Lignins and Pentoses

biorefinery waste streams or chemical intermediates?

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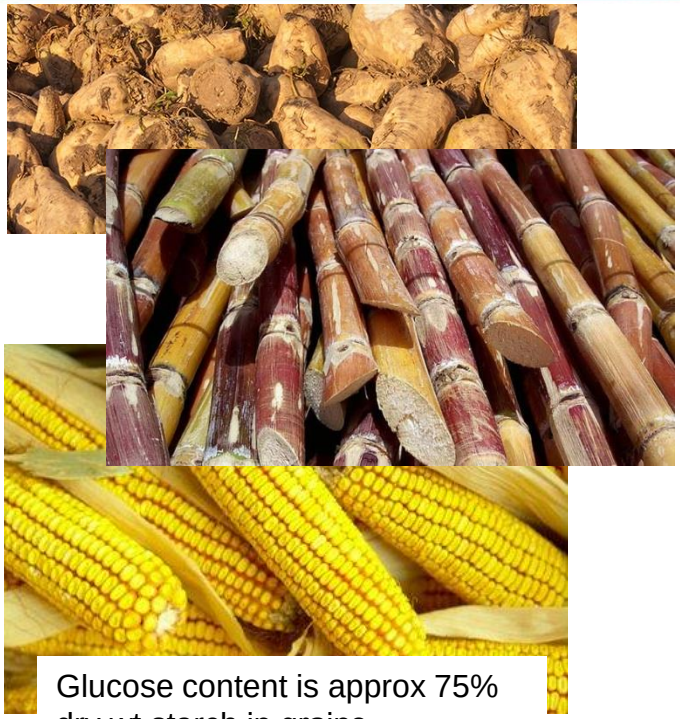
Summary

1. Introduction
2. A few words on biomass pretreatment
3. A little about lignins
4. Spotlight on the pentose field
5. Developing pentose-specific tools
6. Conclusions

INTRODUCTION

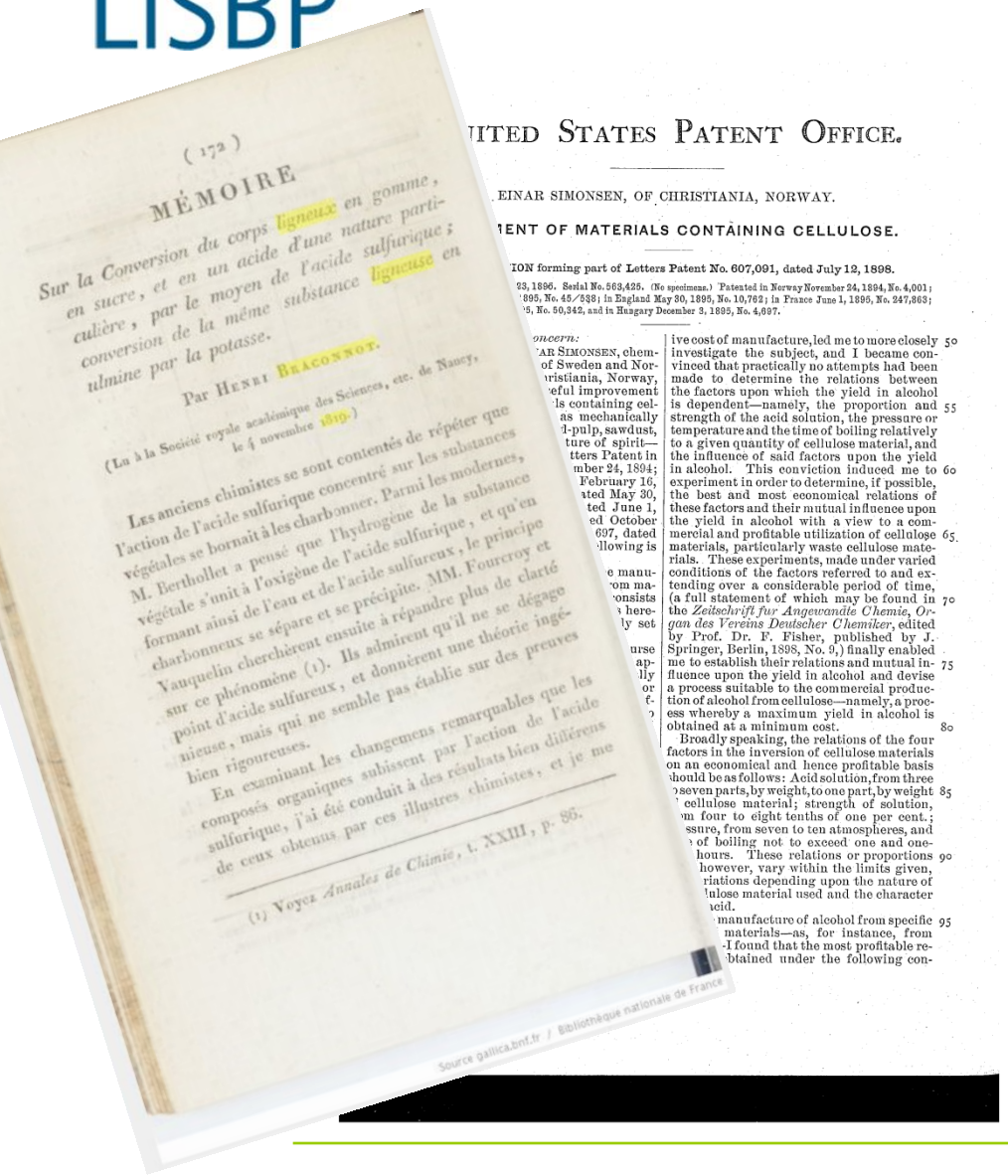
1st to 2nd: a big leap

- Sugarcane and beet ethanol involves a single catalytic step (Baker's yeast)
  - Essentially crushing, separation technology and fermentation
- Grain ethanol is made with crushing/milling, enzyme hydrolysis and fermentation
  - Well-developed amylases
  - Baker's yeast
- Cellulosic ethanol requires
  - Chopping/grinding
  - Catalytic pretreatment
  - Extensive enzymatic hydrolysis
    - a more recent field of development
  - The use of advanced yeast



Glucose content is approx 75% dry wt starch in grains…….

Cellulosic ethanol is an old story


(172)

MÉMOIRE

Sur la Conversion du corps ligneux en gomme, en sucre, et en un acide d'une nature particulière, par le moyen de l'acide sulfurique; conversion de la même substance ligneuse en ulmine par la potasse.

Par Henri Braconnot.

(Lu à la Société royale académique des Sciences, etc. de Nancy, le 4 novembre 1819.)

Les anciens chimistes se sont contentés de répéter que l'action de l'acide sulfurique concentré sur les substances végétales se bornait à les charbonner. Parmi les modernes, M. Berthollet a pensé que l'hydrogène de la substance végétale s'unit à l'oxigène de l'acide sulfurique, et qu'en formant ainsi de l'eau et de l'acide sulfureux, le principe charbonneux se sépare et se précipite. MM. Fourcroy et Vauquelin cherchèrent ensuite à répandre plus de clarté sur ce phénomène (1). Ils admirent qu'il ne se dégage point d'acide sulfureux, et donnèrent une théorie ingénieuse, mais qui ne semble pas établie sur des preuves bien rigoureuses.

En examinant les changemens remarquables que les composés organiques subissent par l'action de l'acide sulfurique, j'ai été conduit à des résultats bien différens de ceux obtenus par ces illustres chimistes, et je me

(1) Voyez *Annales de Chimie*, t. XXIII, p. 86.

UNITED STATES PATENT OFFICE.

EINAR SIMONSEN, OF CHRISTIANIA, NORWAY.

ENT OF MATERIALS CONTAINING CELLULOSE.

ION forming part of Letters Patent No. 607,091, dated July 12, 1898.

23, 1896. Serial No. 563,426. (No specimens.) Patented in Norway November 24, 1894, No. 4,001; 1895, No. 45/338; in England May 30, 1895, No. 10,762; in France June 1, 1895, No. 247,863; 5, No. 50,342, and in Hungary December 3, 1895, No. 4,697.

oncern:

AR SIMONSEN, chem- of Sweden and Nor- hristiania, Norway, eful improvement ls containing cel- as mechanically l-pulp, sawdust, ture of spirit— tters Patent in mber 24, 1894; February 16, ated May 30, ted June 1, ed October 697, dated llowing is

e manu- om ma- onsists s here- ly set urse ap- lly or f-

ive cost of manufacture, led me to more closely investigate the subject, and I became convinced that practically no attempts had been made to determine the relations between the factors upon which the yield in alcohol is dependent—namely, the proportion and strength of the acid solution, the pressure or temperature and the time of boiling relatively to a given quantity of cellulose material, and the influence of said factors upon the yield in alcohol. This conviction induced me to experiment in order to determine, if possible, the best and most economical relations of these factors and their mutual influence upon the yield in alcohol with a view to a commercial and profitable utilization of cellulose materials, particularly waste cellulose materials. These experiments, made under varied conditions of the factors referred to and extending over a considerable period of time, (a full statement of which may be found in the *Zeitschrift fur Angewandte Chemie, Organ des Vereins Deutscher Chemiker*, edited by Prof. Dr. F. Fisher, published by J. Springer, Berlin, 1898, No. 9,) finally enabled me to establish their relations and mutual influence upon the yield in alcohol and devise a process suitable to the commercial production of alcohol from cellulose—namely, a process whereby a maximum yield in alcohol is obtained at a minimum cost.

Broadly speaking, the relations of the four factors in the inversion of cellulose materials on an economical and hence profitable basis should be as follows: Acid solution, from three o seven parts, by weight, to one part, by weight cellulose material; strength of solution, m four to eight tenths of one per cent.; ssure, from seven to ten atmospheres, and of boiling not to exceed one and one- hours. These relations or proportions however, vary within the limits given, riations depending upon the nature of lulose material used and the character cid.

manufacture of alcohol from specific materials—as, for instance, from I found that the most profitable re- btained under the following con-

- Early technology
  - crude, non-enzymatic
  - targeted glucose
- Still today most biorefinery approaches focus on cellulose

Glucocentric approaches

Glucose (noun glu·cose \ˈglü-ˌkōs, -ˌkōz\)
a crystalline sugar $C_6H_{12}O_6$**:** the sweet colorless soluble dextrorotatory form that occurs widely in nature and is the usual form in which carbohydrate is assimilated by animals

Centric (*adjective* cen·tric \ˈsen-trik\)
1 located in or at a centre <a *centric* point>
2 concentrated about or directed to a centre ’a *centric* activity’

Europe's 2G biorefinery

- Critical questions
  - Can sugar be made at competitive cost?
    - Sugar is currently at €220 per tonne
  - Are GHG emissions significantly reduced?
  - Is proper use made of carbon and molecular complexity?

Crescentino (Italy)

The advantages of Proesa™

- The [illegible] ared to oil [illegible] €/kg
- The balance between the CO_2 produced in the industrial cycle and that absorbed by the biomass feedstock is approximately neutral, with reference to Directive 2009/28/EC.
- The separated lignin is used to obtain energy.
- During the processing, biogas is generated as another energy source

$49 per barrel this morning

From betarenewables.com/proesa/what-is

Recalling hard facts

	Av. µg/mg
Cellulose	300
Arabinoxylan	186
Other neutral sugars	18.5
Uronic acid	31.6
Lignin	169

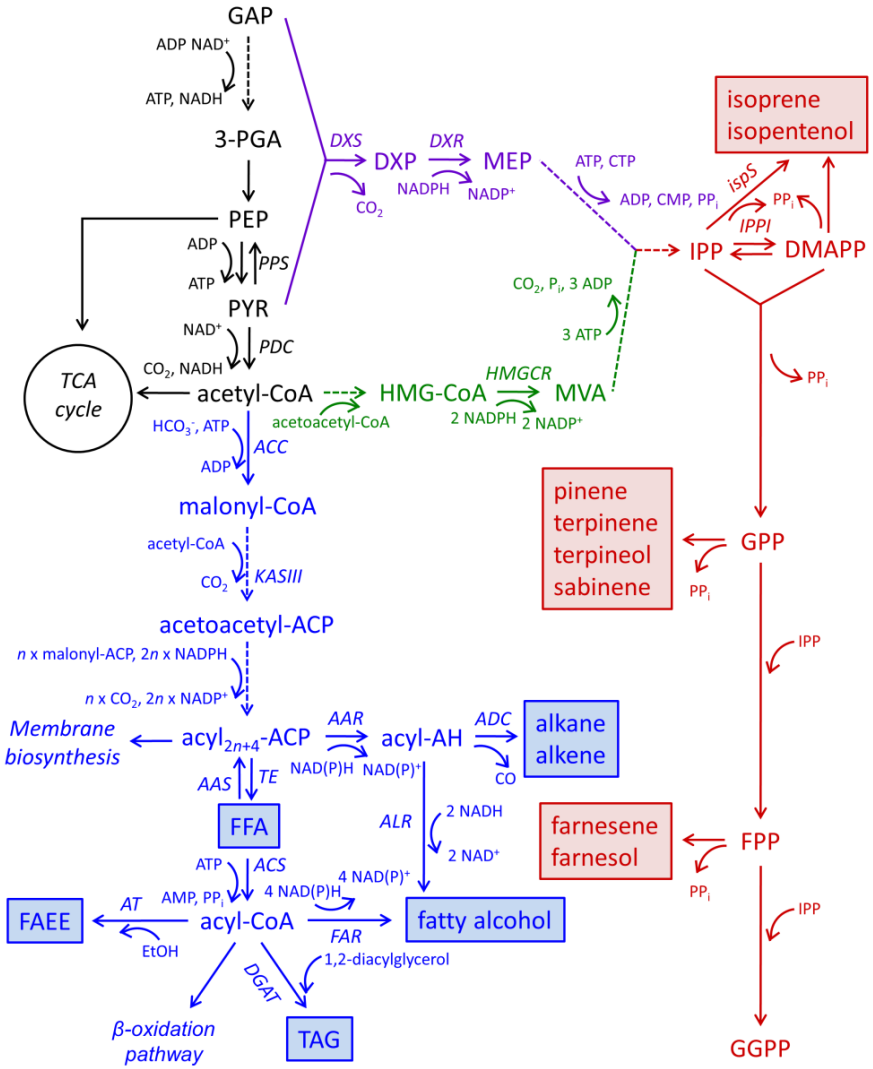
Variation in the chemical composition of wheat straw: the role of tissue ratio and composition
Collins et al. (2014) Biotech Biofuels 7:21

Combined lignin and hemicelluloses constitute approx the same proportion of biomass as glucose

Is it feasible to design solely glucocentric technology?

Why are we glucocentric?

- Glucose is intrinsically linked to energy-yielding metabolic pathways
- Many functions can be made from glucose
  - But how much does it cost?

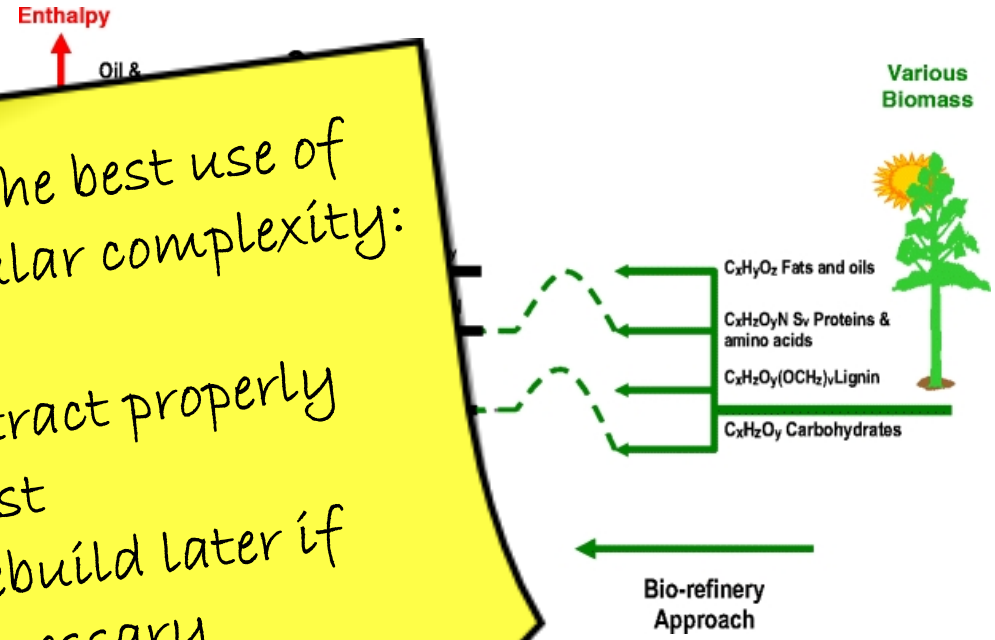


Biomass complexity

- Biomass components are complex molecules
  - Sugars are poly
  - Lignins are fun aromatics
  - Amino acids alre contain N

Make the best use of molecular complexity:

- Extract properly first
- Rebuild later if necessary



of industrial products—the use of

Appl Microbiol Biotechnol. 75(4): 751–762

A FEW WORDS ON BIOMASS PRETREATMENT

Isolating lignin and pentoses

Pretreatment process	Con		vantage
Acid treatment	Dilute minu temp		sis of furfural/hydroxymethyl need for recycling/costly
Alkaline treatment	Alkal		on of salts of calcium and sium
Ammonia treatment	Amm temp		al of ammonia/costly
Steam explosion	180° acid		destruction of pentoses and ion of inhibitors
Organosolv	Orga and I		s inhibit enzymatic sis/costly

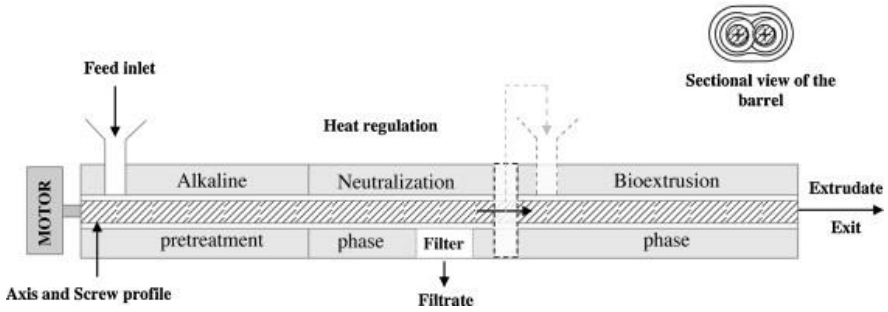
- Alkaline pretreatments break ester bonds and solubilize lignin
  - 1.5% w/w NaOH on wheat straw, residence time of 144 h at 20°C, released 60% release of lignin and 80% of hemicellulose

Influence of alkaline pre-treatments on the cell wall components of wheat straw
Sun et al (1995) Indus Crops Prod 4(2):127–145

- Many pretreatments lead to a mixture of glucose and pentoses
  - Industrial separation of monosaccharides is not easy
  - Co-fermentation technology is necessary

Extrusion technologies

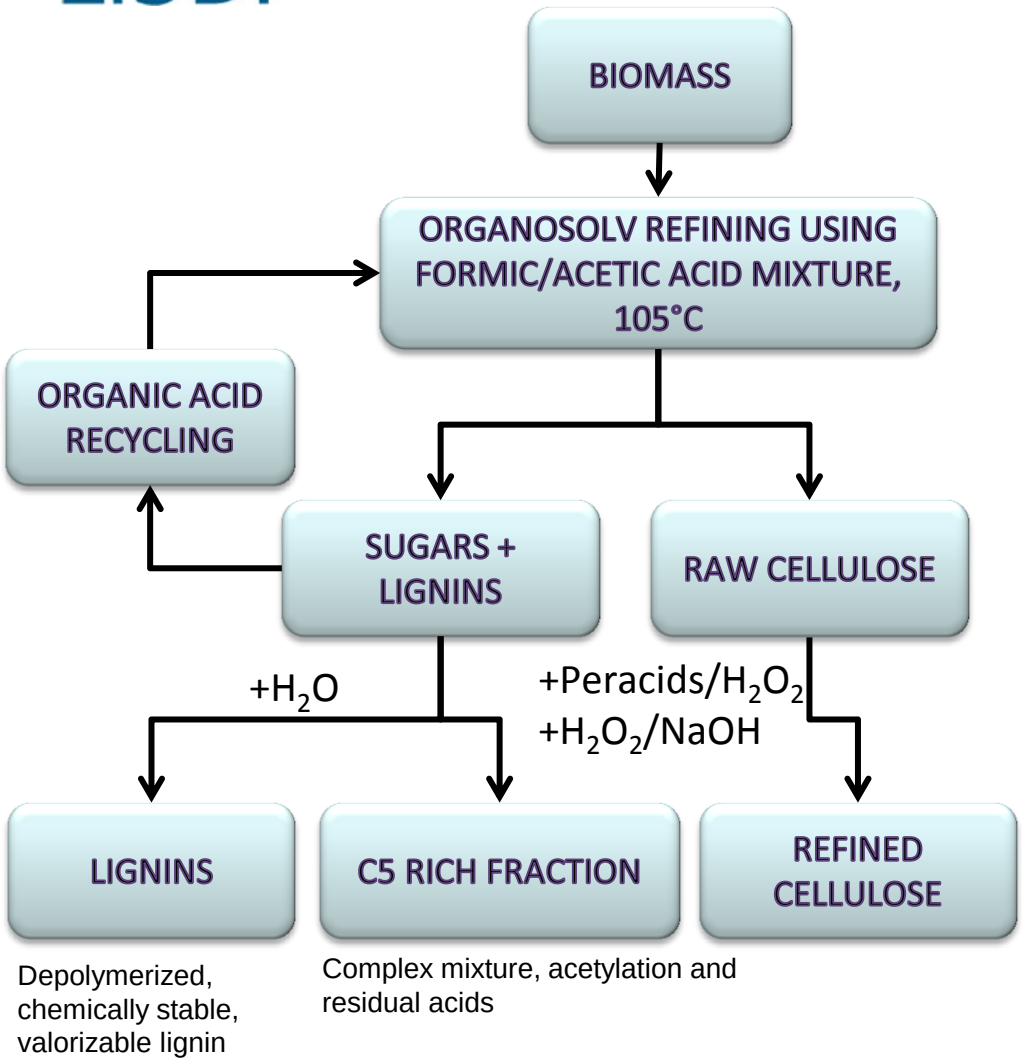
- Extrusion
  - Used in plasturgy
  - Food industry
- Force biomass through a die using a screw apparatus
  - High shear effects cause defibrillation
  - Dynamic filtering and solids content
  - Rapid thermal transfer
  - Mixing effect
- Often combined with alkaline treatment
- Combination with enzymes has been explored



A new lignocellulosic biomass deconstruction process combining thermo-mechano chemical action and bio-catalytic enzymatic hydrolysis in a twin-screw extruder
V. Vandenbossche et al. Industrial Crops and Products 55 (2014) 258–266

- Some lignin and HC released
  - but 7-13% alkali is low
- Further release of HC could be achieved by staged bioextrusion and a second filtration step

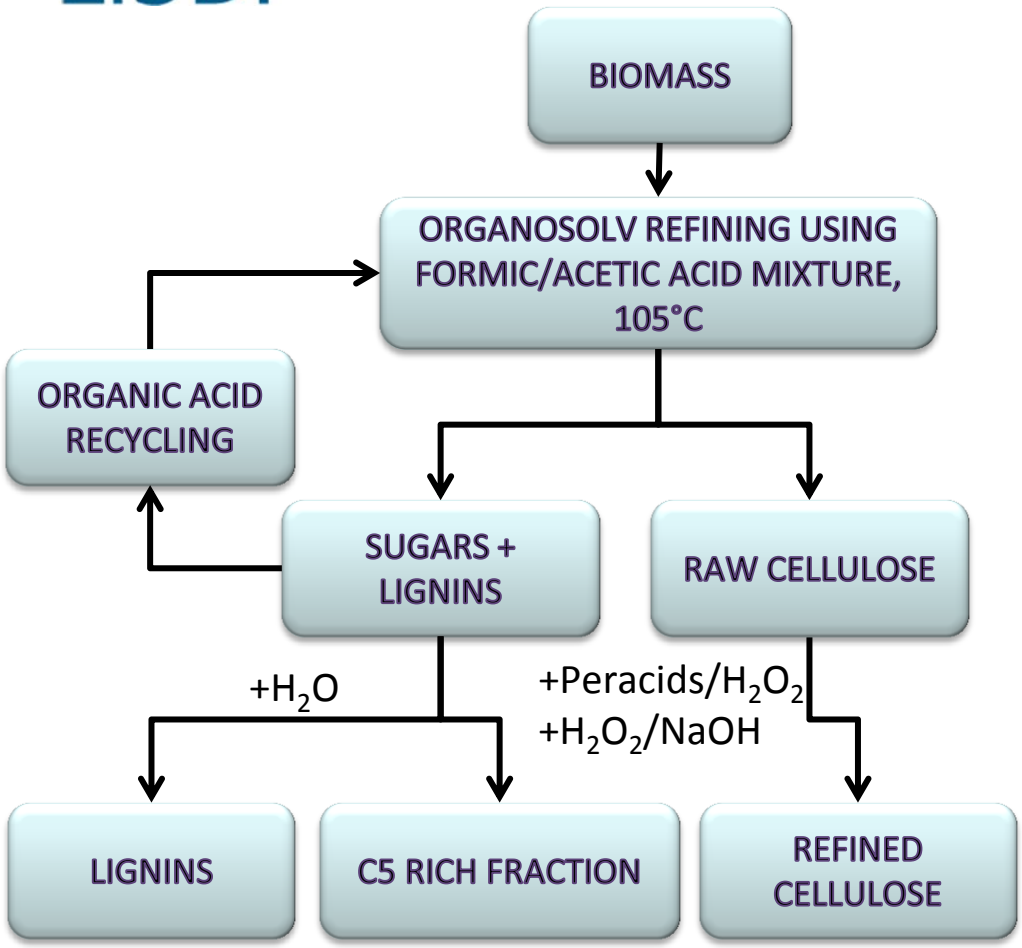
Organosolv technology



Depolymerized, chemically stable, valorizable lignin

Complex mixture, acetylation and residual acids

Organsolv technology



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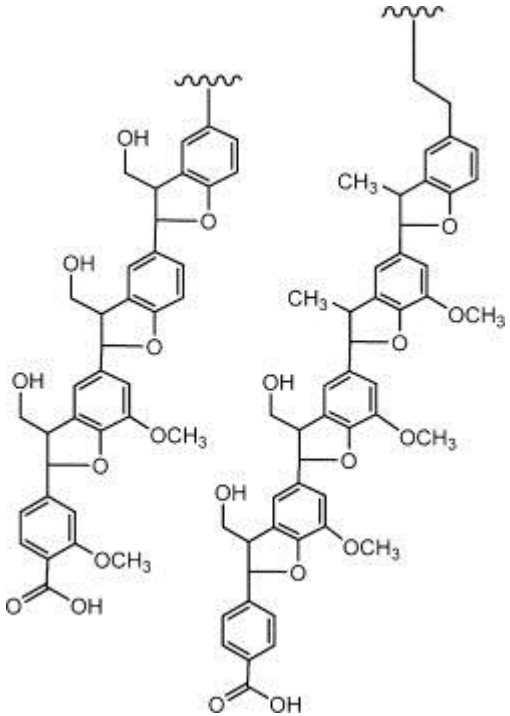


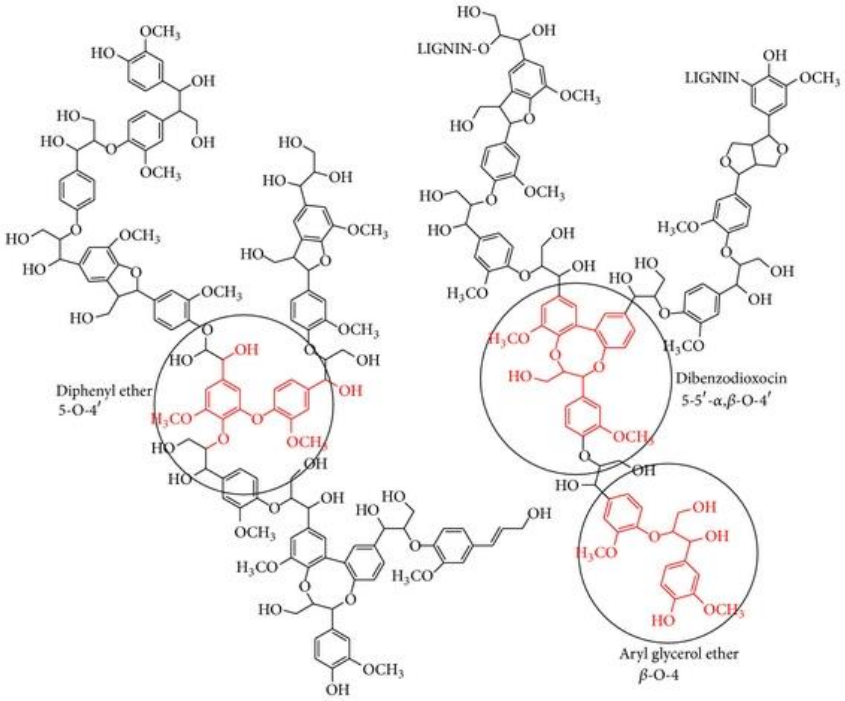
Functionality of wheat straw lignin extracted in organic acid media
Delmas et al. J Appl Polymer Sci 121(1): 491-501

A LITTLE ABOUT LIGNINS

Lignin : a great combustible

'You can do anything with lignin except make money'

- Over 50 Mt lignin produced and burned per annum
  - >1 Mt commercialized
  - 26.7 MJ/kg (Kraft)
  - <€52 per ton (based on natural gas price)
- The process determines the product and the application
  - With or without sulphur
  - HMW or LMW
  - Water soluble or insoluble
  - Condensed or not

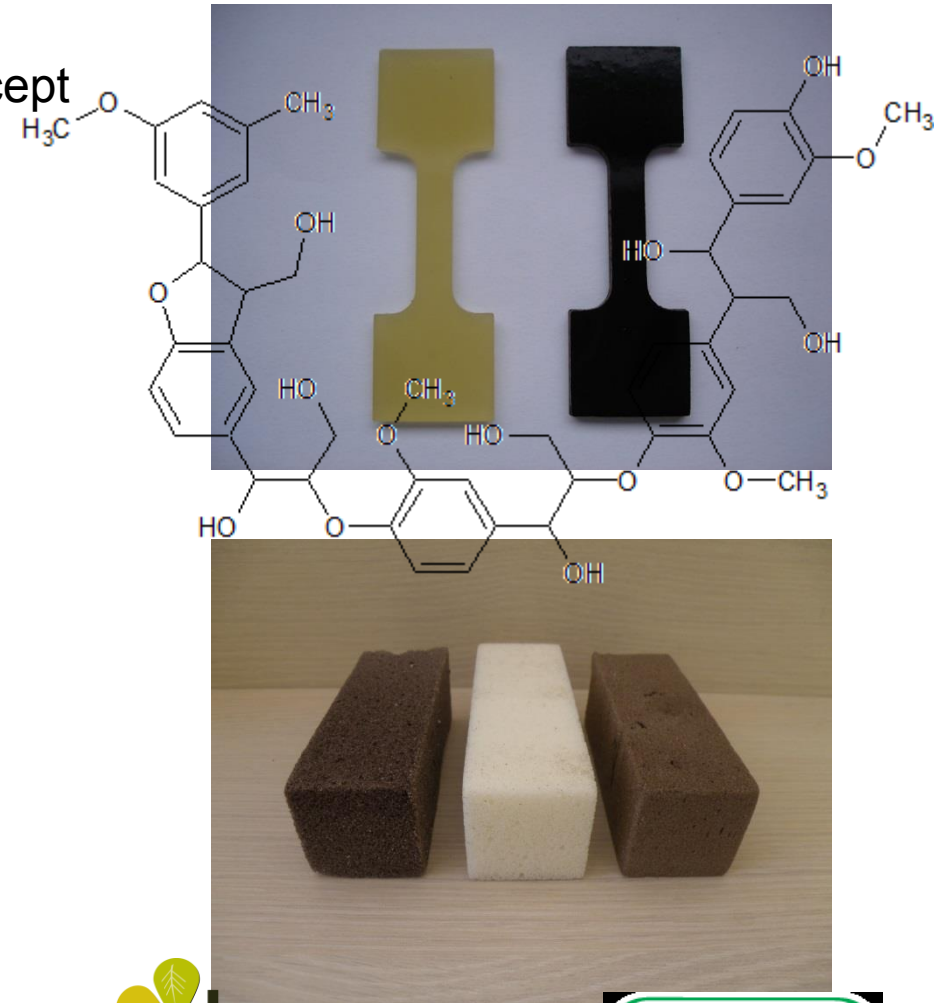


Wang et al. Journal of Applied Chemistry (2013) http://dx.doi.org/10.1155/2013/838645

Intermediates to products

'You can do anything with lignin except make money'

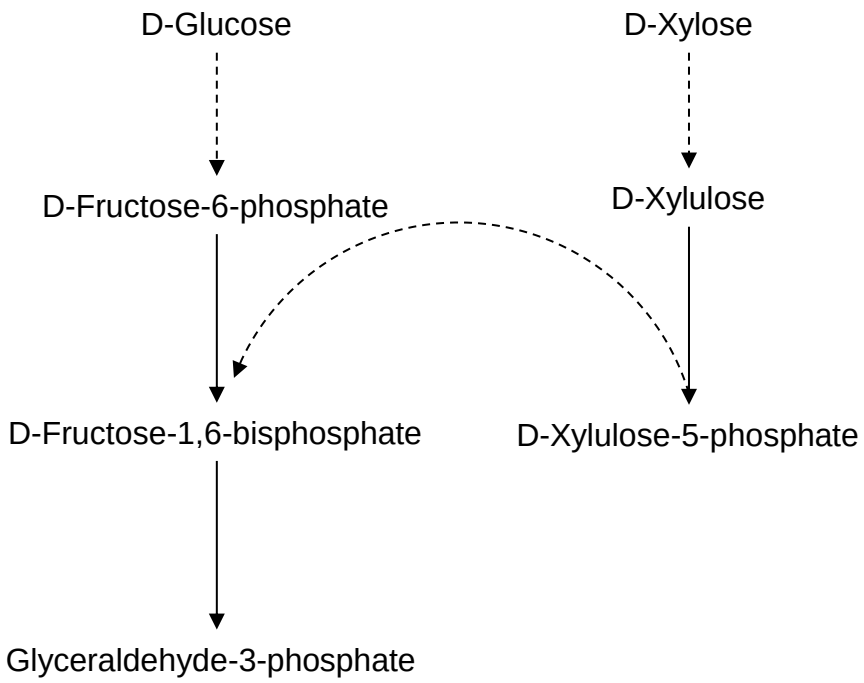
- Organosolv lignins can be useful chemicals
  - Polyurethane components
  - Active bulking agents
  - Carbon black substitute
- Lignin can be a source of precious phenolics
  - Market price of phenol is >€1000 per tonne



SPOTLIGHT ON THE PENTOSE FIELD

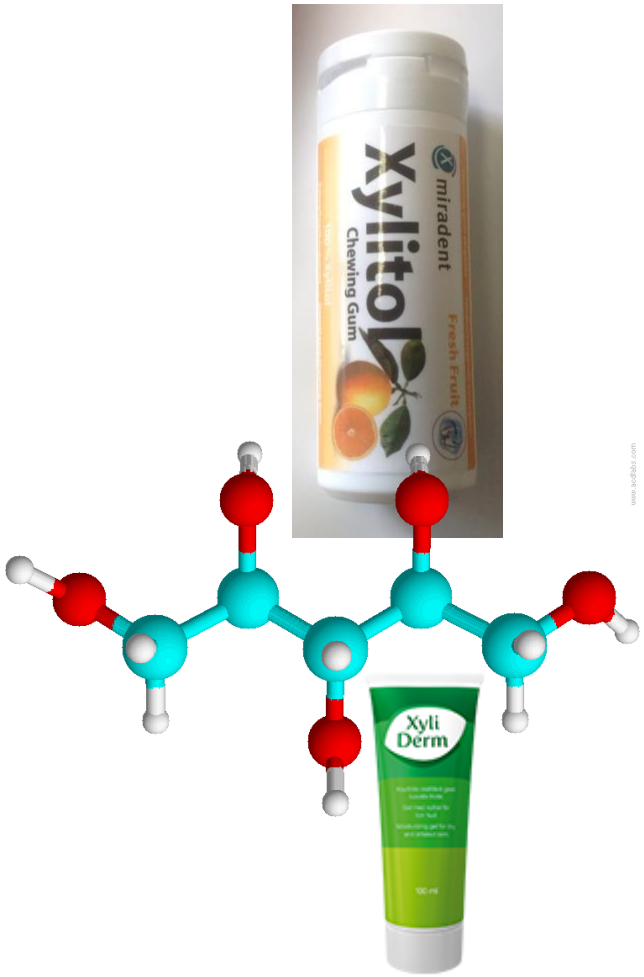
Pentoses, poor man's glucose?

- Xylose is generally considered as a top-up for glucose
  - Approx 1/3 more ethanol
  - Important in view of policy frameworks
- Even in Nature xylose backs up glucose
  - Pentose phosphate pathway
  - Diauxic phenomenon
- But you have to be rich to purchase it!
  - Circa €4500 t^{-1}



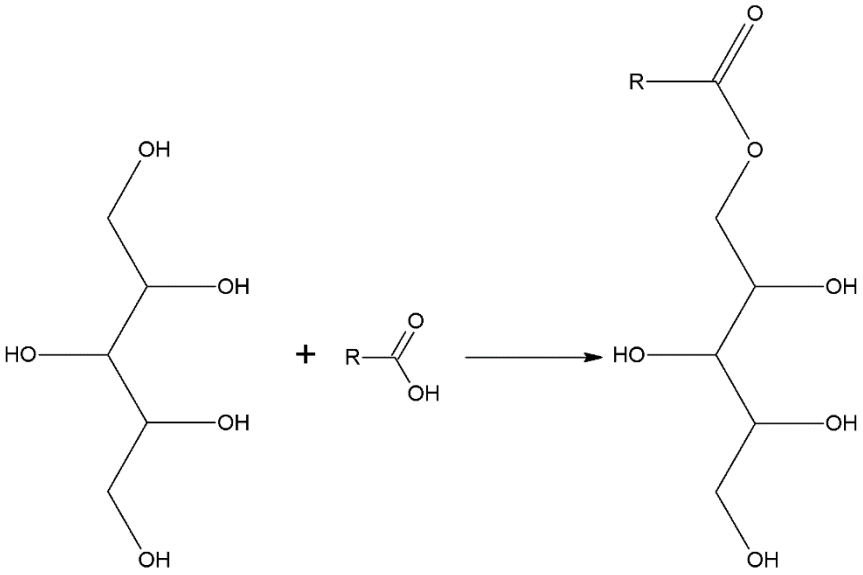
Pentitols:valuable intermediates?

- Xylitol
  - One of the top 12 chemicals from biomass
  - 200,000 $t.yr^{-1}$, circa €5000 t^{-1}
  - Hydrogenation of xylose
    - Raney Ni catalysis
    - Bioconversion (1,01 g/g D-xylose and no CO_2 is produced)
    - VTT possess strains producing 160 $g.L^{-1}$, 2 $g.L^{-1}.h^{-1}$ and >0.9 g/g D-xylose (>200 $g.L^{-1}$ for economic viability)
  - Multiple markets
    - low calorie sweetener
      - confectionary and pharmaceuticals
    - toothpaste, fluoride tablets and mouthwashes
    - polymers



Xylitol monoesters

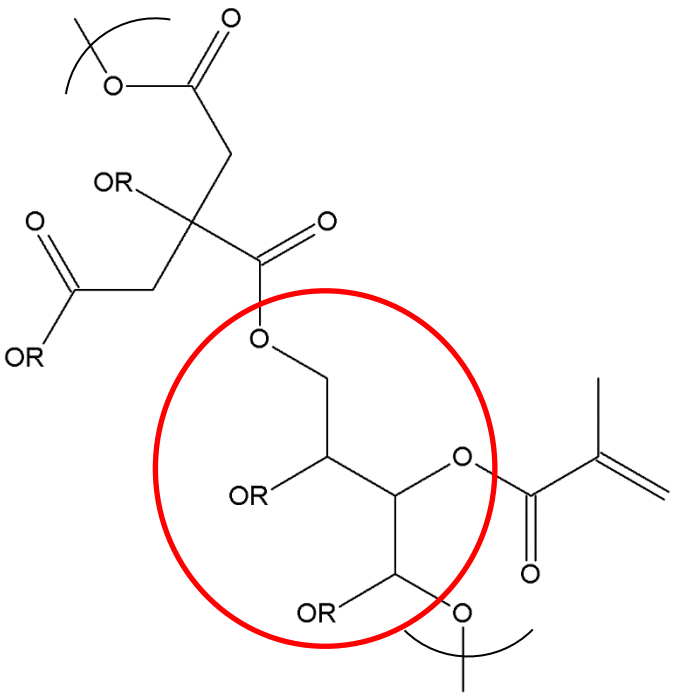
- Xylitol monoesters
  - nonionic surfactants with high emulsifying properties
  - Possibly therapeutic properties (e.g for cancer)



Rufino et al (2009) J. Chem Technol Biotechnol 84:957-960

Xylitol-based polymers

- Xylitol as a polymer building block
  - Biocompatible hydrogels
  - Biodegradable polyesters
    - Copolymerization

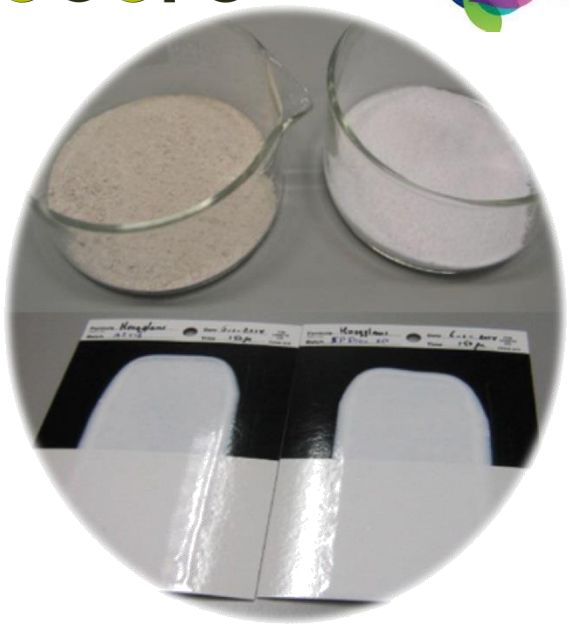


PXC –xylitol and citric acid-based acrylate
Bruggeman et al. (2008) Adv. Mat. 20:1922-1927

Pentose bioconversions

D-xylose $\longrightarrow$ Itaconic acid+ H_2 + H_2O
Theoretical yield of 0.87 g itaconic/g xylose

- Certain bioconversions make good us of 5-carbon sugars
  - No CO_2 produced
  - Uptake of xylose and arabinose remains a challenge
  - Currently 1000 $t.yr^{-1}$

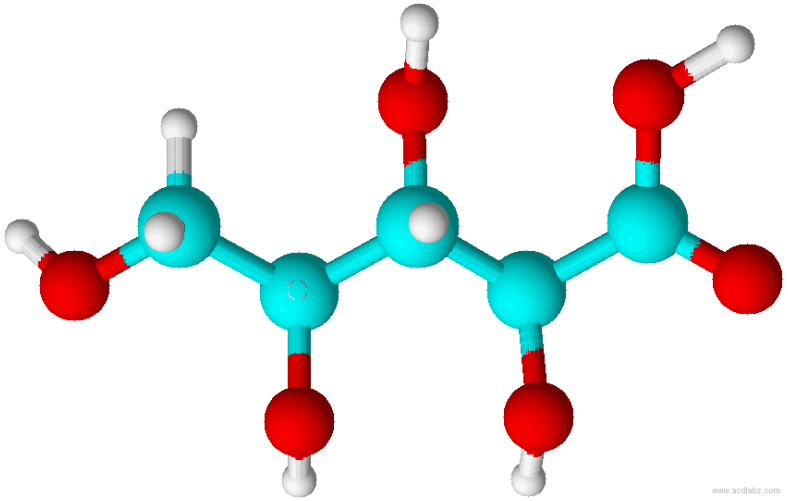


Curtesy of DSM – the BIOCORE project

October 6 2015, Leaf technologies and Dutch DNA announced a R&D collaboration to produce itaconic acid

Pentose bioconversions

- Xylonic acid
  - Among top 30 chemicals from biomass
  - food industry (substitute for gluconic acid)
  - cement additive
  - building block chemical (ex. polyamide)
  - Precursor for 1,2,4-Butanetriol
  - VTT possess strains producing160 $g.L^{-1}$, 1.4 $g.L^{-1}.h^{-1}$ and >0.9 g/g D-xylose
    - Operate even at pH 3.0



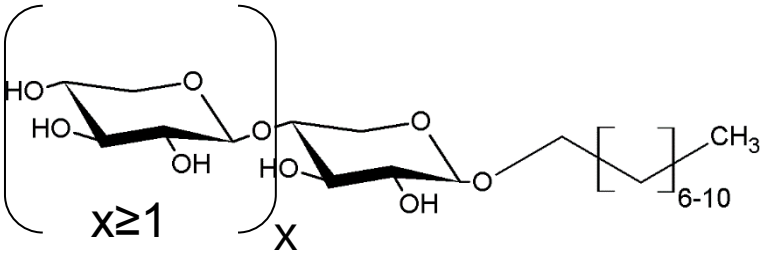
Alkylpolypentosides

- APGs – alkylpolyglycosides
  - Mostly based on glucose
  - 50,000 $t.yr^{-1}$
  - Surfactants used in cosmetics and body care products
- Synthesis using Fischer glycosylation
  - Synthesis of APPs requires less energy (80°C instead of 120°C for glucose)

Development of Agriculture Left-Overs: Fine Organic Chemicals from Wheat Hemicellulose-Derived Pentoses
Martel et al. (2010) Top Curr Chem 294: 79–115

Evaluation of the transglycosylation activities of a GH 39 β-D-xylosidase for the synthesis of xylose-based glycosides
Muzard et al (2009) J. Mol. Catal. B 58:1–5

- Enzymatic synthesis has been demonstrated
  - Use of glycosyl hydrolases and thermodynamic control
    - BhXyl39, only short fatty alcohols
    - TxXyn11 up to octyl with **hydrophilic moiety DP>1**



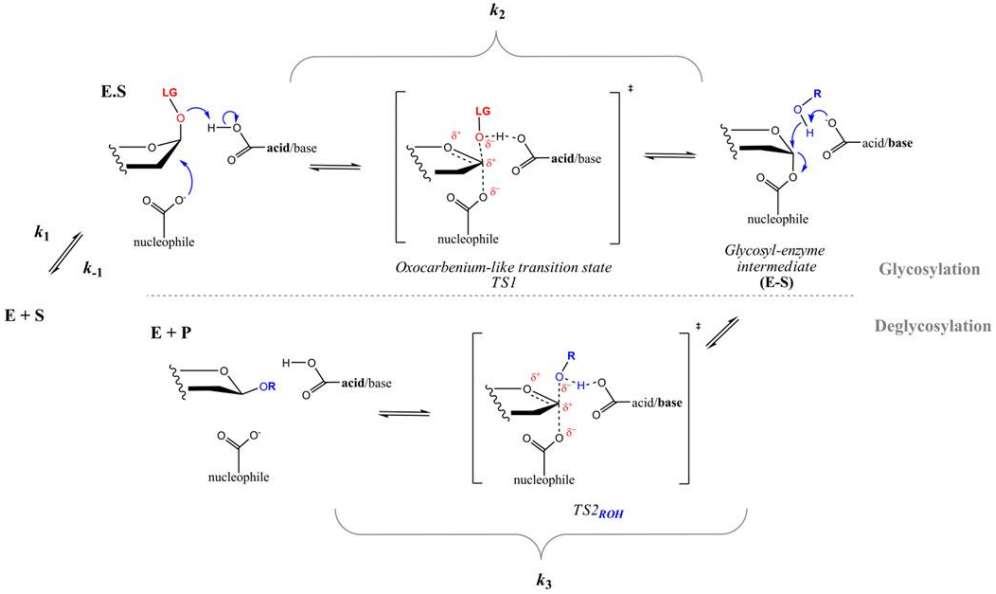
Enzymatic synthesis of alkyl β-D-xylosides and oligoxylosides from xylans and from hydrothermally pretreated wheat bran
Ochs et al (2010) Green Chem 13:2380

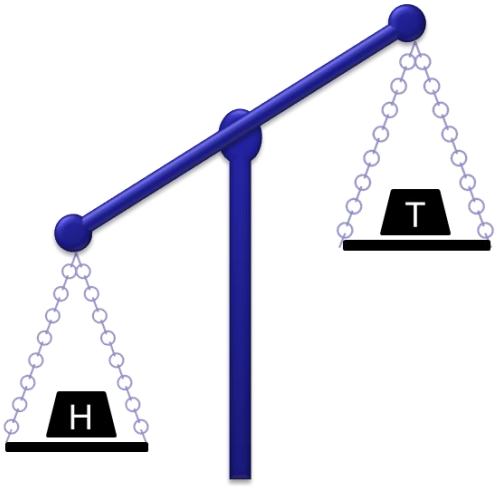
Spotlight on work in Toulouse

DEVELOPING PENTOSE-SPECIFIC TOOLS

Towards better glycosyl transfer

- Many retaining GHs perform glycoslyation
- The majority of GHs are active on hexoses
  - All known naturally occurring non-Leloir transglycosylases are trans**hexosidases** or **hexos**ylases
- Most GH are primarily hydrolytic enzymes





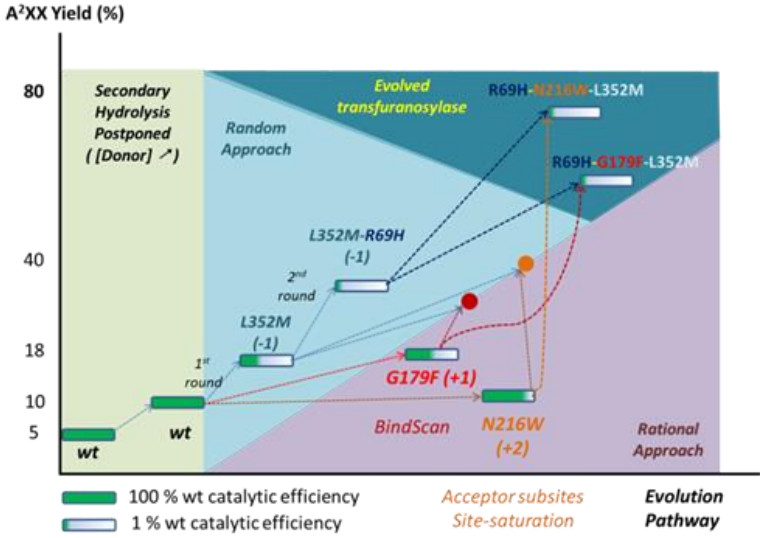
Tipping the H/T balance

Some questions and ideas

- Naturally occurring TGs
  - But how do they work?
  - No obvious structure/function relationships
- Reduce water access?
  - 55M in aqueous solutions
- Increase affinity for other acceptors
  - Favour the docking of fatty alcohols or sugars
- Combine both with reduced potency

Engineering TxAbf

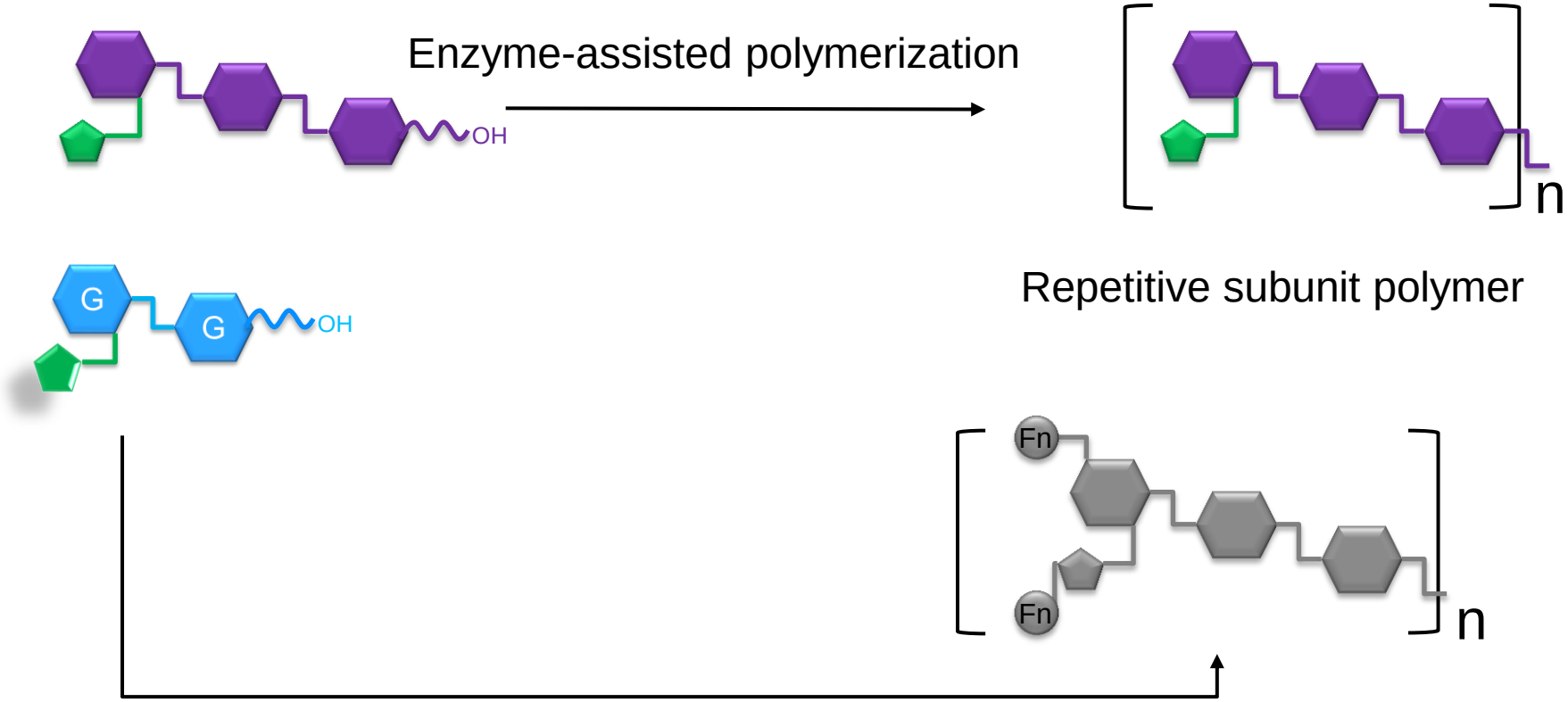
- Combinatorial approach
  - Random mutagenesis to lower hydrolysis
  - Targeted mutagenesis to select for better +1 subsite interactions and transglycosylation



Glycosynthesis in a Waterworld: new insight into the molecular basis of transglycosylation in retaining glycoside hydrolases
Bissaro et al (2015) Biochem J. 467, 17–35

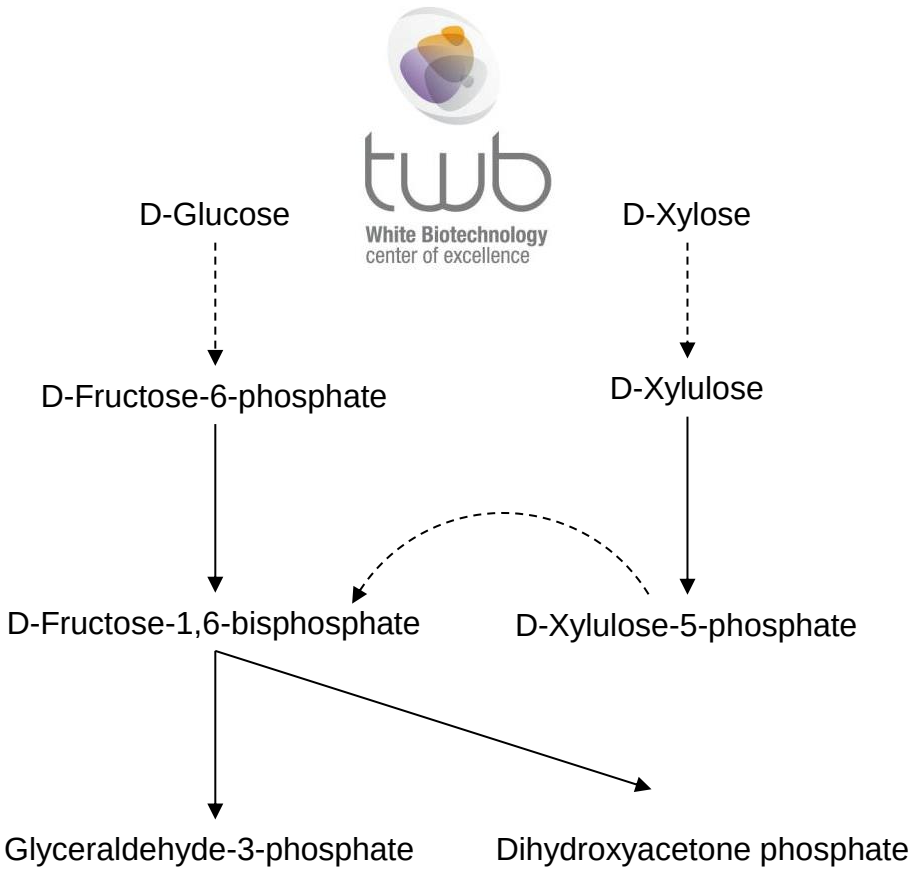
Molecular design of non-leloir furanose-transferring enzymes From an α-l-arabinofuranosidase: A rationale for the engineering of evolved transglycosylases
Bissaro et al (2015) ACS Catal. 2015, 5, 4598−4611

Towards tailored polymers?



Stepping out of the box

- Synthetic biology provides the means to move out of the natural framework
  - Disconnect xylose metabolism from glucose metabolism



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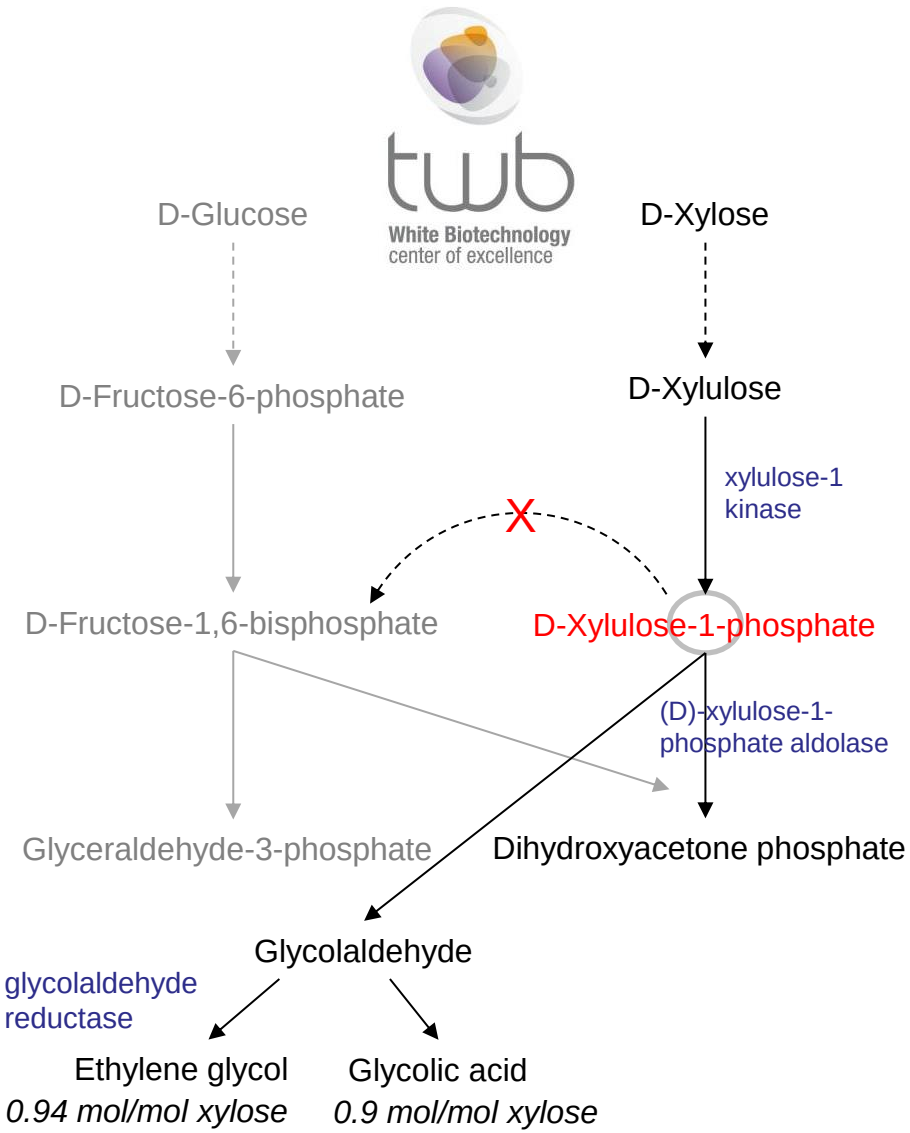

Engineering of a Synthetic Metabolic Pathway for the Assimilation of (d)-Xylose into Value-Added Chemicals
Cam et al. (2015) ACS Synth Biol (ePub)
Optimization of ethylene glycol production from (D)-xylose via a synthetic pathway implemented in Escherichia coli
Alkim et al (2015) Microb. Cell Fact 14:127

Conclusions

"C5 sugars …(xylose and arabinose) have the potential to be outstanding building blocks for commodity chemicals. One challenge will be getting a relatively clean feed stream of these sugars"

- Xylose (and arabinose) production is insufficient
  - Most is burnt (black liquor)
  - Cellulosic ethanol industry will consume it directly
    - No policy incentive to do otherwise
    - Most technologies won't allow alternatives
  - Current price >€1500 t^{-1}
    - Unrealistic simply due to scarcity
- Progress in biotechnology will be a source of innovation
  - Ceative use of synthetic biology
  - New enzyme technologies

THANK YOU FOR LISTENING

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LISBP LABORATOIRE D'INGÉNIERIE DES SYSTÈMES BIOLOGIQUES ET DES PROCÉDÉS

INRA SCIENCE & IMPACT

cnrs dépasser les frontières

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