BECOOL

Brazil-EU Cooperation for Development of Advanced Lignocellulosic Biofuels

Benchmarking of lignocellulosic feedstock for efficient biochemical processing

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Innovations in Lignocellulosic Biomass Value Chains for Advanced Biofuels
EUBCE Workshop
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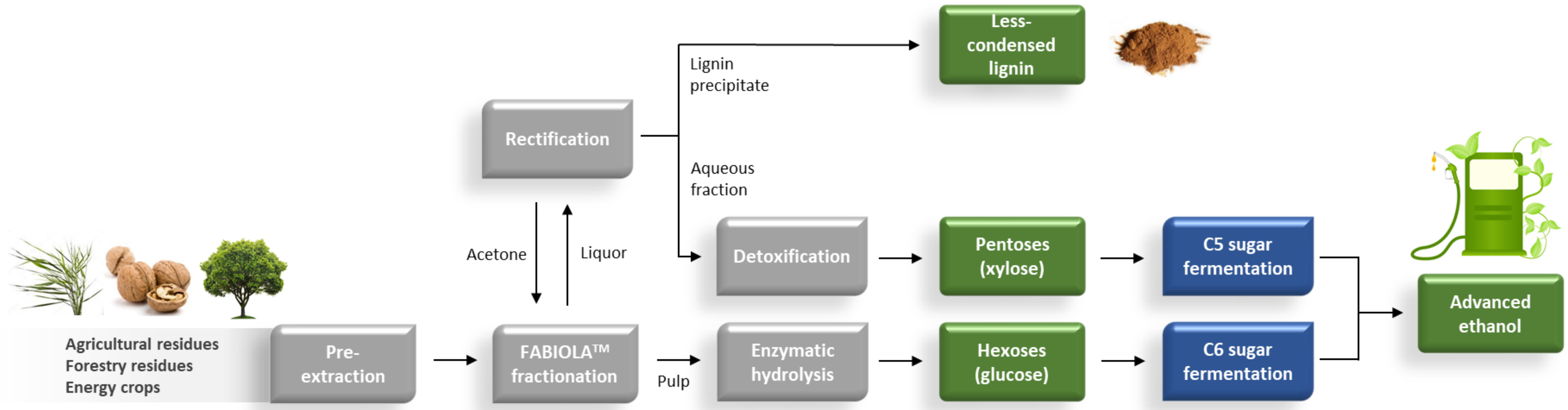
Advanced ethanol via Organosolv fractionation

- TNO's FABIOLA™ organosolv pretreatment technology: Acetone-water fractionation under mild conditions, yielding cellulose-rich pulp, C5 sugars hydrolysate and less condensed lignin.
- Process developed for conventional hardwoods and some agricultural residues, but performance influenced by biomass composition.
- Pre-extraction of lignocellulosic feedstocks: Reduce variability in fractionation performance.
- **Main objective:** Screening of wide range of lignocellulosic feedstocks and evaluate fermentability towards ethanol.



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Methodologies

Selected feedstocks

Herbaceous

Hemp, sunn hemp, sorghum, giant reed

Agri-residues

Wheat straw, corn stover

Reference

Beech wood

Methodology

- Biochemical characterization of feedstocks and products
- Acetone-gradient solvent extraction and acetone mild organosolv fractionation (2L-scale)
- Detoxification of C5 sugars hydrolysate using 4% GAC
- Enzymatic saccharification of cellulosic pulp
- Ethanol fermentation: *Saccharomyces cerevisiae* Ethanol Red® (Leaf – Lesaffre) for C6 sugars, *Spathaspora passalidarum* CBS 10155T for C5 sugars, cultivation in flasks in nutrient- and salt-supplemented media

Pretreatment of lignocellulosic feedstocks

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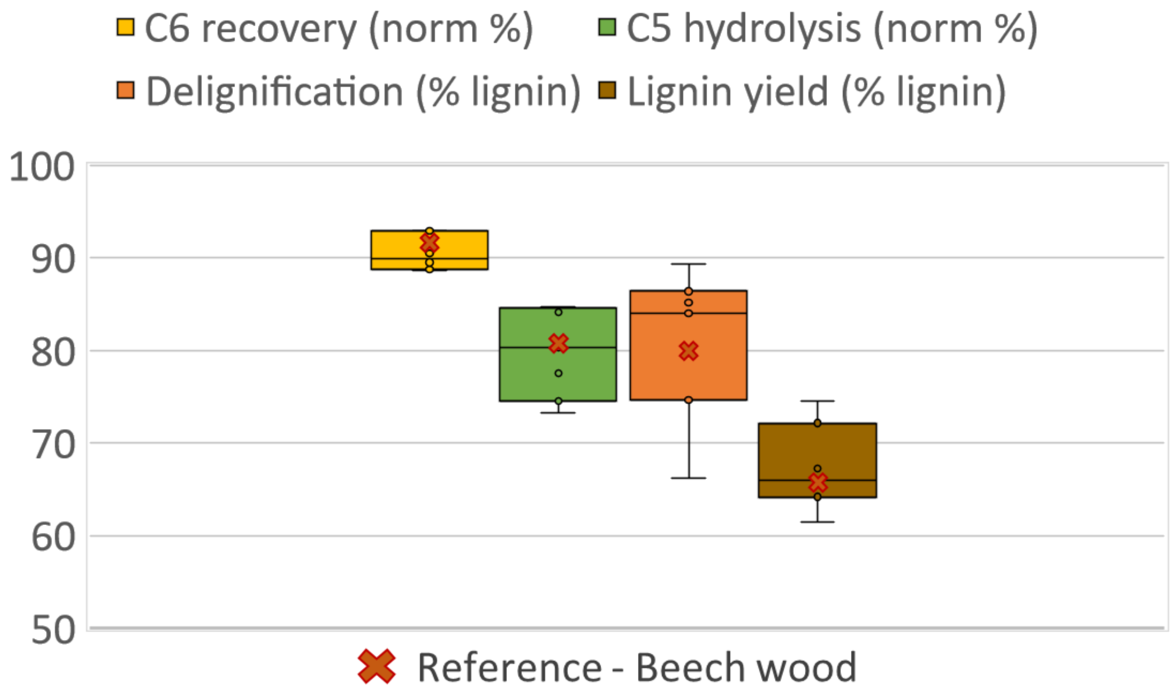
Pre-extraction:

- Robust to treat the wide range of biomasses.
- Reduction of ash/extractives content in most feedstocks.
- Higher lignocellulose content (70 ⇨ 82 % dw).

Fractionation:

- High C6 sugar recovery in product pulps from all feedstocks.
- C5 recovery, delignification and lignin yield still quite variable among feedstocks, but comparable to reference biomass.
- Certain feedstocks require higher acid dosages: Hemps.



C6 sugars – Enzymatic hydrolysis of pulps

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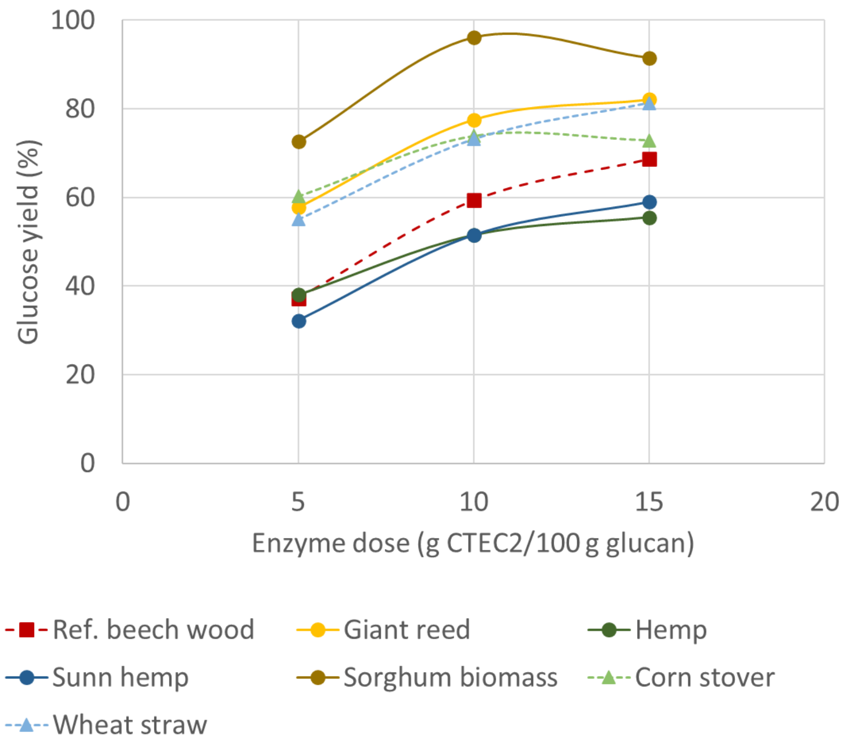
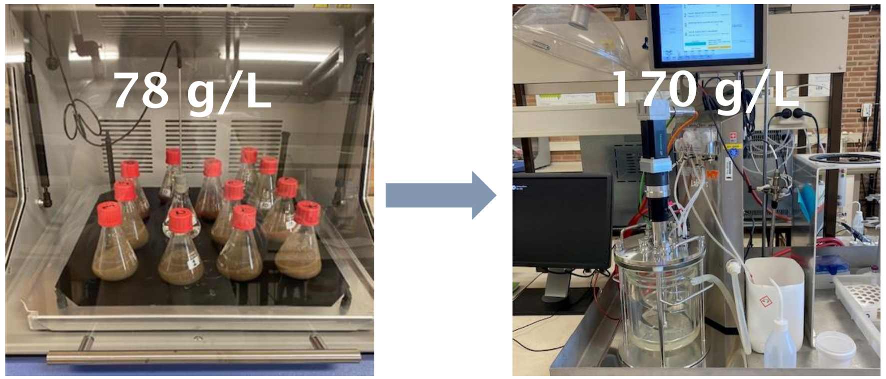
- Enzymatic hydrolysis of FABIOLA™ pulps using commercial enzyme cocktail (Cellic CTEC2, Novozymes) at standard consistency (10% w/v).
- Pulps from BECOOL feedstocks gave rise to high glucose yields (>70%) after 48 h, except hemps.
- Additional work: High consistency hydrolysis (25% w/v) readily feasible with corn stover in fed-batch operation: Up to 170 g/L glucose from corn stover pulp.





C6 sugars – Ethanol fermentation

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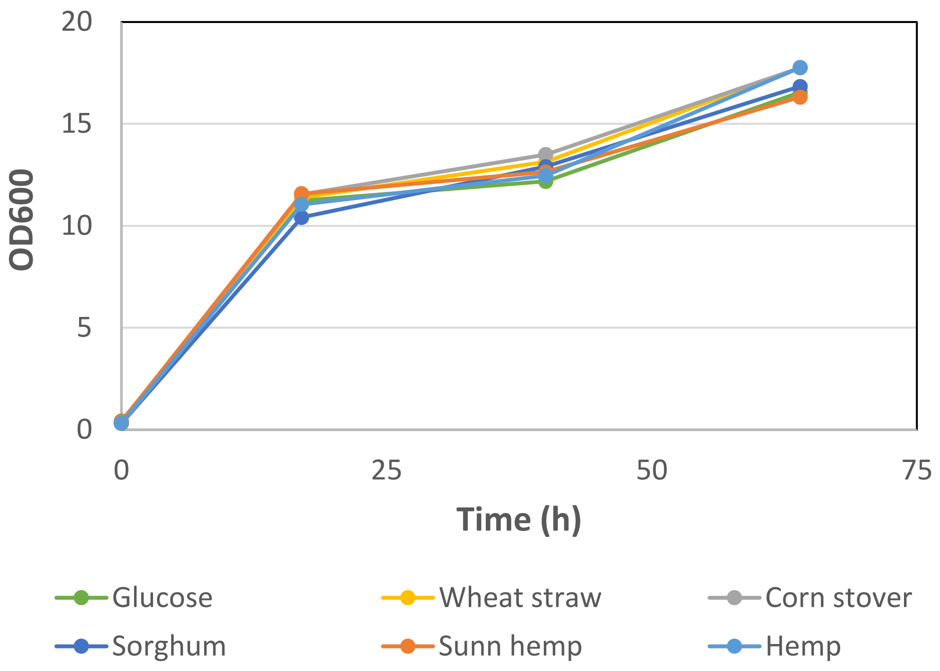
- Good growth with all C6 substrates.
- Ethanol yield on glucose (control): 0.41 g/g = 80% of theoretical maximum.
- Ethanol yield only slightly lower on feedstocks: Not all sugars consumed (xylose ~5%).



Substrate	Total sugars	Sugar consumption	Ethanol production	Ethanol yield on total sugars
	g/L	%	g/L	% of control
Glucose (control)	117	100	48.3	100
Wheat straw	93	96	37.5	97
Maize stover	93	95	36.6	95
Sorghum	88	95	34.6	95
Sunn hemp	109	94	41.4	92
Hemp	84	96	33.0	95

C5 sugars – Detoxification for inhibitors removal

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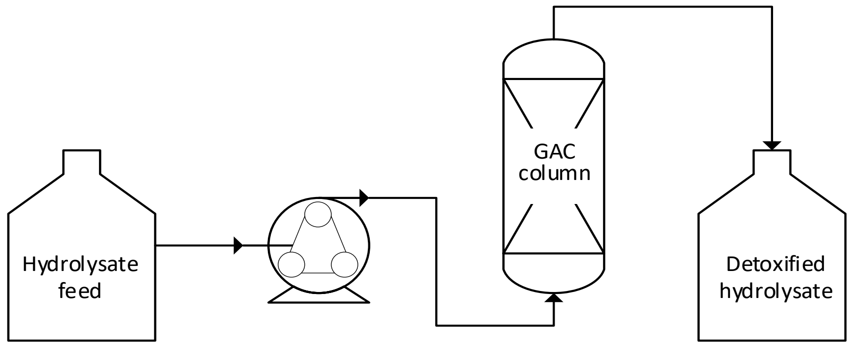
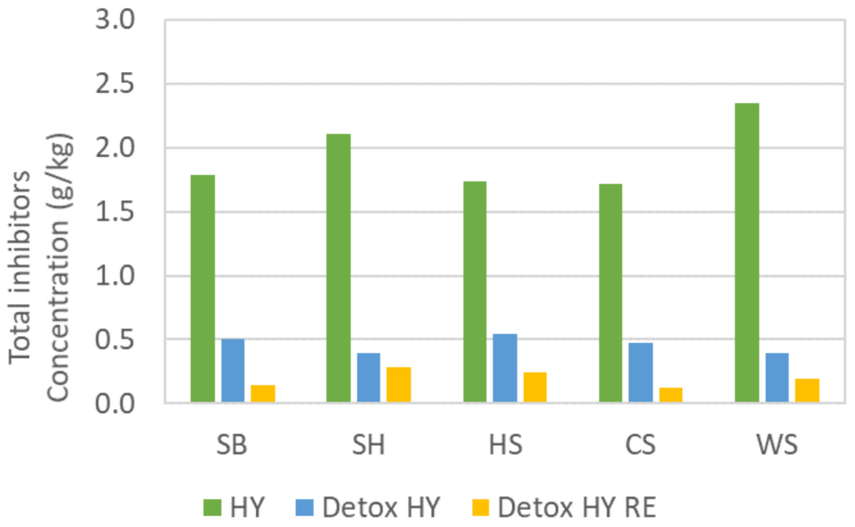
- Between 6-11% C5 sugars degradation to furfural, presence of ppm levels of phenolics
- Carbon adsorption provides a potential route for removal of these inhibitory compounds prior to fermentation
- Fixed bed of granular activated carbon as an scalable alternative: Sizing based on adsorption capacities characterized with representative hydrolysate
- Detoxified hydrolysates further evaporated under vacuum to reach sufficiently high sugar concentrations (30 g/L ⇨ 60 g/L)



Scheme of detoxification setup



C5 sugars – Ethanol fermentation

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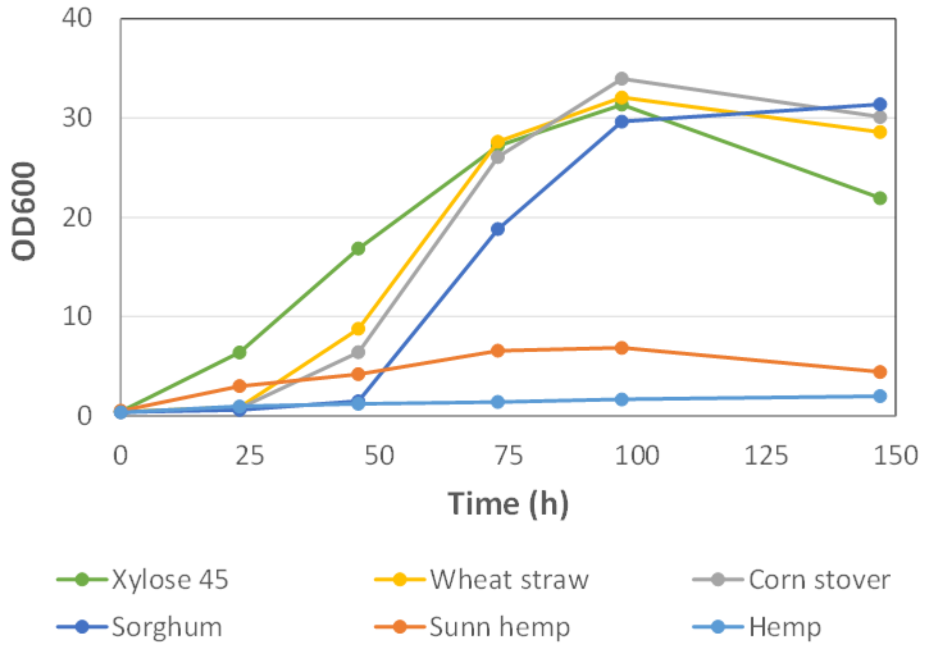
- Good growth in most C5 substrates expect sunn hemp and hemp (further dilution required).
- Ethanol yield on xylose (control): 0.38 g/g = 75% of theoretical maximum.
- Lag phase due to initial acetic acid (5-7 g/L).
- Ethanol yield lower on feedstocks: Ethanol is not produced from C6 sugars (mannose, galactose, glucose), so not all sugars consumed.



Substrate	Total sugars	Sugar consumption	Ethanol production	Ethanol yield on total sugars
	g/L	%	g/L	% of control
Xylose (control)	45	100	17.2	100
Wheat straw	55	91	17.0	82
Maize stover	60	91	15.9	69
Sorghum	54	89	16.0	79
Sunn hemp	33	96	8.1	87
Hemp	17	94	5.6	88

Concluding remarks and outlook

- Agricultural residues and herbaceous biomasses readily pretreated through pre-extraction and mild acetone organosolv fractionation.
- High fractionation product yields: 90% glucan in pulp, 82% C5 in hydrolysate, 68% lignin as final product.
- Effective enzymatic hydrolysis and detoxification to produce C6 and C5 sugar streams: Still challenges with hemp biomasses (enzymatic recalcitrance, higher inhibitor content).
- Fermentations were successful but challenges: Remaining sugars and acetic acid content.
- Future work:
  - Sequential and co-cultivation of strains using mixed C6/C5 streams to allow 1-step fermentation
  - Adaptation of the process to substrates
  - Optimisation of upstream conditioning with fermentation routes

Partners

Thank you!

Join the EUBCE session:

5CO.10.1 Production Of Bio-Ethanol From Beech Wood Pellets Via Mild Acetone Organosolv Fractionation

Ana Lopez-Contreras WUR

Wednesday, 11 May 2022

16:15 - 17:15 CEST

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