneous) Chemical Structure

**Feedstock
Challenge:**
Lignocellulose
biomass
recalcitrance and
heterogeneity is an
issue!



Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapfle M, Ladisch MR, 2004.

Main composition:

Cellulose, Hemicellulose , Lignin, Proteins, Pectins, Extractives, Ash

Polysaccharides & Lignin Composition

Cellulose (30-50%)

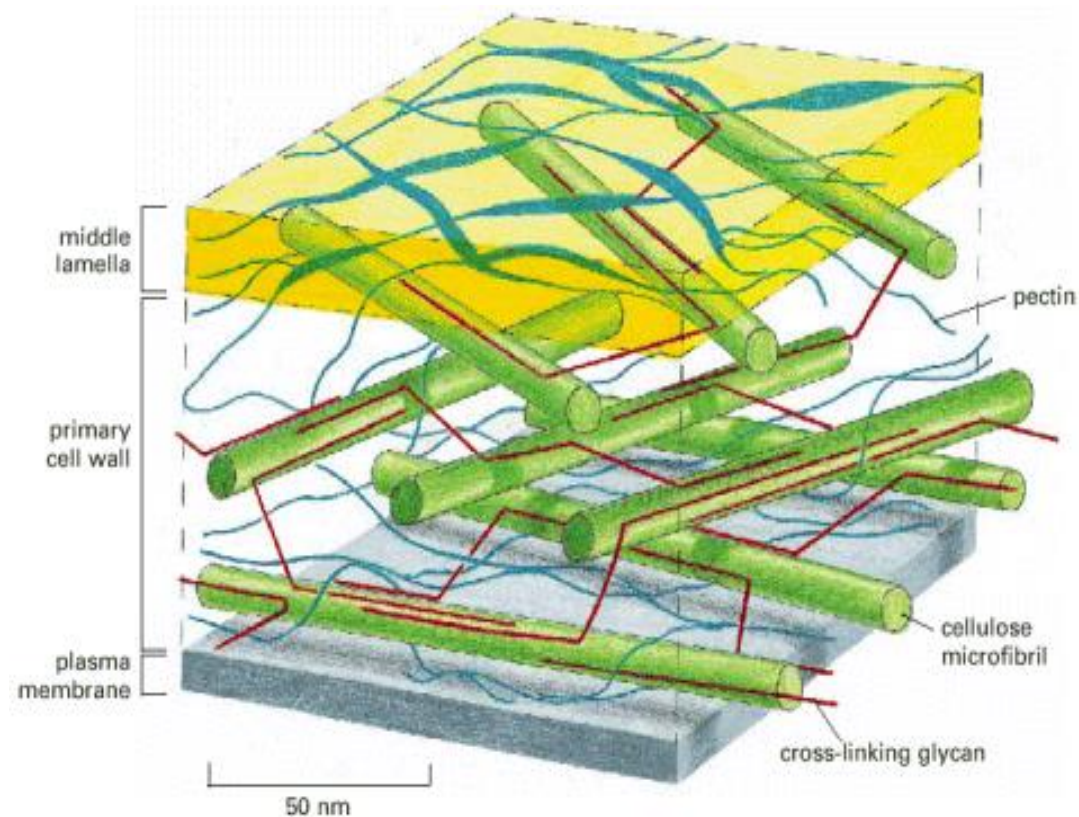
- ❖ crystalline polymer of glucose;
- ❖ difficult to undergo chemical hydrolysis but susceptible to enzymatic attack by cellulases.

Hemicellulose (15-30%)

- ❖ heteropolymer of pentoses and hexoses;
- ❖ variably substituted (acetyl, uronic);
- ❖ more easily depolymerized.

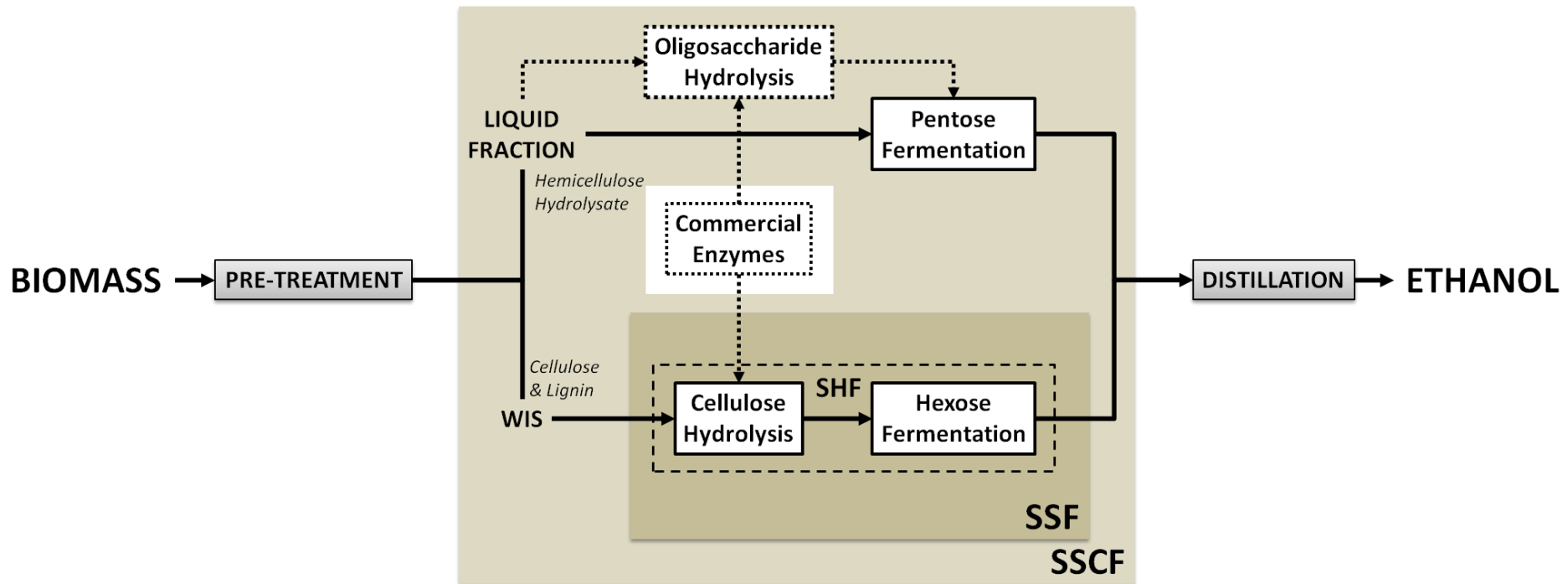
Lignin (10-25%)

- ❖ complex aromatic structure;
- ❖ resistant to biochemical conversion;
- ❖ different depolymerization chemistry.

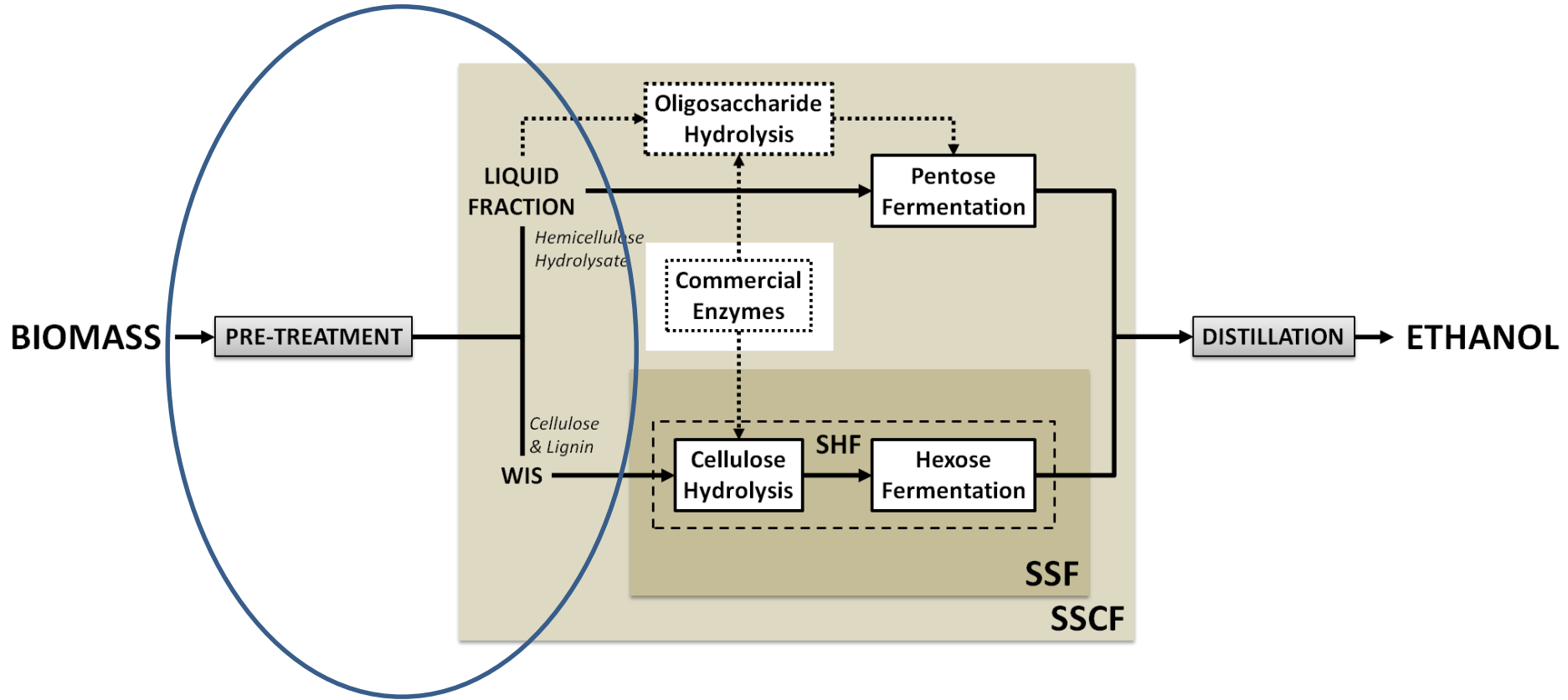


(In: Alberts *et al.*, 2002, *Molecular Biology of the Cell* 4th ed.)

Advanced Biofuel Production from LC Biomass



Advanced Biofuel Production from LC Biomass



Biomass (Deconstruction) Pretreatments

Pretreatments → *Essential to disrupt the complex structure of lignocellulosic biomass*

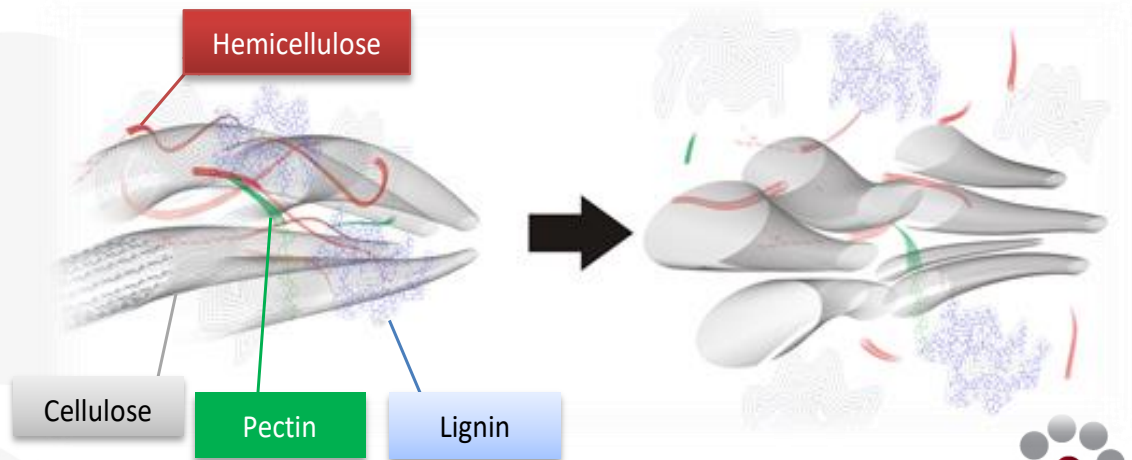
Hemicellulose solubilization
↑ Extraction of lignin
↓ Crystallinity of cellulose

Wide range of conventional LC Biomass

Pretreatment Technologies, e.g.:

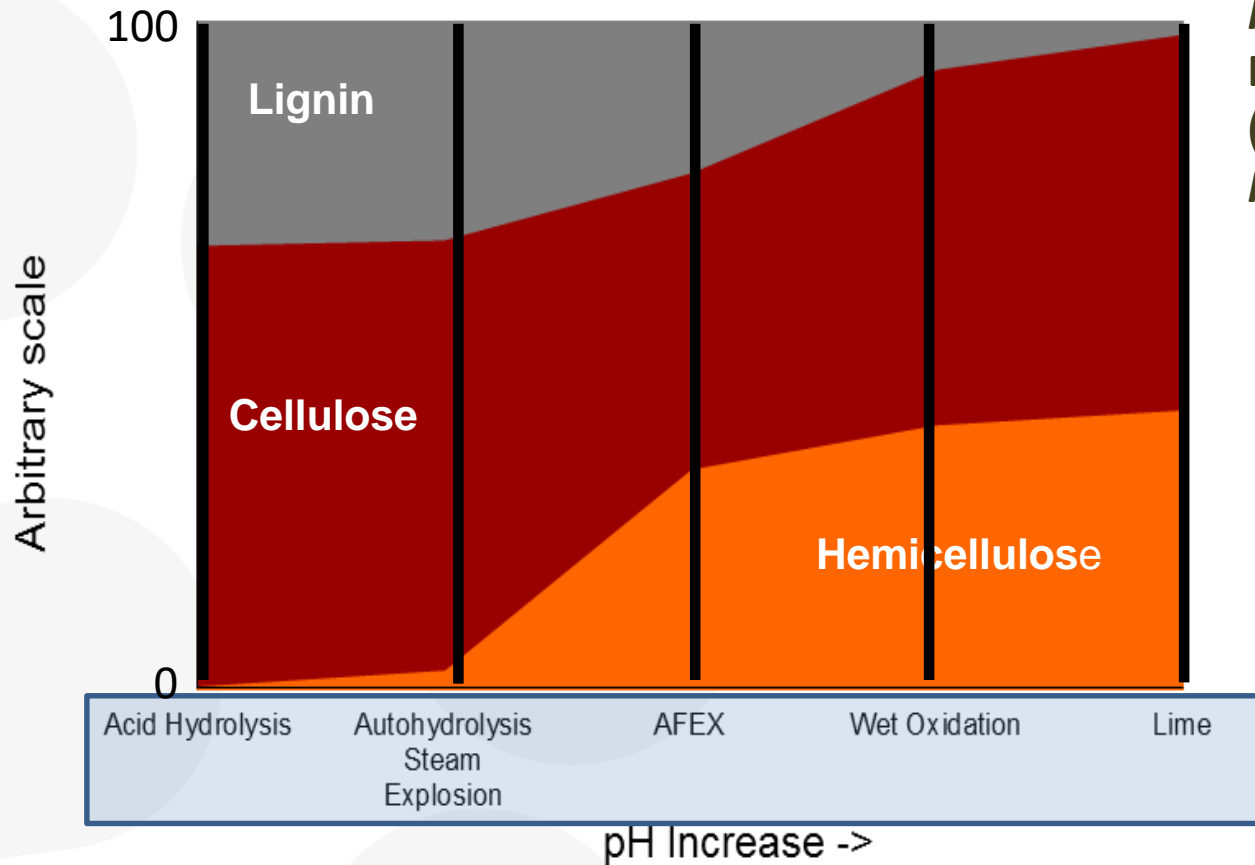
- Natural pulping
- Autohydrolysis (Hydrothermic) (LHW/Steam Explosion)
- Dilute acid hydrolysis
- Organosolv (acetone, ethanol)
- Alkaline (AFEX, etc)

↓
↑ Surface area for enzyme binding and attack



Biomass deconstruction pretreatments

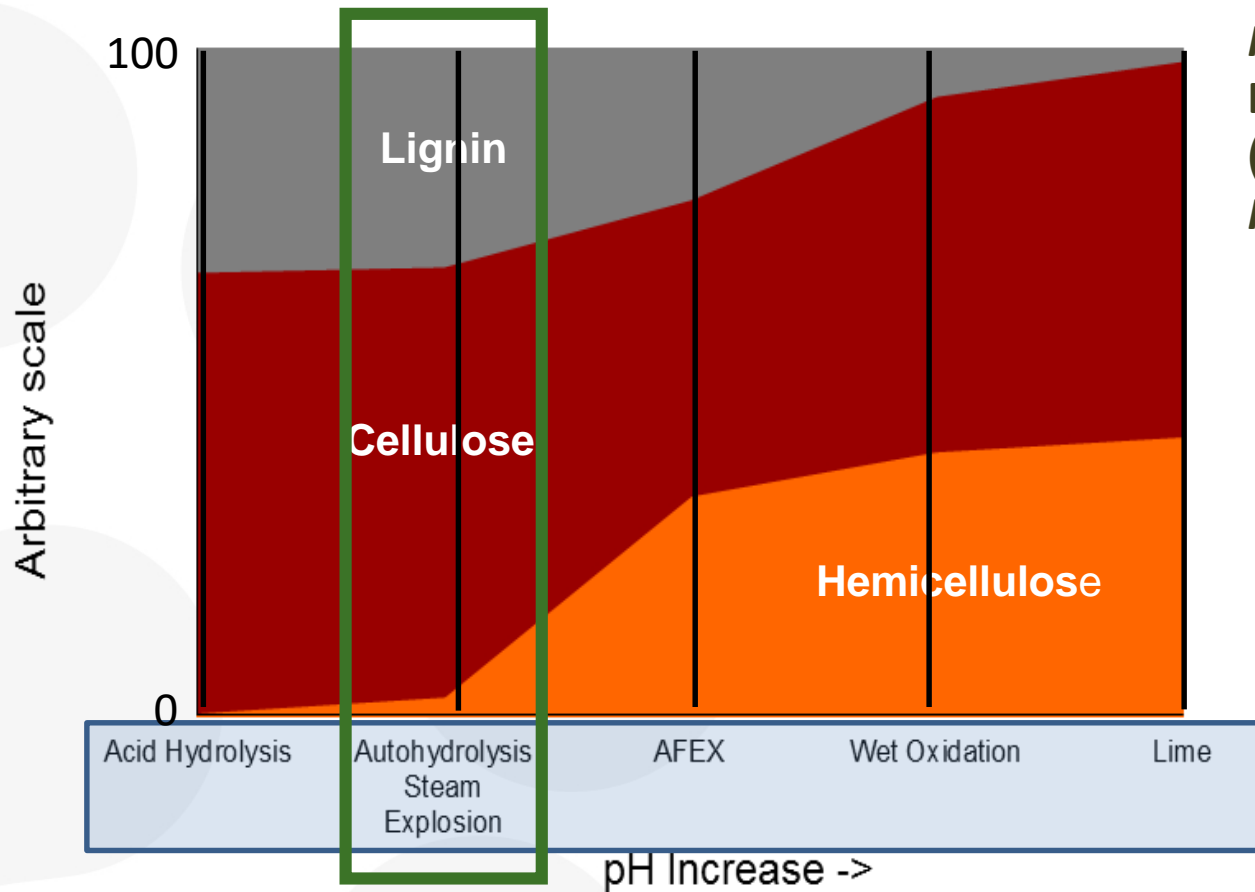
Pretreated Biomass Composition



In: Carvalho, F., Duarte, L.C., Gírio, F. M. (2008). J. Scientific & Ind. Res., 67, 849-864

Biomass deconstruction pretreatments

Pretreated Biomass Composition



In: Carvalho, F., Duarte, L.C., Gírio, F. M. (2008). J. Scientific & Ind. Res., 67, 849-864

Hydrothermal pretreatments

Autohydrolysis or liquid hot water (LHW)

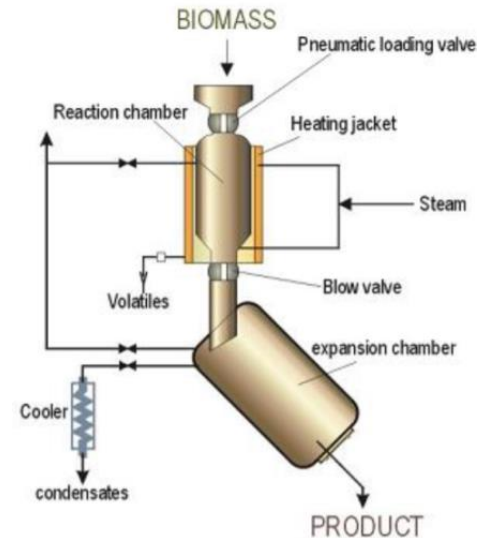
- Biomass mixed with water heated to a defined temperature under pressure (150-230°C, minutes-hours) – liquid hot water.



Biomass Deconstruction LHW Facilities, Unit of Bioenergy at LNEG - Lisbon, Portugal

Steam explosion (uncatalyzed)

- Saturated steam (< 240°C, seconds-minutes)
- Biomass is wetted by steam at high pressure and then exploded when pressure within the reactor is rapidly released
- Disaggregation of lignocellulosic matrix, breaking down inter- and intra-molecular linkages (forces resulting from decompression), ultrastructure modification



¹ Adapted from: "Lignocellulosic ethanol" (2013), D. Chiamonti, A. Giovannini, R. Janssen, R. Mergner, WIP Renewable Energies

BetaRenewables (Proesa®)

Local: Crescentino (Italy)

270,000 ton/yr straw; 60 000 ton/year of ethanol , 13MWe generated from lignin. Production process: **uncatalysed steam explosion** (Proesa®), EH + Co-Fermentation C5+C6 sugars; since Oct 2013.



1st Commercial

Clariant (SunLiquid®)

Local: Straubing - Munique (Germany)

Cereal straw, agricultural waste. 500 tons/year Ethanol + Lignin for CHP.

Production process: **uncatalyzed steam explosion**, enzymatic hydrolysis and co-fermentation of C₅ and C₆ ; since 2012.

Demo



Raízen

Local: Piracicaba - SP, Brazil

sugar cane bagasse and straw; 32,000 ton/year Ethanol + electricity. Co-location with an existing 1G bioethanol plant from sugar cane. Production process: logen's technology – acid-catalysed steam explosion, EH and Fermentation; since 2015.

1st Commercial

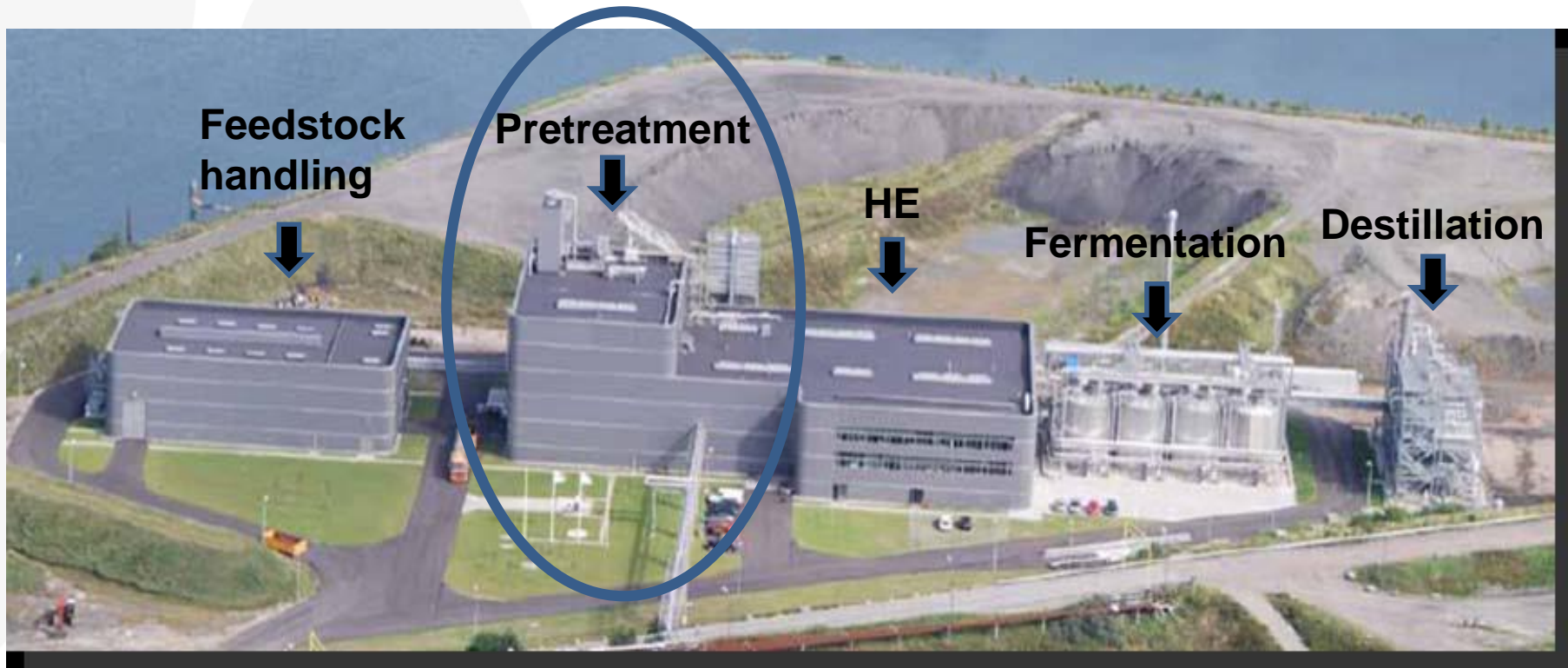


Inbicon (Dong Energy)

Local: Kalundborg (Denmark)

30.000 ton/year wheat straw, 5.000 ton/year Ethanol + Lignin for CHP.

Production process: **Autohydrolysis (LHW)**, enzymatic hydrolysis and co-fermentation of C_5 and C_6 ; since 2009.



Demo

www.inbicon.com

UNEG

Biomass deconstruction pretreatments

State-of-the-art for Conventional Biomass Pretreatments:

- ❑ Insufficient (or no) separation of cellulose and lignin



↓ Effectiveness of enzymatic cellulose hydrolysis

- ❑ Formation of by-products that inhibit fermentation



- Sugars → furans
- Lignin → phenolic compounds

- ❑ Use of chemicals and energy-intensive

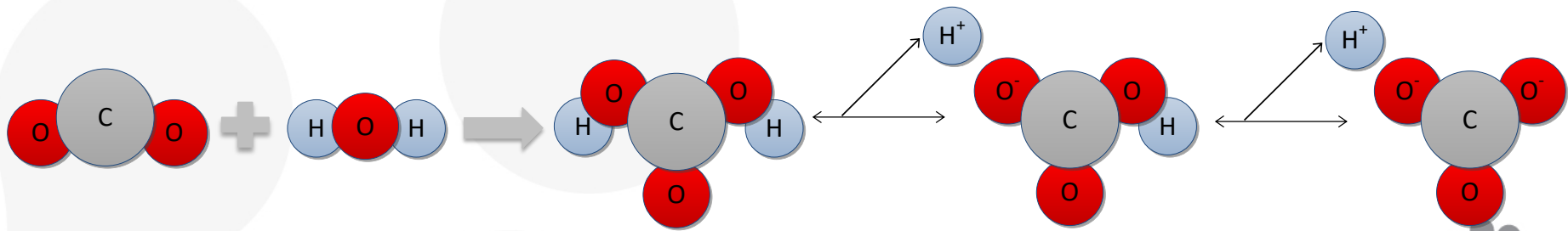
- ❑ In general, **no clean technology (waste streams production)** (e.g. for dilute acid AFEX pretreatments)

- ❑ In general, **high costs of corrosion-resistant equipment** (e.g. for dilute acid pretreatment)

Alternative technology

Sub-/Supercritical Fluids

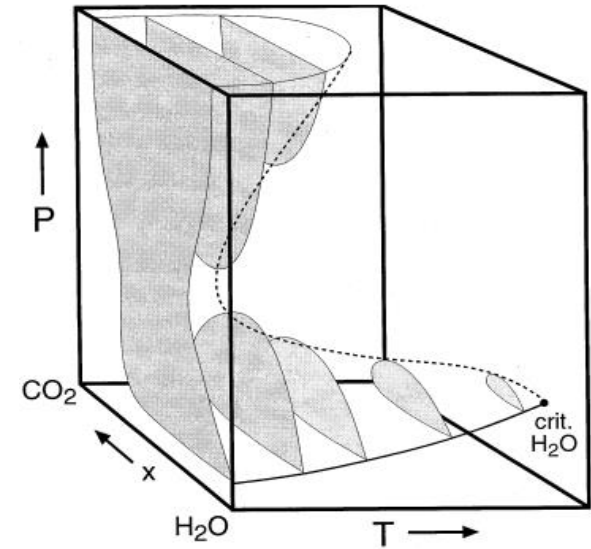
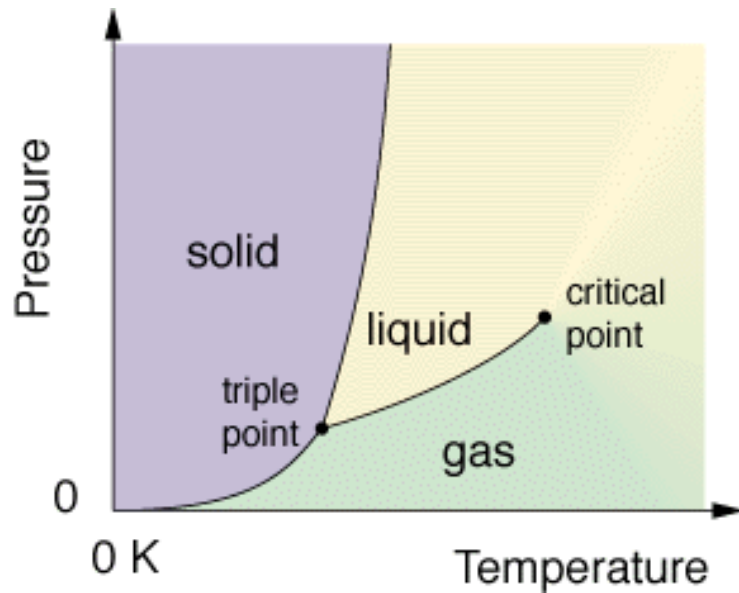
High-pressure $\text{CO}_2\text{-H}_2\text{O}$ biphasic system



Properties of supercritical fluids

Typical supercritical solvents: CO₂, H₂O, propane, butane

- GRAS - generally regarded as safe (scCO₂ and water)
- cheap, non-toxic (scH₂O, scCO₂)
- chemically inert, odourless, tasteless
- non-flammable, non-explosive
- reaction gases (H₂, O₂) totally miscible
- reaction and separation step integrated



Phase diagram of CO₂ + H₂O mixture
(Geochim Cosmochim AC, 2000, 64, 1753-1764)

	Density (g/mL)	viscosity (P)
gas	$\sim 10^{-3}$	$0.5-3.5 \cdot 10^{-4}$
scF	0.2-0.9	$0.2-1.0 \cdot 10^{-3}$
liquid	0.8-1.2	$0.3-2.4 \cdot 10^{-2}$

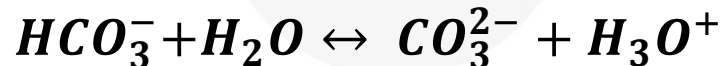
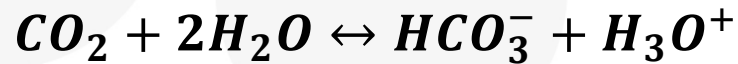
Hydrothermal vs. High Pressure CO₂-H₂O mixture?

1) Hydrothermal (e.g. autohydrolysis)



2) CO₂ + H₂O biphasic system

☐ Mixture becomes more acidic



↑ Hydrolysis of hemicellulose
↑ Enzymatic digestibility of cellulose

Estimated (lower) pH

$$\text{pH} = 8.00 \times 10^{-6} \times T^2 + 0.00209 \times T - 0.216 \times \ln(P_{\text{CO}_2}) + 3.92^*$$

50 bar of CO ₂	20/35 bar of CO ₂	Hydrothermal
3.72	3.78	5.5
pH @ T = 200 °C		



High-pressure CO₂-H₂O biphasic system

Experimental set-up

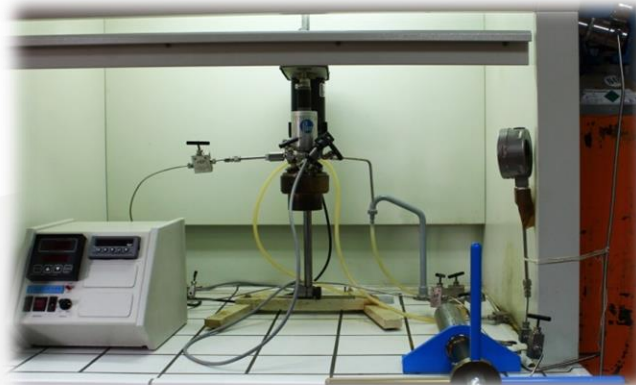


LC Biomass



H₂O

& CO₂



High-pressure reaction system



Solid fraction

Cellulose and Klason lignin



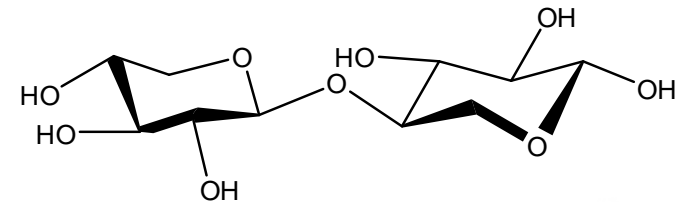
Gas fraction

Volatile furfural



Liquid fraction

C₅-sugars-rich liquor



Xylose oligomers



Studied experimental conditions:

T = 160-220 °C

Initial pCO₂ = 20-50 bar

t = 0 to 90 min

LSR = 10 g/g

High-pressure CO₂-H₂O biphasic system

Experimental set-up



LC Biomass



H₂O

& CO₂



High-pressure reaction system



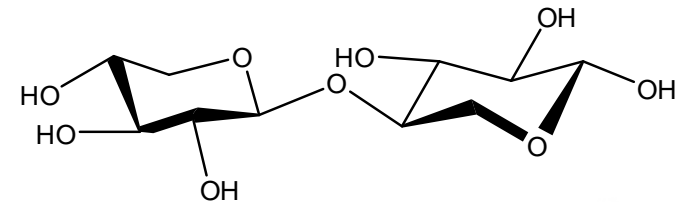
Gas fraction

Volatile furfural



Liquid fraction

C₅-sugars-rich liquor

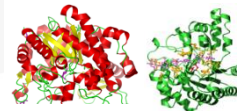


Xylose oligomers



Solid fraction

Cellulose and Klason lignin



Cellulase and β -glucosidase

High saccharification yields

Studied experimental conditions:

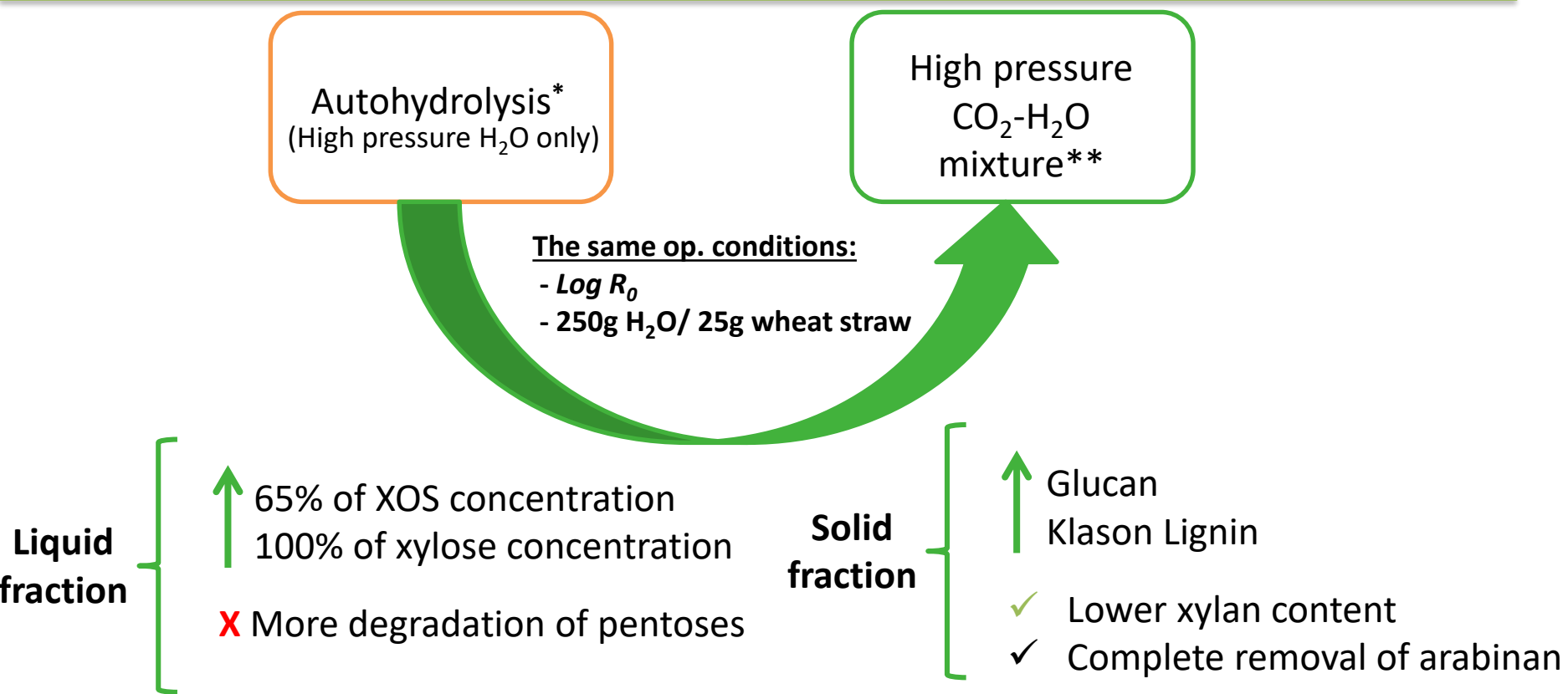
T = 160-220 °C

Initial pCO₂ = 20-50 bar

t = 0 to 90 min

LSR = 10 g/g

Effect of CO₂ addition to autohydrolysis



The *in-situ* formed **carbonic acid** enhances the **hydrolysis** of hemicellulose

*Carvalho et al. Appl. Biochem. Biotechnol., 2009, **153**, 84-93

** Green Chemistry



PAPER

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The CO₂-assisted autohydrolysis of wheat straw

Cite this: *Green Chem.*, 2014, **16**, 238

Sara P. Magalhães da Silva,^{a,b} Ana Rita C. Morais^a and Rafat Bogel-Lukasik^a

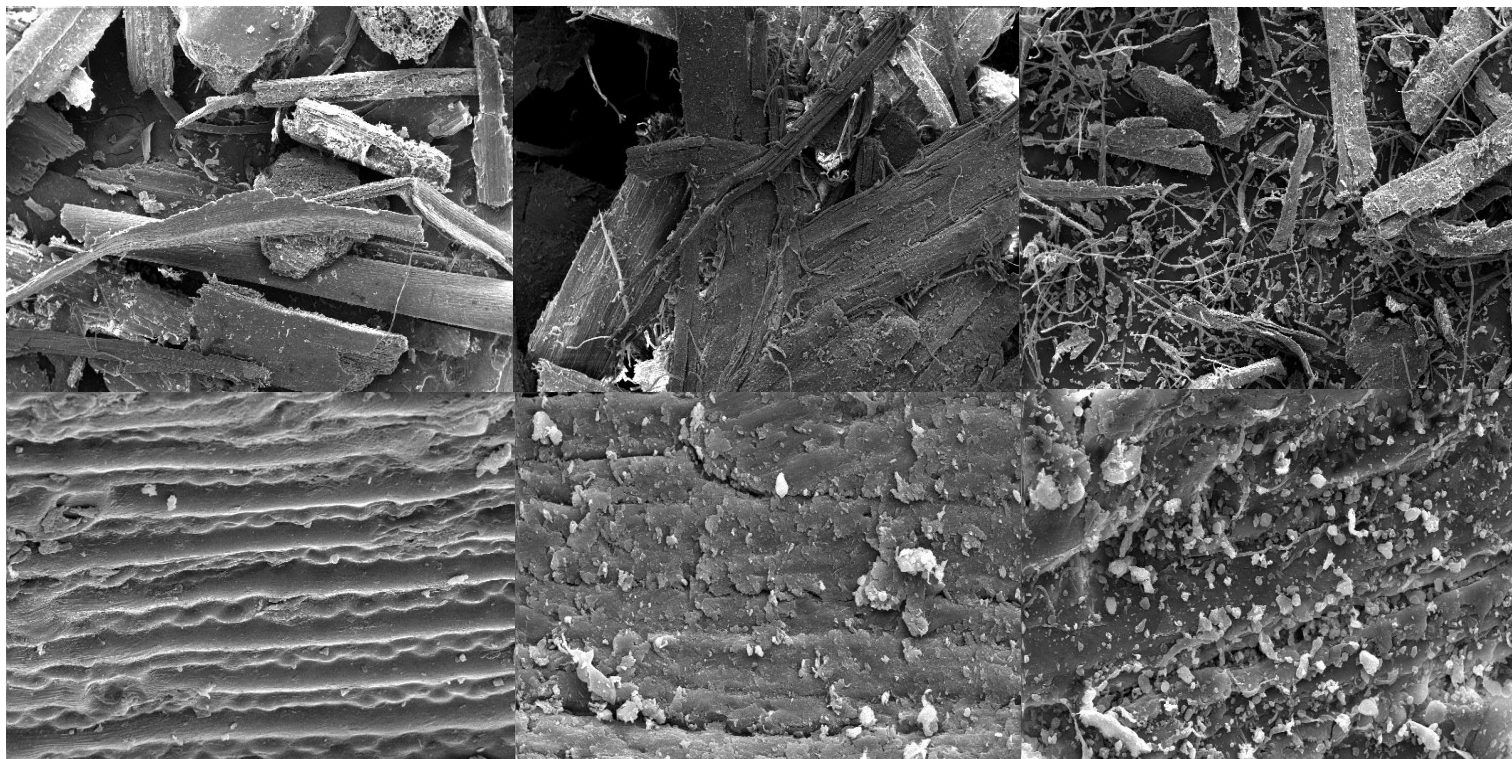
Effect of CO₂ addition on the morphology of residue

Scanning electron microscopy

Untreated

Autohydrolysis^a

High-pressure CO₂/H₂O^{a,b}



^aT = 225°C; ^binitial CO₂ pressure of 60 bar

Addition of CO₂ to water promotes **advanced disruption of structure** of processed solids in comparison to autohydrolysis.

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Cite this: *Green Chem.*, 2014, 16, 4312

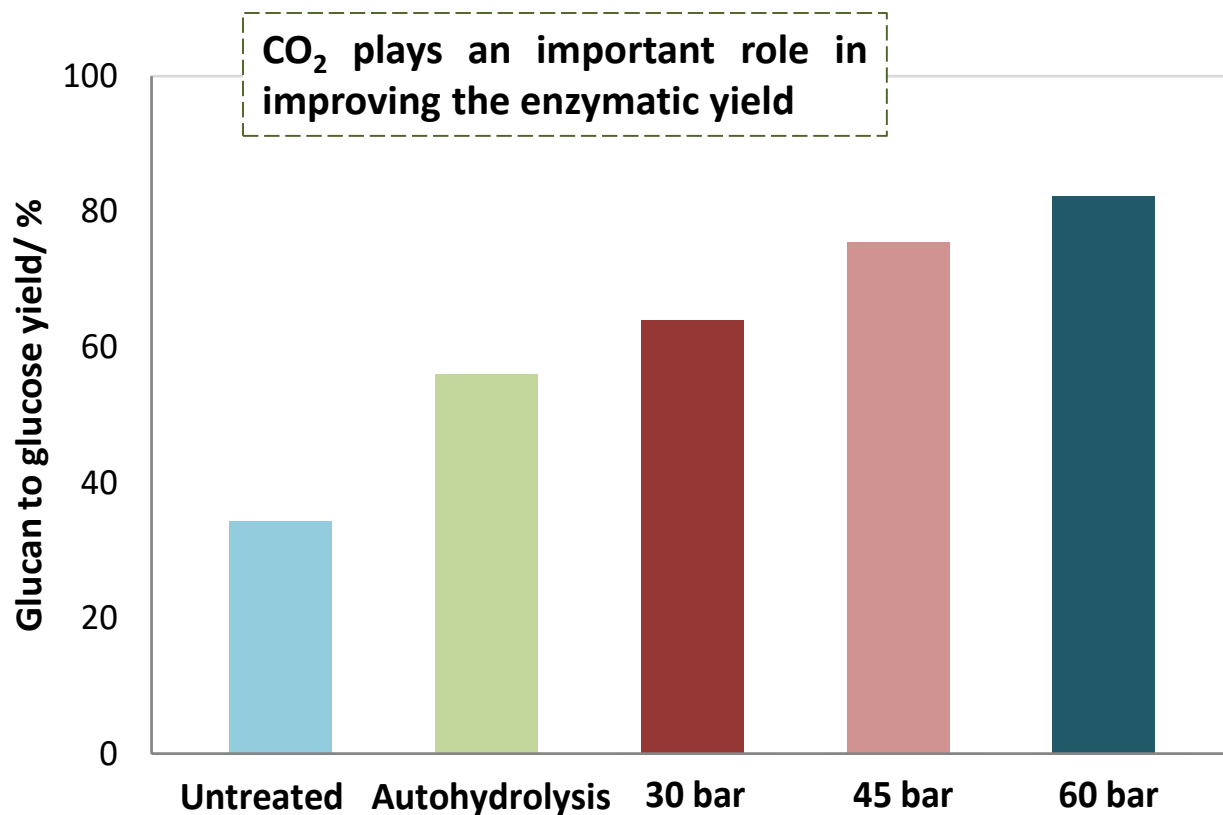
Integrated conversion of agroindustrial residue with high pressure CO₂ within the biorefinery concept

Ana R. C. Morais, Ana C. Mata and Rafal Bogel-Lukasik*



Effect of CO₂ addition and pressure

Enzymatic hydrolysis



Reaction conditions:

T = 225°C

t = non-isothermal conditions

p_{CO_2} = 50 bar

LSR = 10 g/g

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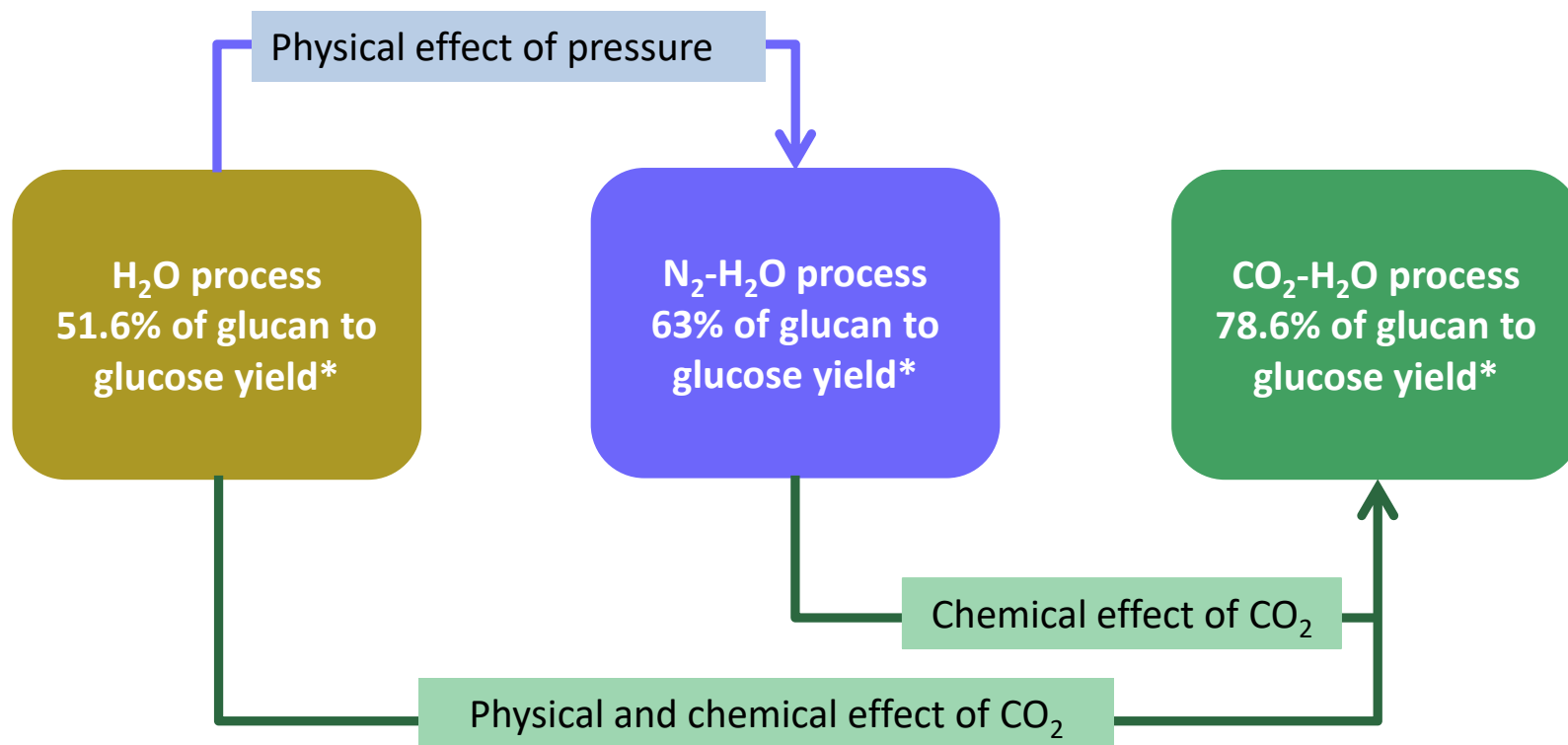
Cite this: *Green Chem.*, 2014, 16, 4312

Integrated conversion of agroindustrial residue with high pressure CO₂ within the biorefinery concept

Ana R. C. Morais, Ana C. Mata and Rafal Bogel-Lukasik*

Enzymatic conditions: Celluclast® 1.5 L (64 FPU/g) and Novozym 188 (60 FPU/g); 0.1 M sodium citrate buffer (pH = 4.8) and 2 % (w/w) sodium azide solution, 250 rpm and 50 °C

Double role of CO₂ pressure



- Pressure influenced positively the enzymatic digestibility of the processed materials.

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Integrated conversion of agroindustrial residue with high pressure CO₂ within the biorefinery concept

Ana R. C. Morais, Ana C. Mata and Rafal Bogel-Lukasik*

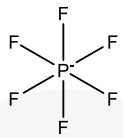


Novel Deconstruction Pretreatments

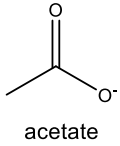
➤ Ionic Liquids

Anions

Cations

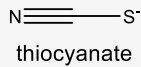


hexafluorophosphate



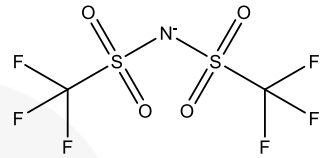
acetate

Cl⁻
chloride

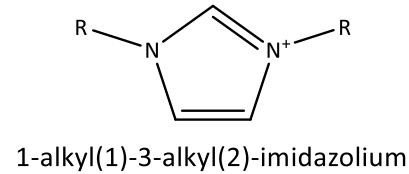


thiocyanate

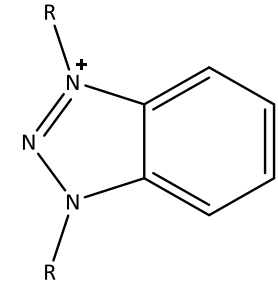
I⁻
iodide



bis(trifluoromethanesulfonyl)amide

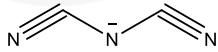


1-alkyl(1)-3-alkyl(2)-imidazolium

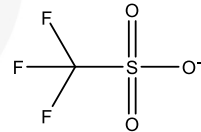


1-alkyl(1)-3-alkyl(2)-benzotriazolium

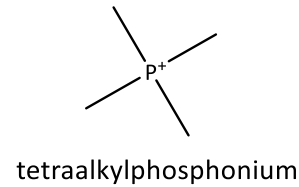
Br⁻
bromide



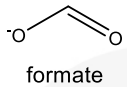
dicyanamide



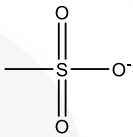
trifluoromethanesulfonate



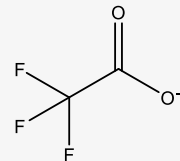
tetraalkylphosphonium



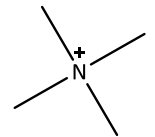
formate



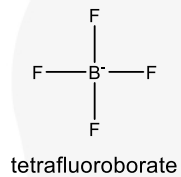
methanesulfonate



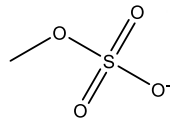
trifluoroacetate



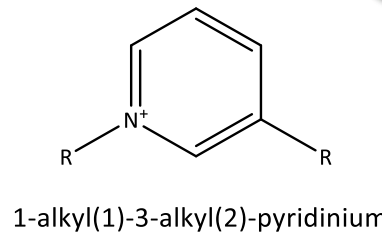
tetraalkylammonium



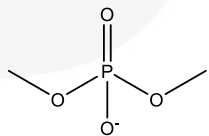
tetrafluoroborate



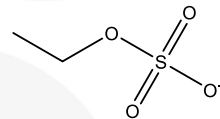
methylsulfate



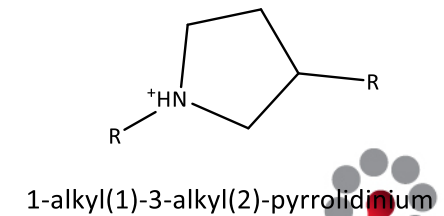
1-alkyl(1)-3-alkyl(2)-pyridinium



dimethylphosphate



ethylsulfate



1-alkyl(1)-3-alkyl(2)-pyrrolidinium

Novel Deconstruction Pretreatments

➤ Ionic Liquids

General properties

- ❖ High polarity
- ❖ Negligible volatility
- ❖ Thermal stability
- ❖ High conductivity
- ❖ Large electrochemical window



Tailored properties

- ❖ Density and Viscosity
- ❖ Lipophilicity and Polarity
- ❖ Acidity and Basicity
- ❖ Solubility

Pre-treatment of LC biomass with ILs

- 1) Alter the physicochemical properties of the biomass macromolecular components;
- 2) Extract a specific macromolecular fraction;
- 3) Perform different fractionation approaches after dissolution.

Advantages:

- ✓ ↓ Cellulose crystallinity;
- ✓ ↑ Extraction of lignin
- ✓ Less degradation of monosaccharides;
- ✓ Recyclability and reuse of ILs.



Pre-treatment of LC biomass with ILs

da Costa Lopes *et al.* *Sustainable Chemical Processes* 2013, **1**:3
<http://www.sustainablechemicalprocesses.com/content/1/3/3>



SUSTAINABLE
CHEMICAL PROCESSES

REVIEW

Open Access

Ionic liquids as a tool for lignocellulosic biomass fractionation

Andre M da Costa Lopes¹, Karen G João¹, Ana Rita C Morais¹, Ewa Bogel-Łukasik² and Rafał Bogel-Łukasik^{1*}

RSC Advances

RSC Publishing

PAPER

Novel pre-treatment and fractionation method for lignocellulosic biomass using ionic liquids

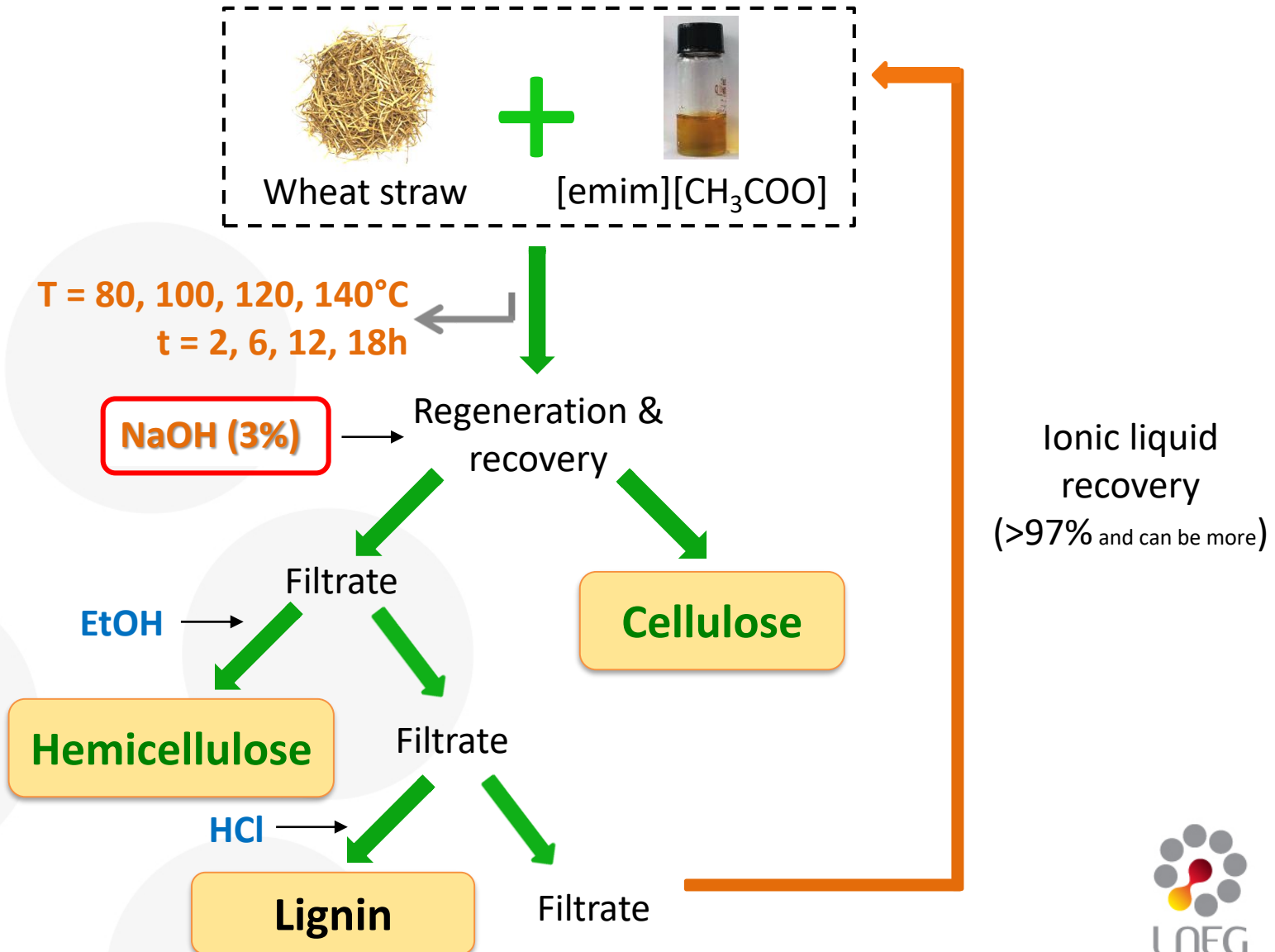
Cite this: *RSC Advances*, 2013, **3**, 16040

Sara P. Magalhães da Silva,^{ab} André M. da Costa Lopes,^a Luisa B. Roseiro^a and Rafał Bogel-Łukasik^{*a}

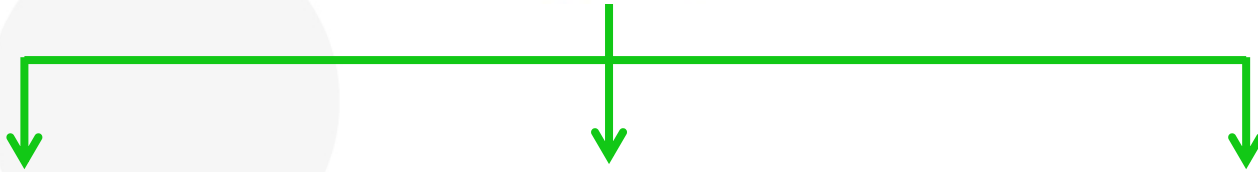


LNEG

Three-step fractionation with ILs



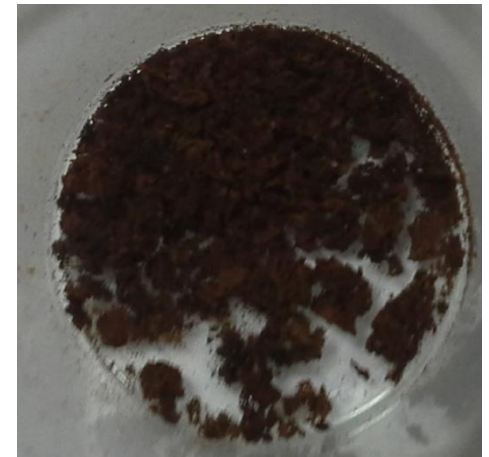
Biomass deconstruction with ILs



Cellulose

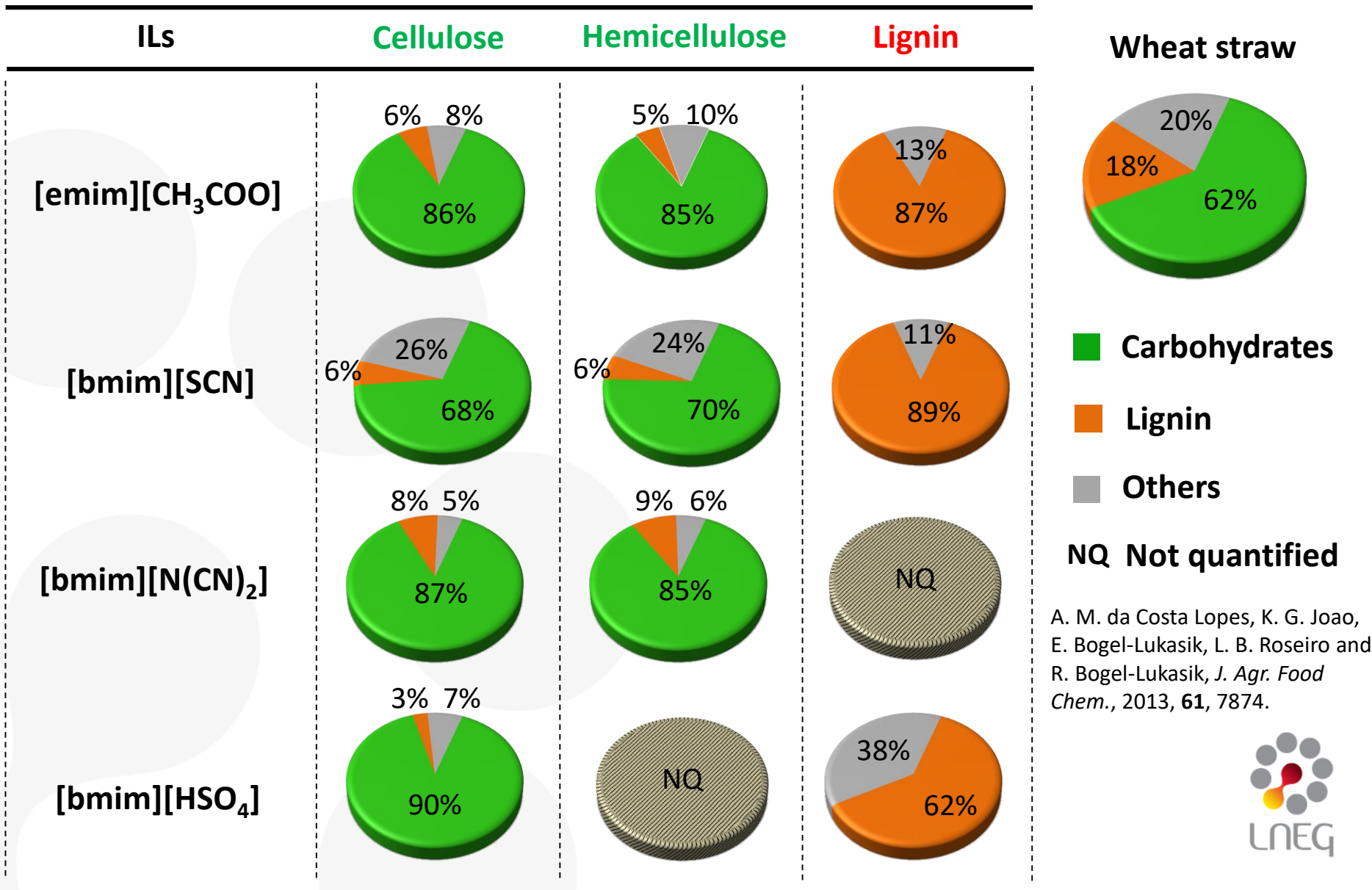


Hemicellulose

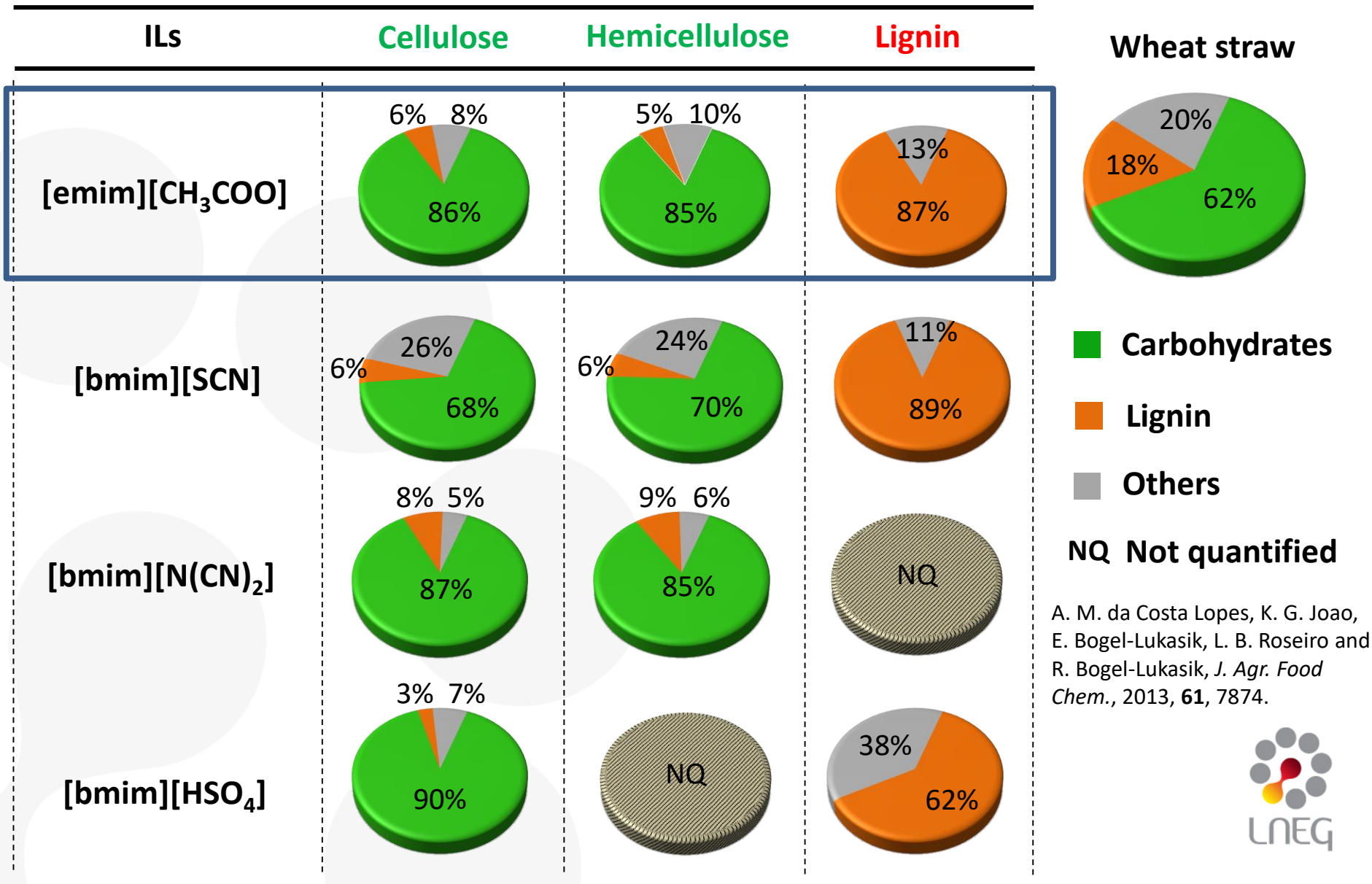


Lignin

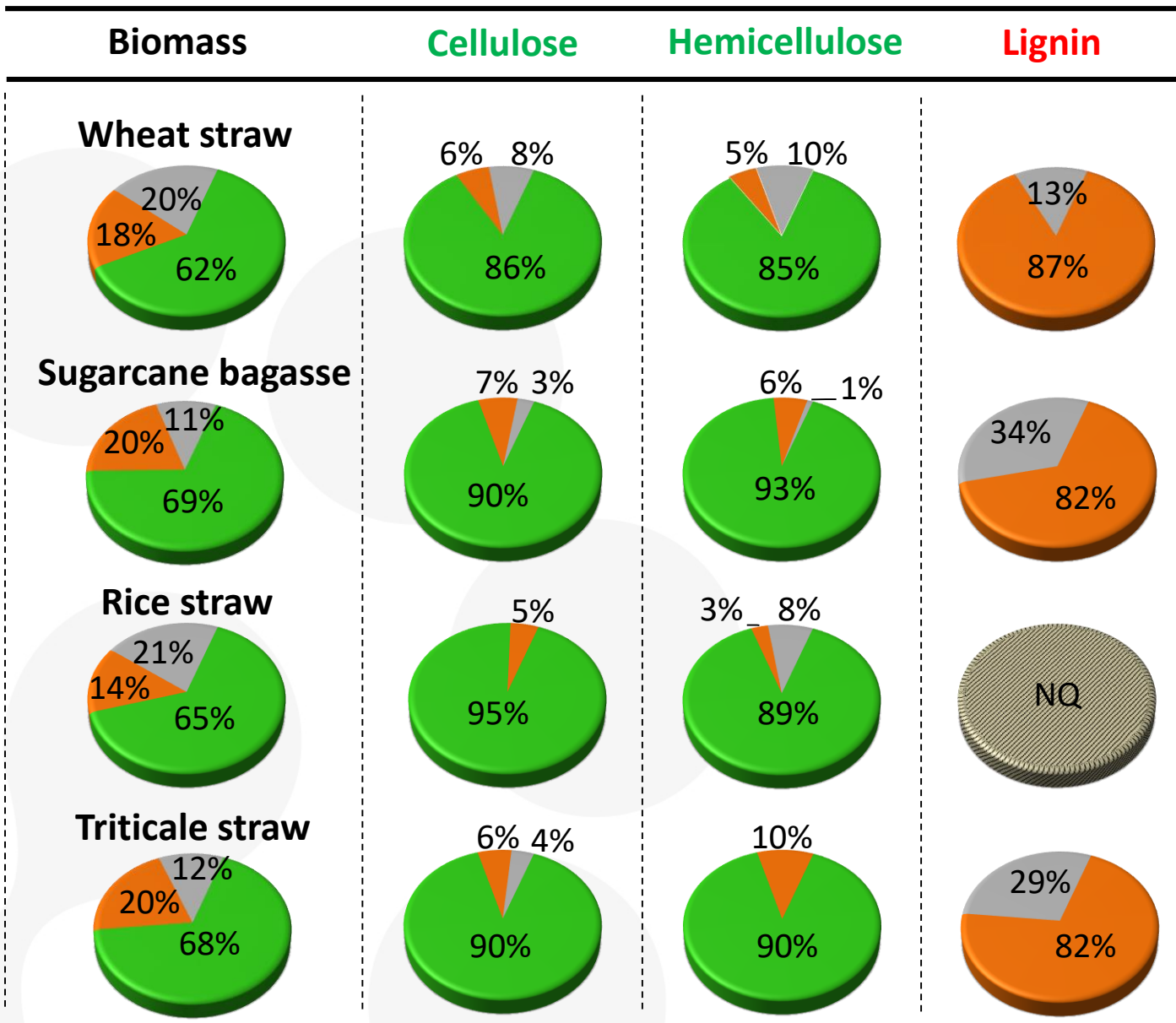
Biomass main components purity after ILs



Biomass main components purity after ILs

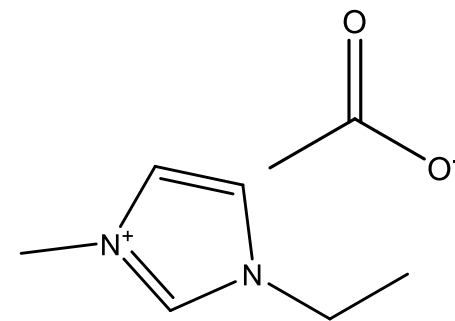


Several LC Biomass tested



- Carbohydrates
- Lignin
- Others

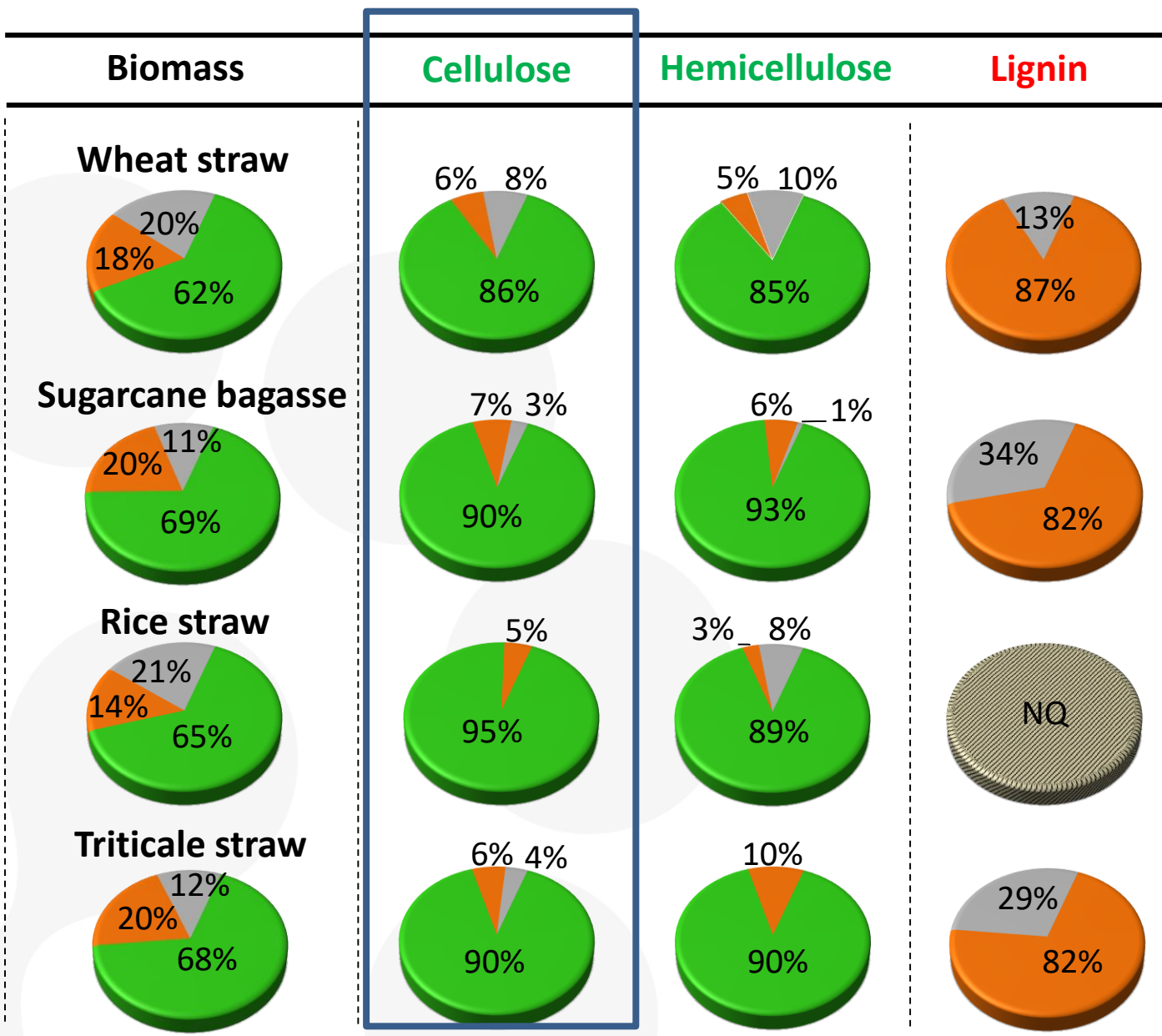
NQ Not quantified



[emim][CH₃COO]

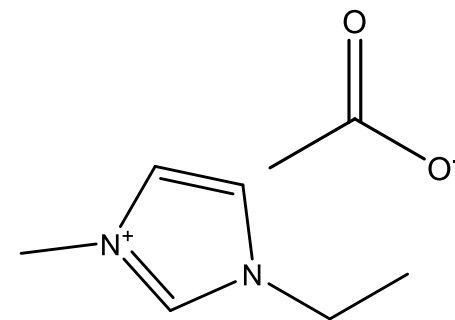


Several LC Biomass tested



- Carbohydrates
- Lignin
- Others

NQ Not quantified



[emim][CH₃COO]



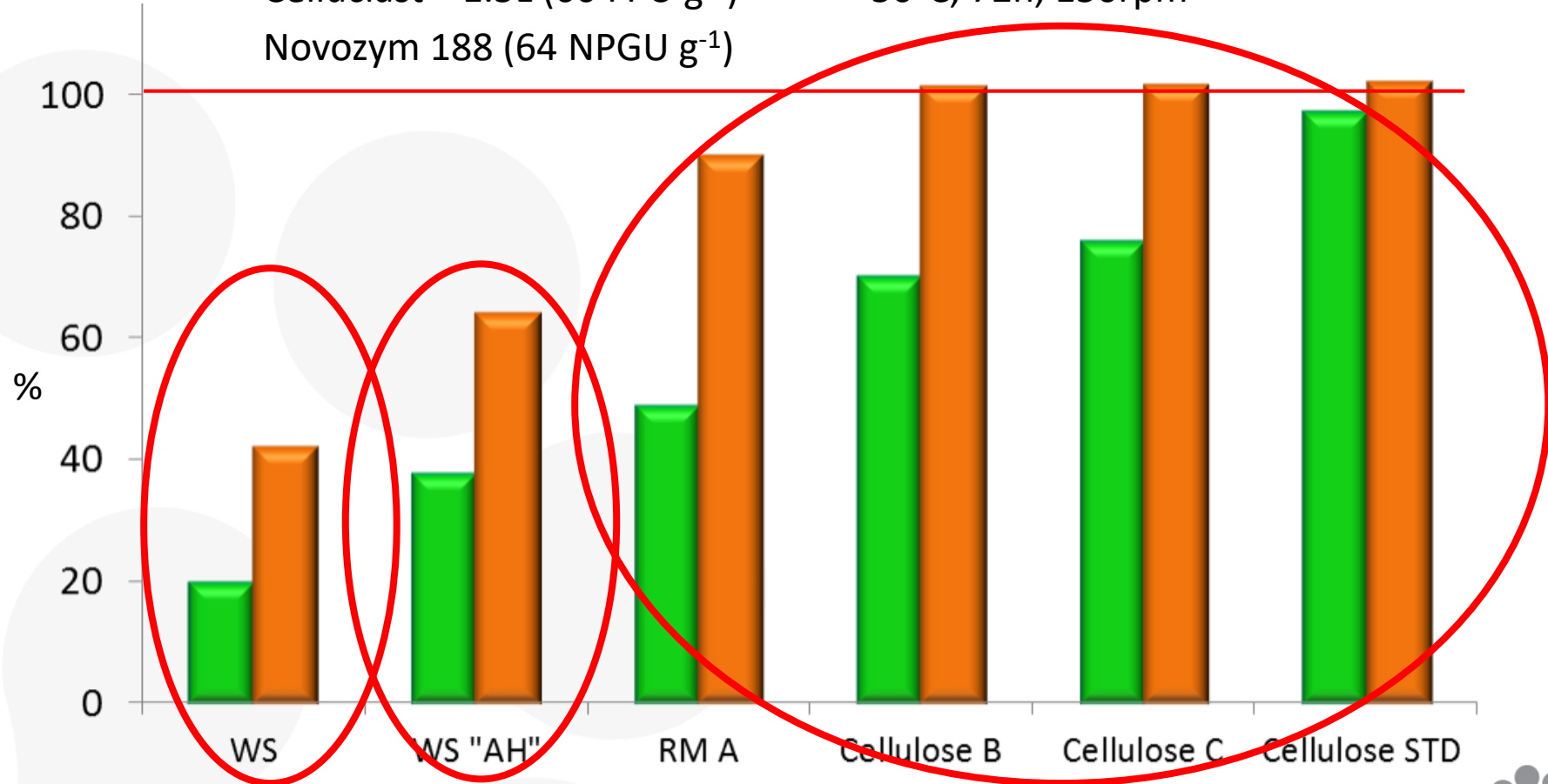
IL's Promotes Cellulose Enzymatic Digestability

Enzymes:

Celluclast[®] 1.5L (60 FPU g⁻¹)
Novozym 188 (64 NPGU g⁻¹)

Conditions:

50°C, 72h, 150rpm

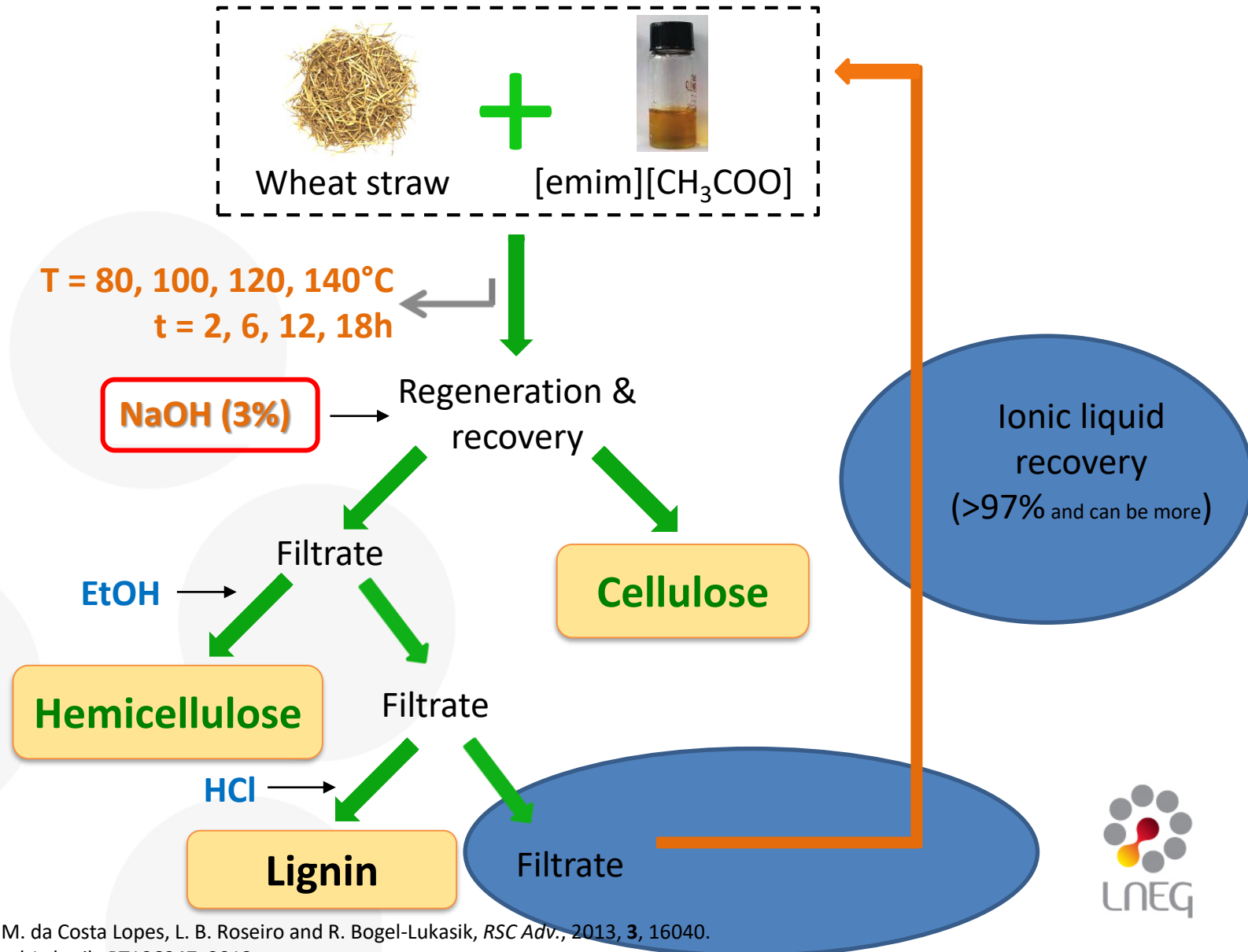


■ Glucose yield (% w/w biomass) ■ Total sugar yield (% w/w)

WS – wheat straw; AH – acid hydrolysed; RM – regenerated material

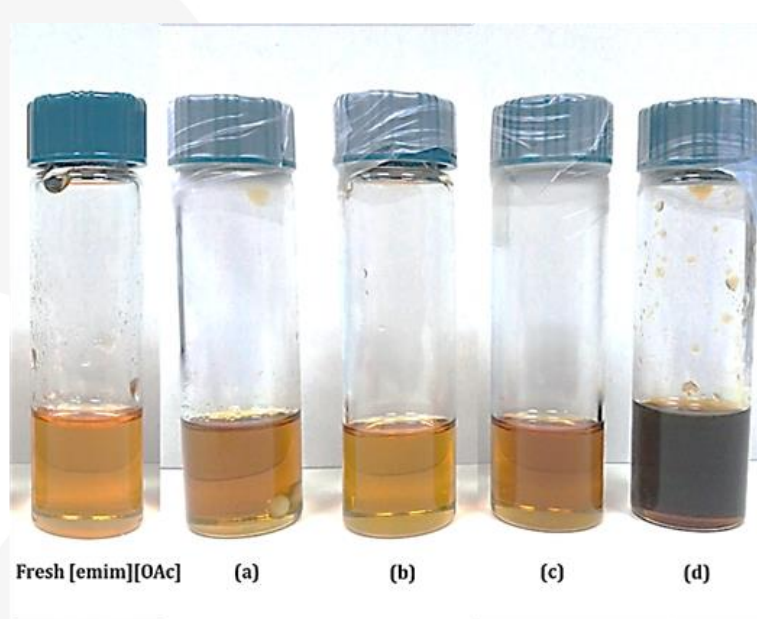


Three-step fractionation with ILs

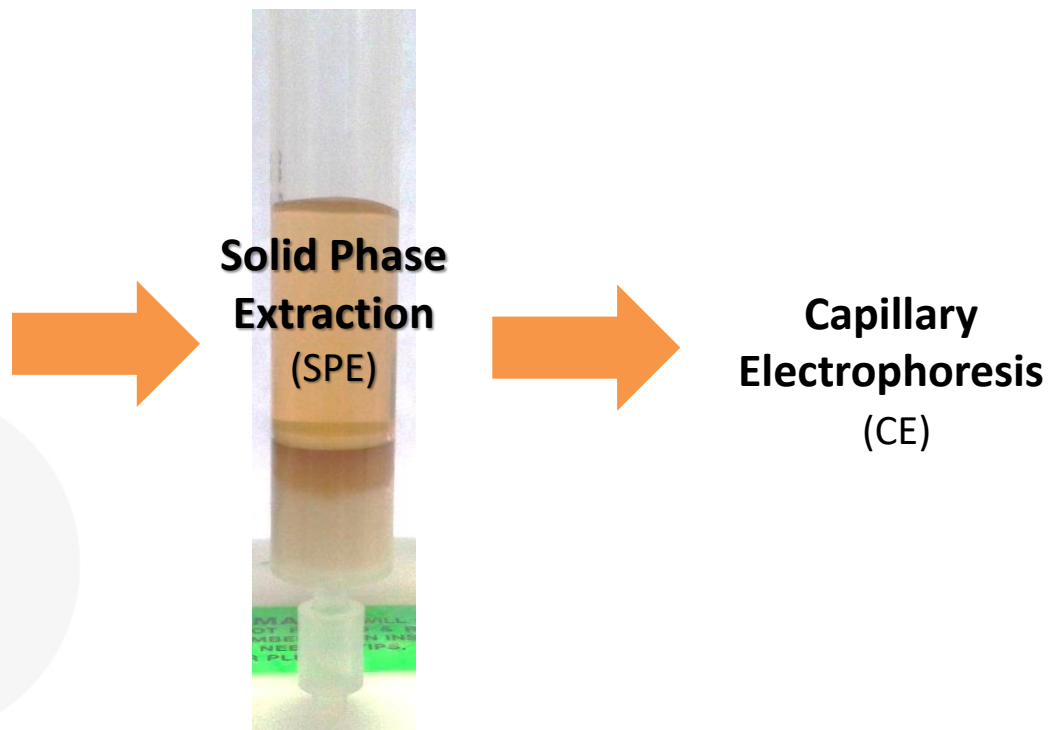


CE analysis of recovered [emim][CH₃COO]

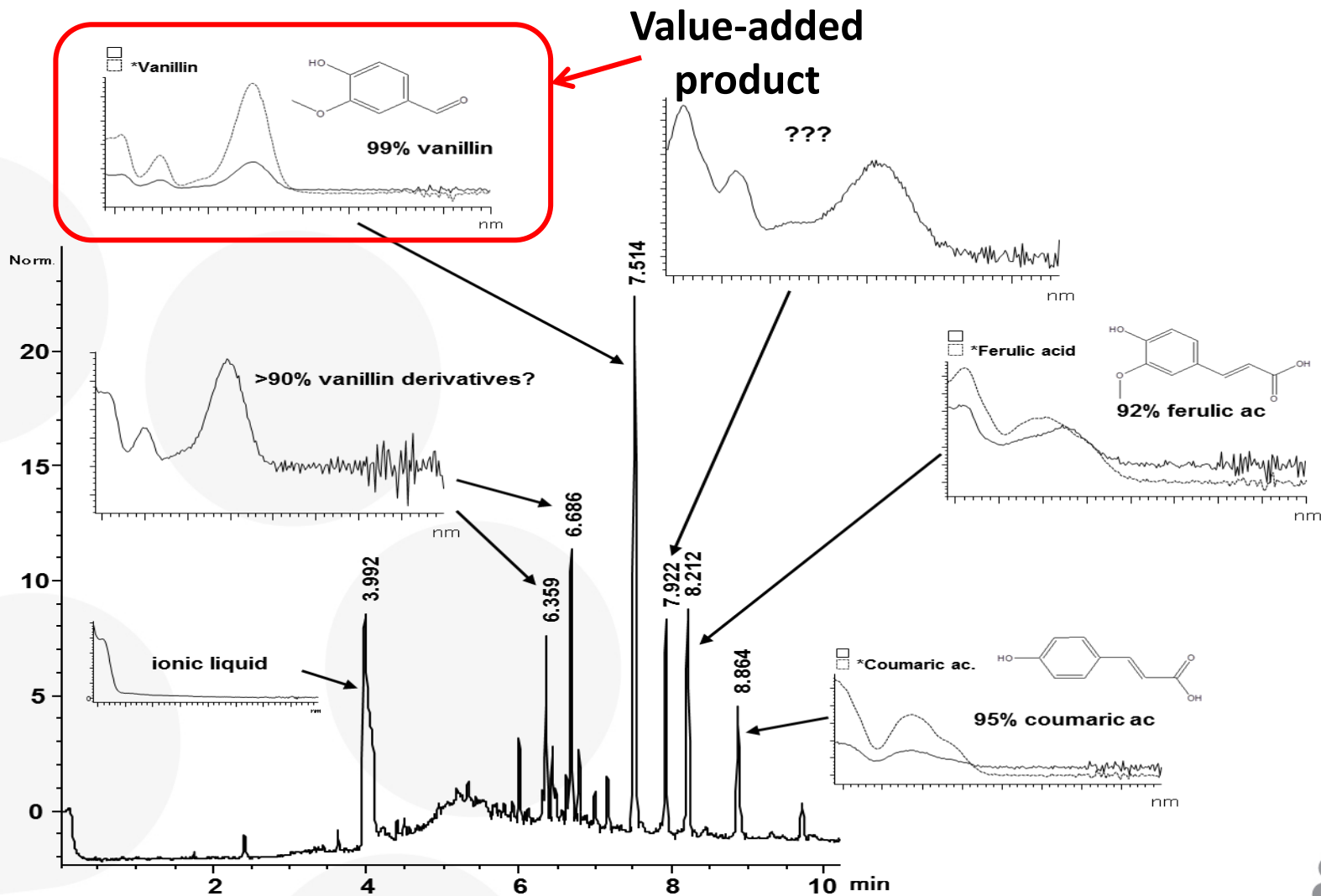
➤ Ionic Liquid Recovery with co-extraction of Phenolics compounds



Colour of the fresh [emim][OAc] and recovered ILs after the wheat straw pre-treatment at different temperatures: **(a)** 80°C; **(b)** 100°C; **(c)** 120°C and **(d)** 140°C.

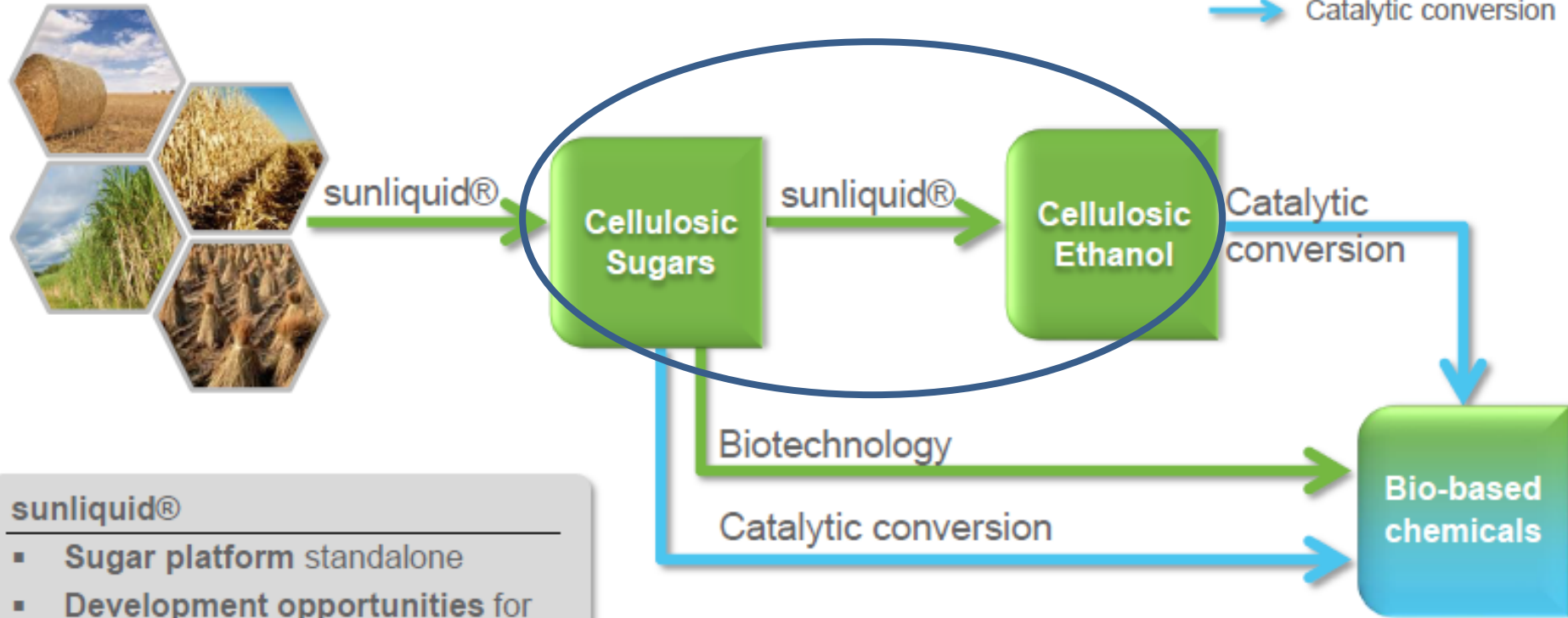


CE analysis of recovered [emim][CH₃COO]



Electropherogram recorded at 320 nm showing the phenolic profile of the recovered IL after pre-treatment at 100°C during 18h.

(Bio)chemicals from LC Biomass



sunliquid®

- **Sugar platform** standalone
- **Development opportunities** for biobased products
- **Extensive know-how** in biocatalysis, strain optimization and heterogenous catalysis

(Bio)chemicals from LC Biomass



sunliquid®

Cellulosic Sugars

sunliquid®

Cellulosic Ethanol

Catalytic conversion

Biotechnology

Catalytic conversion

Bio-based chemicals

→ Biotechnology
→ Catalytic conversion

sunliquid®

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Hemicellulose Platform

Chemical Diversity

Polysaccharide type	Biological origin	Abrev.	Amount ^a	Units			DP	Schematic representation
				Backbone	Side chains	Linkage		
Arabinogalactan	Softwoods	AG	1-3;35*	β -D-Galp	β -D-Galp α -L-Araf β -L-Arap	β -(1→6) α -(1→3) β -(1→3)	100-600	
Xyloglucan	Hardwoods, grasses	XG	2-25	β -D-Glcp β -D-Xylp	β -D-Xylp β -D-Galp α -L-Araf α -L-Fucp Acetyl	β -(1→4) α -(1→3) β -(1→2) α -(1→2) α -(1→2)		
Galactoglucomannan	Softwoods	GGM	10-25	β -D-Manp β -D-Glcp	β -D-Galp Acetyl	α -(1→6)	40-100	
Glucomannan	Softwoods and Hardwoods	GM	2-5	β -D-Manp β -D-Glcp			40-70	
Glucuronoxylan	Hardwoods	GX	15-30	β -D-Xylp	4-O-Me- α -D-GlcpA Acetyl	α -(1→2)	100-200	
Arabinoglucuronoxylan	Grasses and cereals, softwoods	AGX	5-10	β -D-Xylp	4-O-Me- α -D-GlcpA β -L-Araf	α -(1→2) α -(1→3)	50-185	
Arabinoxylans	Cereals	AX	0.15-30	β -D-Xylp	α -L-Araf Feruloy	α -(1→2) α -(1→3)		
Glucuronoarabinoxylans	Grasses and cereals	GAX	15-30	β -D-Xylp	α -L-Araf 4-O-Me- α -D-GlcpA Acetyl	α -(1→2) α -(1→3)		
Homoxylans	Algae	X		β -D-Xylp ^b				

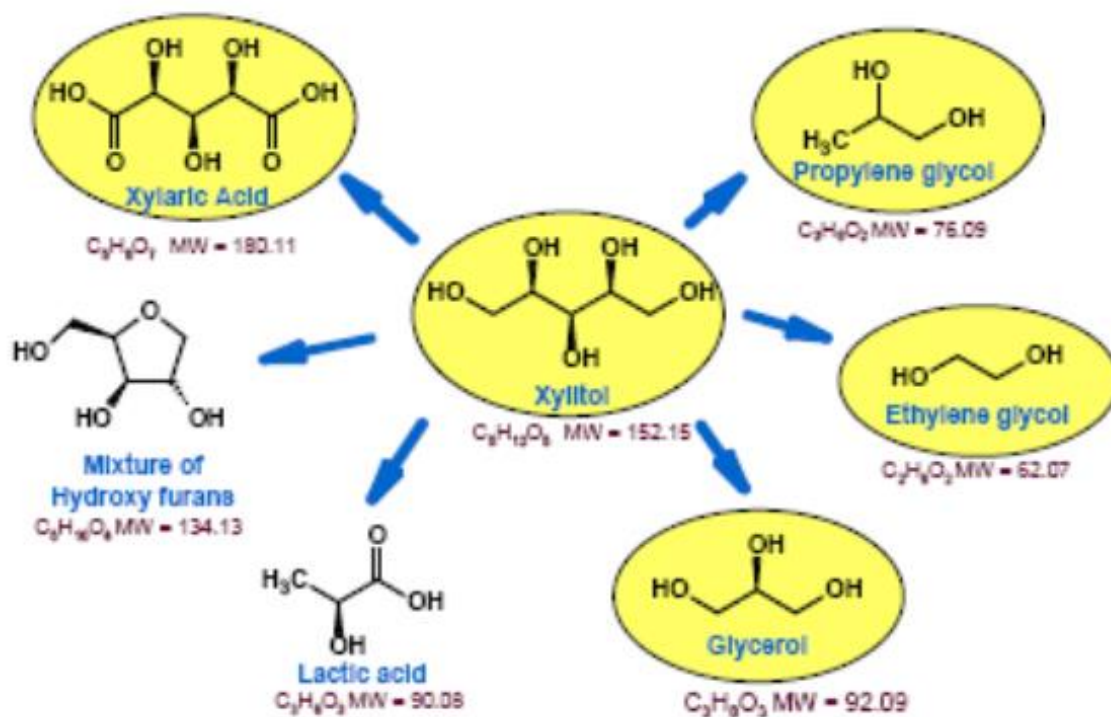
^a% on dry biomass; ^b may also present β -(1→3) linkages on the backbone; * (up to) in the heartwood of larches

In: Gírio FM, Fonseca C, Carvalheiro F, Duarte LC, Marques S & Bogel-Lukasik R (2010), "Hemicelluloses for fuel ethanol: a review", *Bioresource Technology* 101:4775-4800.

(Hemi)cellulose Platform

Top 10 Sugar-derived Products as Building Blocks

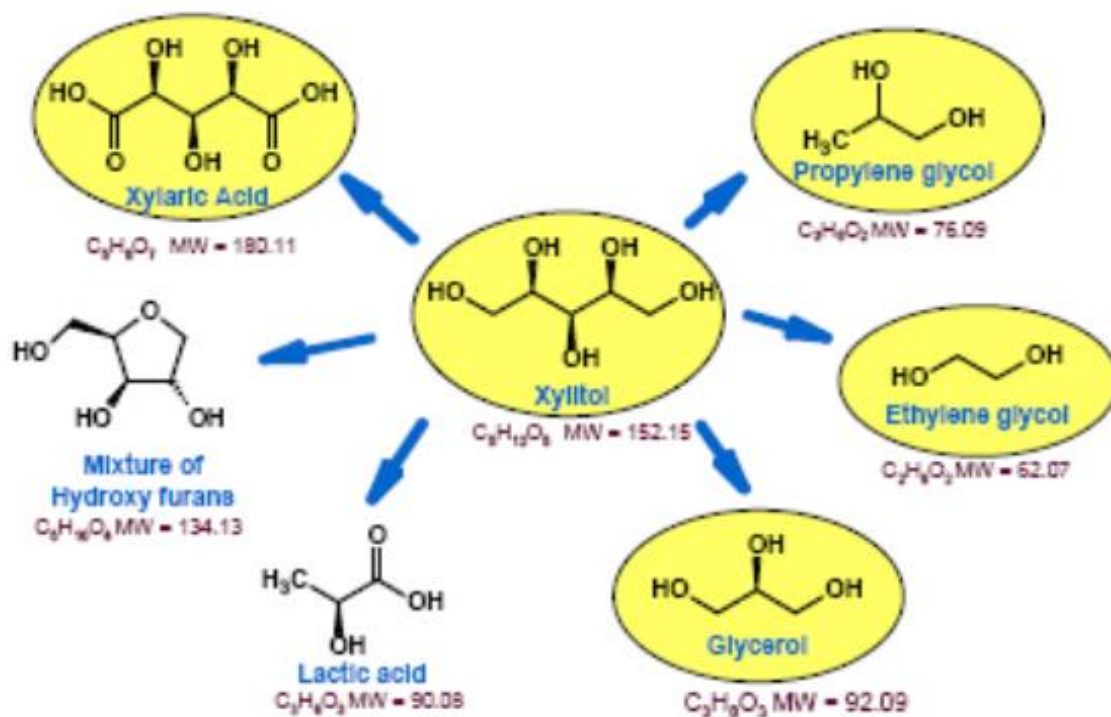
- Ethanol
- Furans (Furfural, HMF, FDCA)
- Glycerol & derivatives
- Biohydrocarbons (isoprene, other)
- Lactic acid
- Succinic acid
- 3- hydroxypropionic acid/aldehyde
- Levulinic acid
- Sorbitol
- Xylitol



(Hemi)cellulose Platform

Top 10 Sugar-derived Products as Building Blocks

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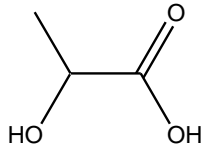


Lactic Acid as Building Block

Applications:

- Food, plastics (polylactates), cosmetic, pharmaceutical, industries
- “Top value chemical”

Bozell & Petersen 2010



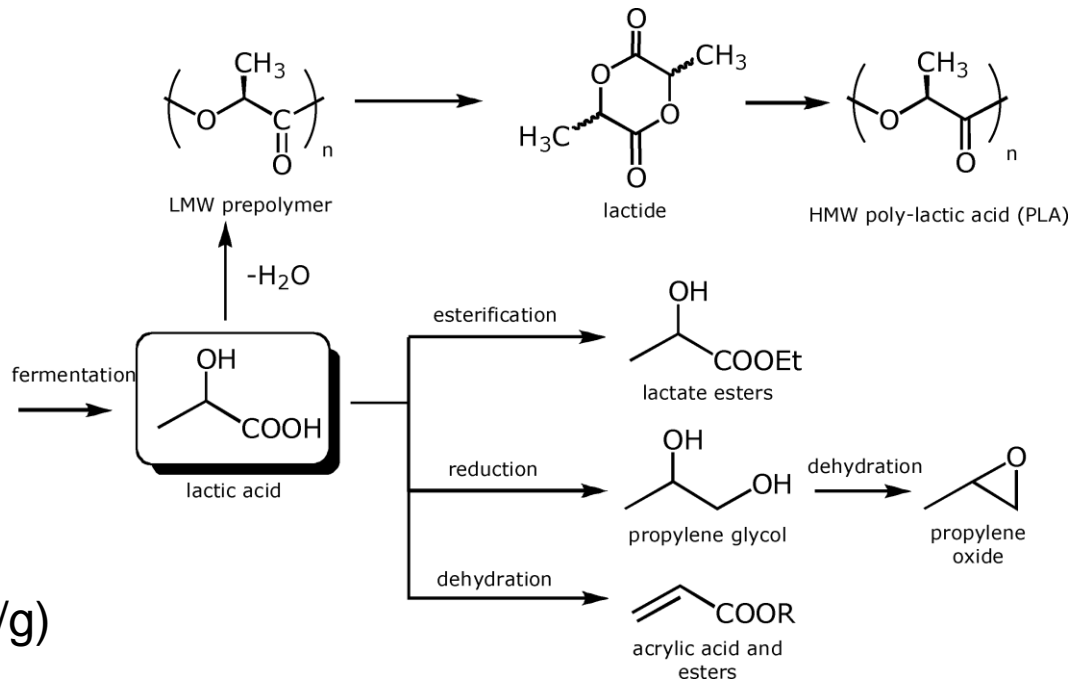
Production:

- Recombinant *E. coli*
 $Y = 0.63$ g/g xyl. Catabolic
De-repressed mutants can
assimilate Xyl +Glc ($Y = 0.73$ g/g)

- Recombinant *Pichia stipitis*

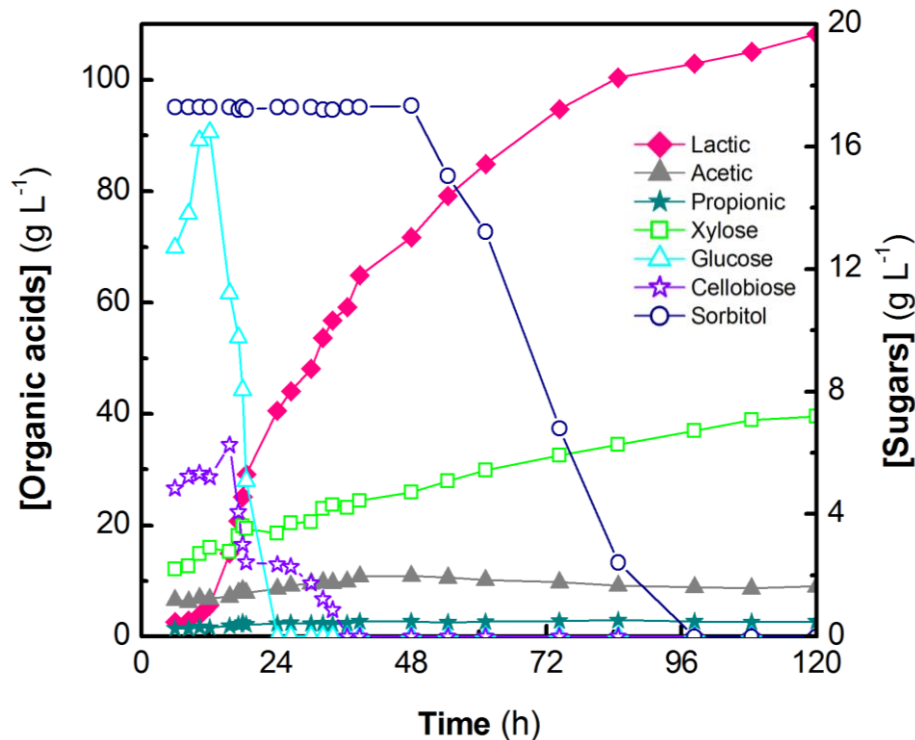
Higher tolerance to product inhibition

That leads to the use of calcium as neutralizing agent, or the use of membranes
(*Simultaneous Fermentation and Separation*)



Lactic Acid as Building Block – LNEG data

- ❑ Simultaneous Saccharification and Fermentation under Pulsed Fed-Batch
- ❑ Feedstock: RECYCLED PAPER SLUDGE (=Cellulose)
- ❑ *Lactobacillus* sp. + Cellulases



Feeding strategy for conversion in Bioreactor

⇒ Fed-Batch Process - addition of more RPS after a 5-h period ⇔ $2 \text{ g}_{\text{Polysac.}}/\text{L/h}$ (\approx initial rate of hydrolysis)

Pulsed Feeding ($n=1-6$) $t_n = n \cdot 5\text{h}$:
 $P_n = 10 \text{ g}_{\text{Polysac.}}/\text{L} = 38.6 \text{ g}_{\text{RPS}}/\text{L}$

Under Optimal Feeding Strategy:

Rate of production ⇒ Maximum = 4.7 g/L/h
Overall = 0.9 g/L/h

Extent ⇒ Yield = 68%

LA concentration = 108.2 g/L (120h)

Marques (2012) L(+)-Lactic Acid Production from Recycled Paper Sludge, PhD Thesis (Biotechnology), IST, Lisboa

Marques S, Matos CT, Gírio FM, Roseiro JC, Santos JAL (2017) Biochem Eng J. 120: 63-72

Marques S, Gírio FM, Santos JAL, Roseiro JC (2017) Biomass Conv Bioref. 7: 127-137

Marques S, Santos JAL, Gírio FM, Roseiro JC (2008) Biochem Eng J. 41: 210-216

Marques S, Alves L, Roseiro JC, Gírio FM (2008) Biomass Bioenergy 32: 400-406

Take-away messages

- ✓ Current breakdown methods for LCB main polysaccharides are not enough robust to get clean and pure sugar streams;
- ✓ Novel deconstruction methods can gain the Bioeconomy race if they succeed to lower the technology cost;
- ✓ Ionic Liquids are a suitable pretreatment to enhance Lignin extraction and to decrease Cellulose cristalinity;
- ✓ High Pressure CO₂-H₂O biphasic system enhances Hemicellulose hydrolysis and also increases Cellulose digestibility;
- ✓ Bio-based products value chains shall benefit from better understanding of the Polysaccharide Chemistry.

ACKNOWLEDGMENTS



www.lneg.pt

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More info:

www.proethanol2g.org

www.smibio.net

www.babet-real5.eu

www.ambition.org

www.brisk2.eu

