BioREFINE-2G Workshop:
Bioplastics from 2nd Generation Biorefineries
York, June 5, 2015

Process evaluation of industrial strains

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Layout of talk

- **Biomass-derived substrates**
  - Biomass
  - Available streams
- **Process evaluation**
  - Comparing strains
- **Conclusions**

Biorefine-2G – overall goals

Novel 2nd generation biorefinery concept using industrial yeast as production organism for the production of diacids and diacid derived biopolymers ***from side and waste streams rich in C5 sugar*** *and mixtures of C5/C6 sugars.*

Side and waste streams..

These are likely to contain:

- Multiple sugars
- Other (unwanted) compounds in the medium
- Particles..

So

- The production organisms needs to work in a multi-sugar mixture with inhibitors under process conditions
- Suitable and tailored modes of fermentation operation are needed
- Efficient methods for recovery and purification of acids from the broth needed

”Biomass” is not a species..

They look different..

Bagasse (from*Saccharum officinarum*)

Eucalyptus

Picea abies

Pinus taeda

Their compositions are different..

Type	Plant	Glucan	Xylan	Arabinan	Mannan	Lignin	Ref.
Hard wood	Poplar	49.9	17.4	1.8	4.7	18.1	Wiselogel et al., 1996
	Eucalyptus	46.1	17.1	0.8	0.4	19.8	Rencoret et al., 2010
Soft wood	Douglas-Fir	43	3.0	1	13.0	28	Mabee et al., 2006
	Spruce	43.4	4.9	1.1	12.0	28.1	Tengborg et al., 1998
Crop residues	Wheat straw	38.2	21.2	2.5	0.3	23.4	Wiselogel et al., 1996
	Corn stover	35.6	18.9	2.9	0.3	12.3	Hayn et al., 1993
	Sugarcane Bagasse	39.0	22.1	2.1	0.4	23.1	DOE, USA
Dedicated crops	Switch grass	31.0	20.4	2.8	0.3	17.6	Wiselogel et al., 1996
	Miscanthus	39.5[c]	19.0[c]	1.8[c]	NR	24.1	Vrije et al., 2002
	Arundo donax L.	39.3	18.4	1.2	0.2	26.2	Bura et al., 2012

What streams are there?

Possible streams include:

- **Streams from the food industry**
  - Less likely to contain much C5.
- **Streams from the forest industry**
  - Black liquor (from Kraft cooking)
    - This is normally burnt for recovery of chemicals
  - Liquor from sulfite cooking (SSL = spent sulfite liquor)
    - This is often fermented. In addition, lignosulphonates are recovered.
- **Streams from 2nd generation ethanol production**

Kraft pulping

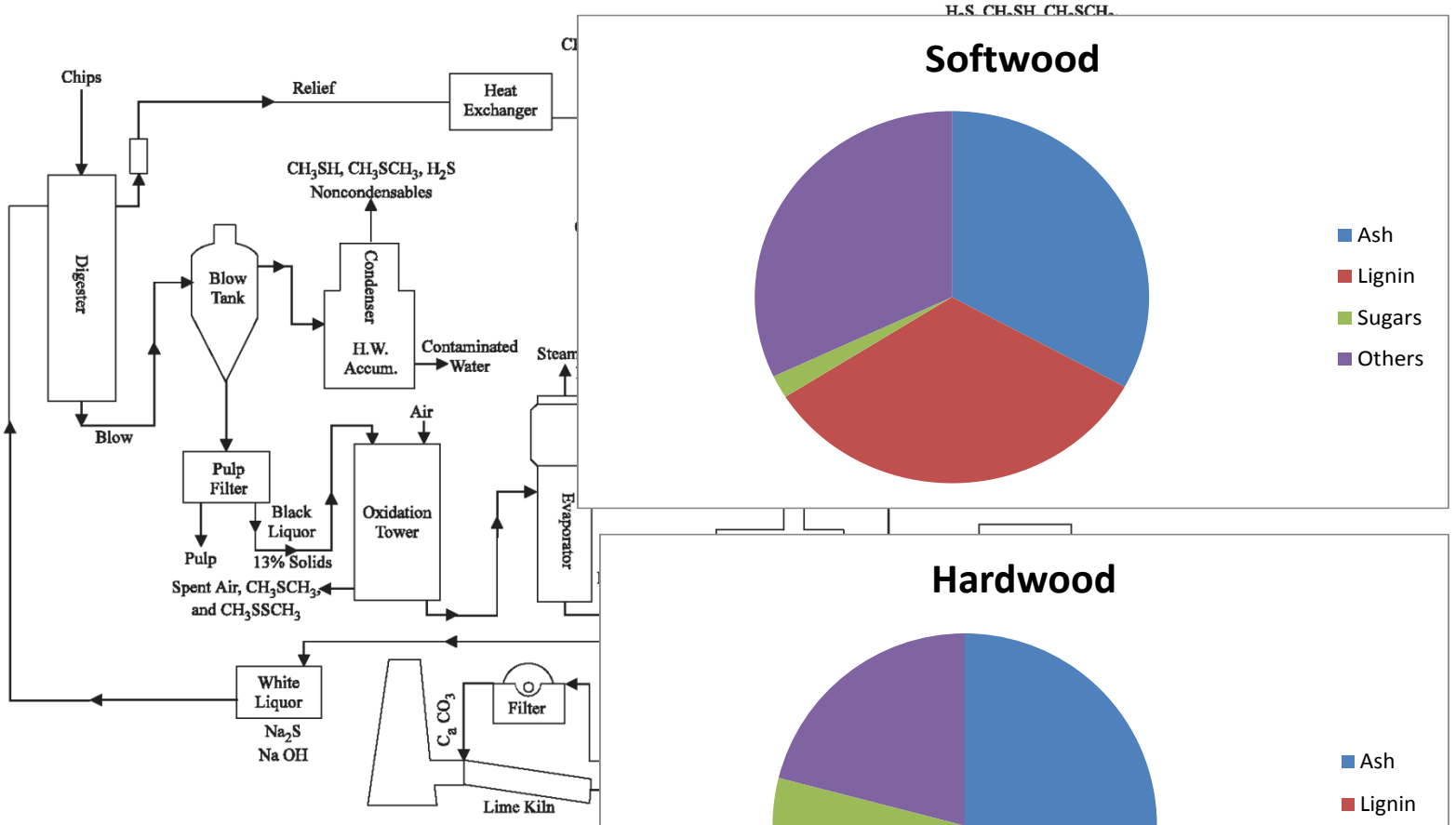
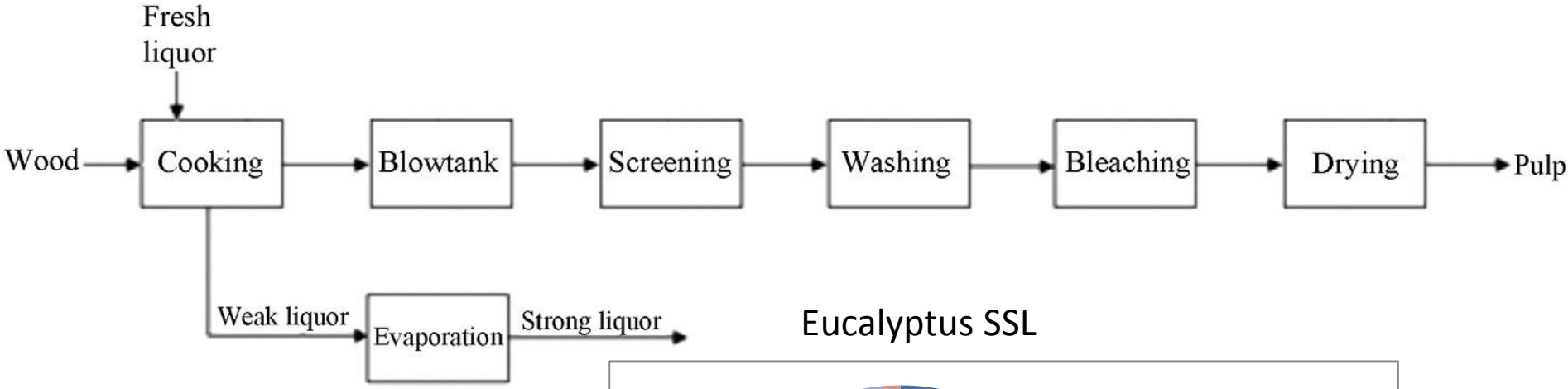
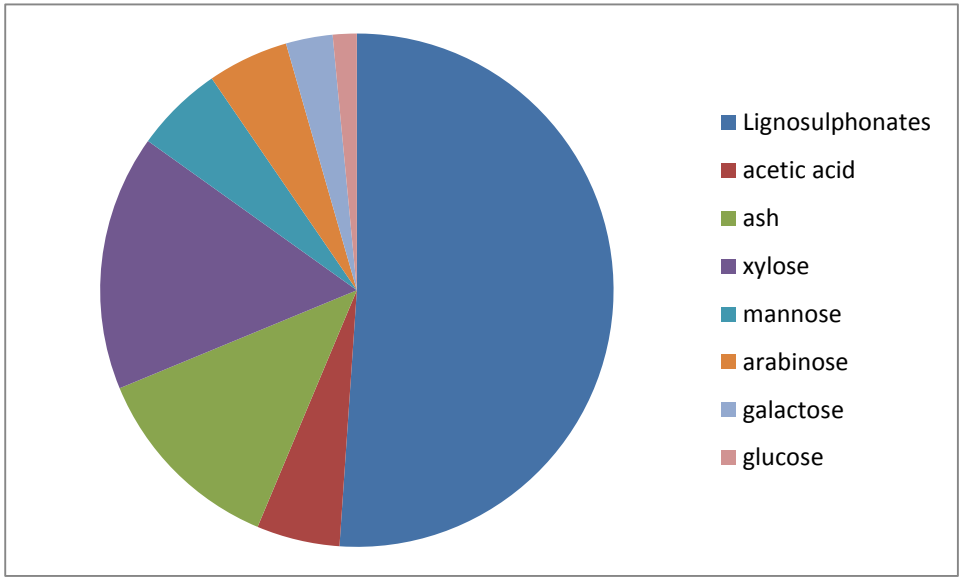


Figure 10.2-1. Typical kraft sulfate

Sulfite process

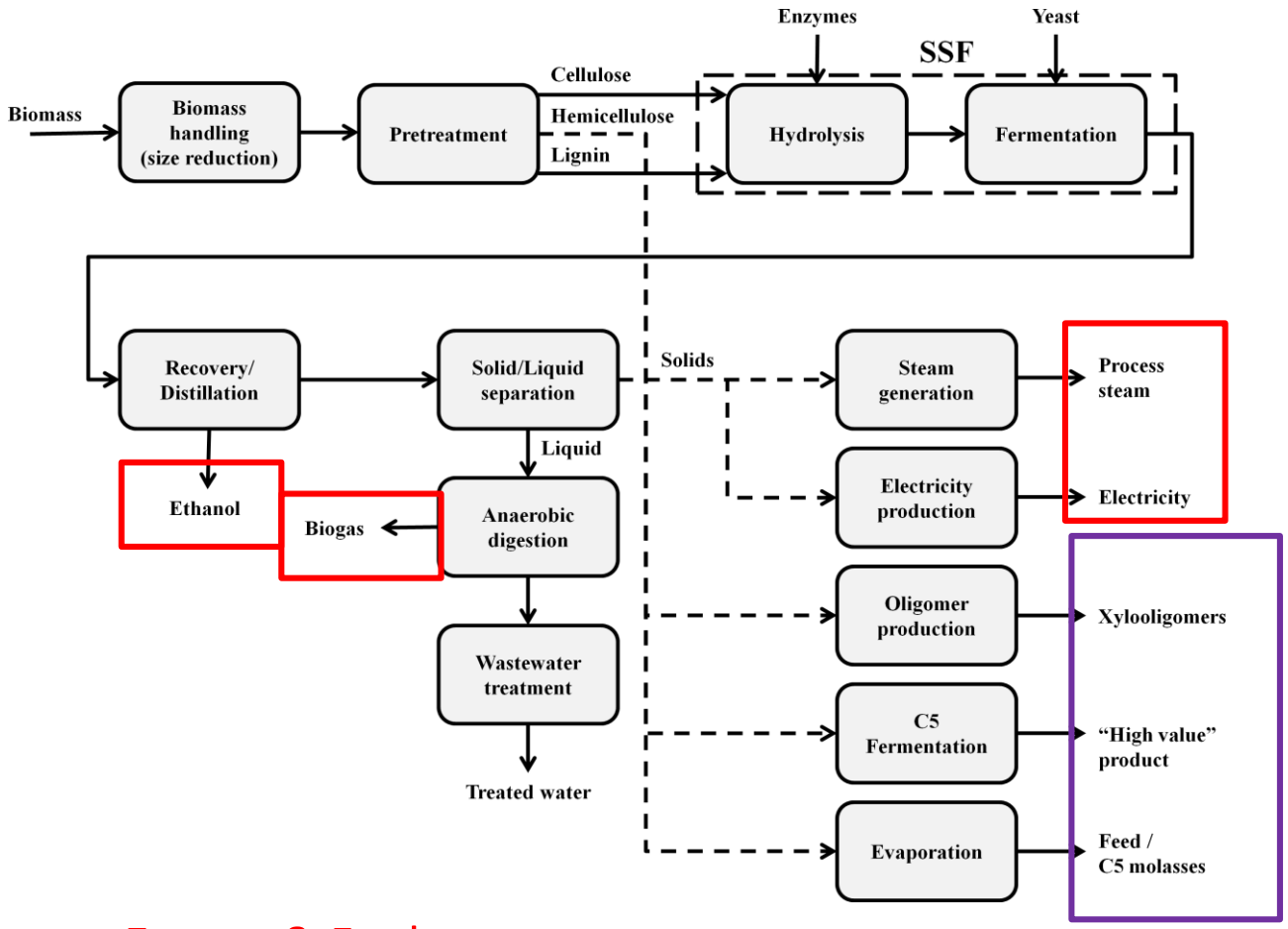


Eucalyptus SSL



Xavier et al, Bioresource Technology 101 (2010) 2755–2761

2nd generation ethanol biorefinery



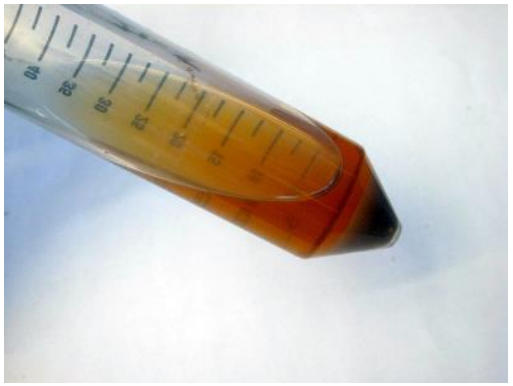
Mutturi et al.,"Bioethanol focused biorefineries" in Advances in Biorefineries,. Woodhead publishing, 2014

BALI™ project in a nutshell

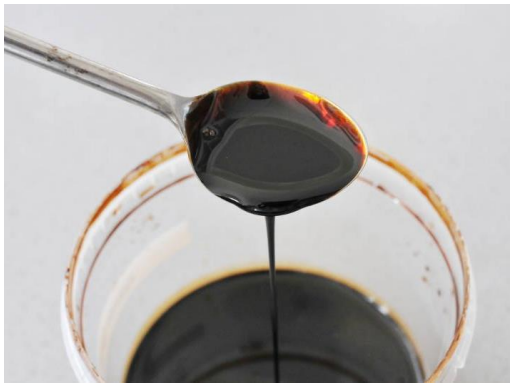
- **The main goal in the BALI™ project is efficient co-production of**
  - Sugar solutions by enzymatic hydrolysis of pretreated biomass (annual plants, hardwood, softwood).
  - commercial lignosulfonates from new feedstocks.

Bagasse

Cellulose with low lignin content

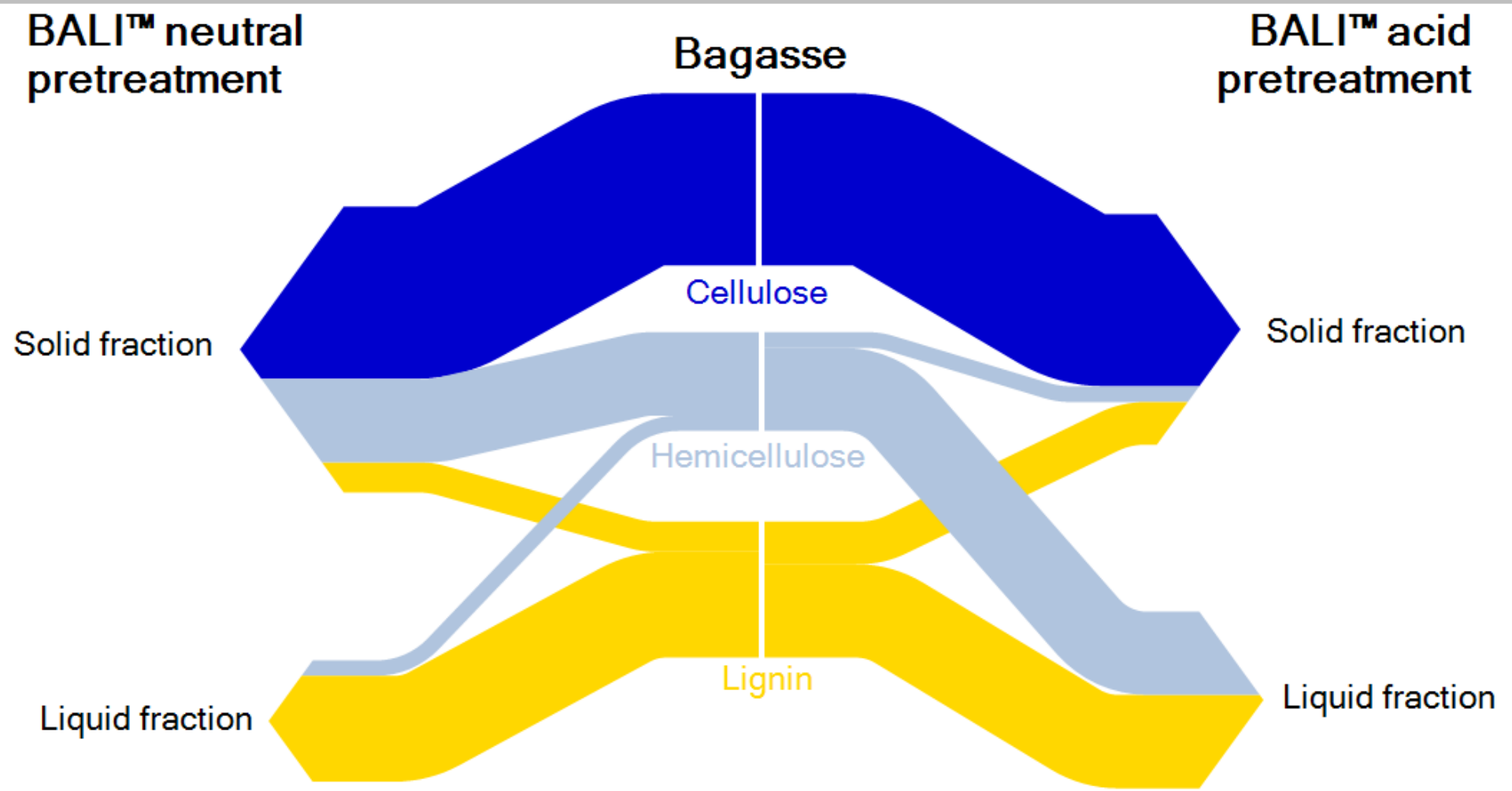


Sugar in solution



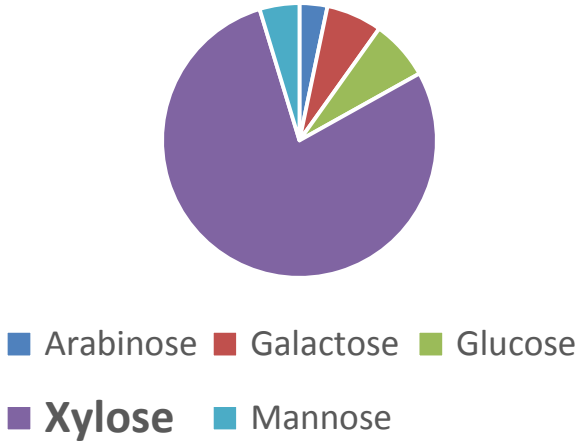
Water soluble lignin

BALI™ neutral vs. acid pretreatment

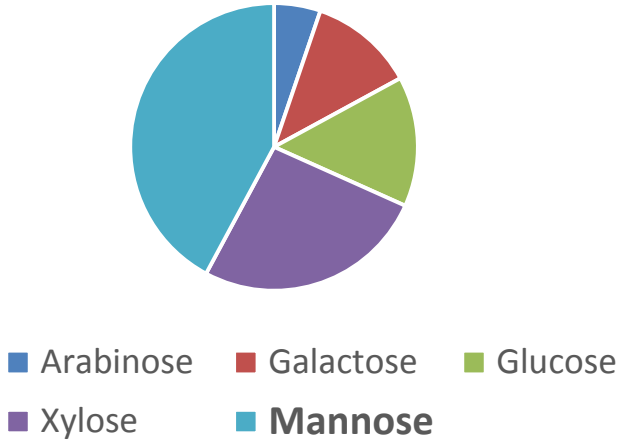


Substrates - relative sugar contents

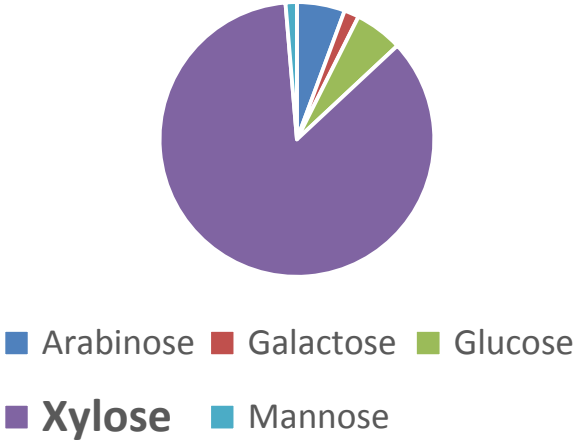
Eucalyptus, SSL



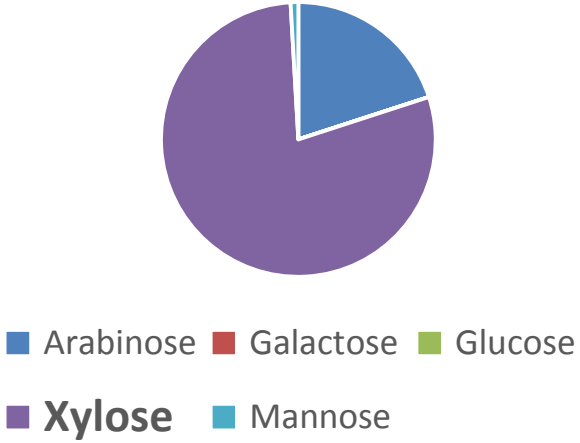
Loblolly pine, BALI



Bagasse, BALI



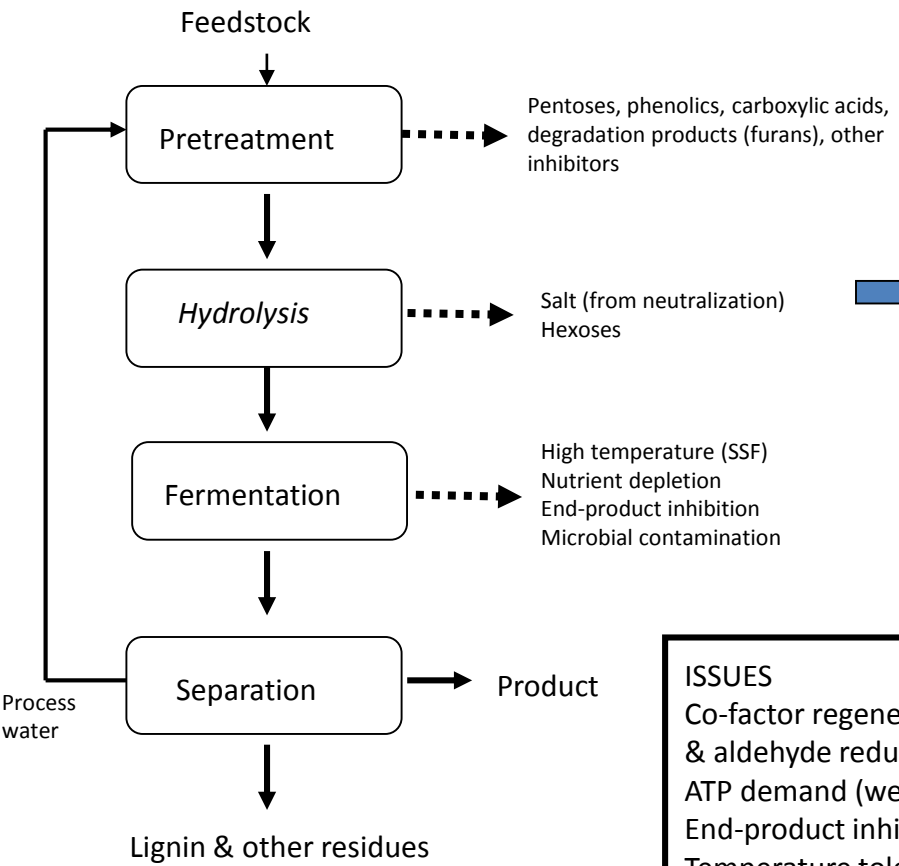
Spruce - fermented SSL

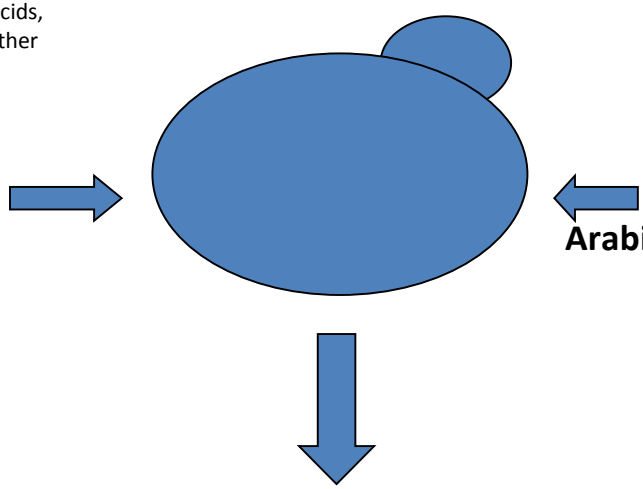


Lignocellulose conversion – Fermentation challenges

Environmental factors

Genetic factors





Xylose pathway

- oxidation/reduction
  XR/XDH/XK
- isomerization
  XI/XK

Arabinose pathway

- Oxidation/reduction
  XR/LAD/LXR/XDH
- Isomerization
  AI/RK/RE

Activation of PPP

ISSUES
Co-factor regeneration (xylose & arabinose pathways & aldehyde reduction)
ATP demand (weak acid & transport)
End-product inhibition
Temperature tolerance
Osmotic stress & compatible solute formation
Other uncharacterized toxic effects
Competition/contamination

Almeida et al. Biotechnol. J, 3, 286-299, 2011

***Saccharomyces cerevisiae* is a species.**

However, strain behaviour can be very different!

Substrate inhibition test

- **The least inhibiting substrate was the fermented spruce SSL, whereas both bagasse and Eucalyptus were more inhibiting.**
- **Relatively large differences between strains were seen.**
- **Out of 8 tested strains, the best strain for each substrate varied.**

Take home message

- **The feedstock determines the properties of the sugar stream!**
- **Pretreatment also..**
- **Selection of strain**
  - The choice of strain is *substrate specific*.
  - You need to assess strains in **YOUR substrate!**
- **Issues to consider**
  - LS
  - Salts (osmotic stress)
  - Acids (ATP decoupling)
  - Furans (not really in SSL..)
  - Phenolics

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