Lignins and Pentoses

biorefinery waste streams or chemical intermediates?

Michael J. O’DONOHUE
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2. A few words on biomass pretreatment
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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

- **Early technology**
  - crude, non-enzymatic
  - targeted glucose

- **Still today most biorefinery approaches focus on cellulose**

**Glucocentric approaches**

Glucose (noun *glu· cose* \(\text{ˈglü-ˌkōs, -ˌkōz}\)) a crystalline sugar \(\text{C}_6\text{H}_{12}\text{O}_6\): 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* \(\text{ˈsən-trık}\))
1 located in or at a centre `<a centric point>`
2 concentrated about or directed to a centre 'a centric activity'
• 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?

The advantages of Proesa™
• The cost of the product is competitive compared to oil $49 per barrel this morning €/kg
• The balance between the CO$_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.

From betarenewables.com/proesa/what-is
Recalling hard facts

<table>
<thead>
<tr>
<th>Component</th>
<th>Av. µg/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>300</td>
</tr>
<tr>
<td>Arabinobioxylan</td>
<td>186</td>
</tr>
<tr>
<td>Other neutral sugars</td>
<td>18.5</td>
</tr>
<tr>
<td>Uronic acid</td>
<td>31.6</td>
</tr>
<tr>
<td>Lignin</td>
<td>169</td>
</tr>
</tbody>
</table>

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 in the manufacture of industrial products—the use of proteins and amino acids

© Springer-Verlag 2007

Biomass components are complex molecules
- Sugars are polyoxygenated
- Lignins are functionalized aromatics
- Amino acids already contain N

Make the best use of molecular complexity:
- Extract properly first
- Rebuild later if necessary

Enthalpy

Oil & Gas

Various Biomass

Bio-refinery Approach

C₆H₁₀O₫ Fats and oils
C₆H₆O₇ N Sr Proteins & amino acids
C₅H₈O₇Lignin
C₆H₁₂O₇ Carbohydrates
A FEW WORDS ON BIOMASS PRETREATMENT
### Isolating lignin and pentoses

<table>
<thead>
<tr>
<th>Pretreatment process</th>
<th>Conditions</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid treatment</td>
<td>Dilute/concentrated acid/few minutes/high and low temperature</td>
<td>Increase in porosity/increased enzymatic hydrolysis</td>
<td>Synthesis of furfural/hydroxymethyl furfural/need for recycling/costly</td>
</tr>
<tr>
<td>Alkaline treatment</td>
<td>Alkali treatment at NTP</td>
<td>Removal of lignin/hemicellulose hydrolysis</td>
<td>Formation of salts of calcium and magnesium</td>
</tr>
<tr>
<td>Ammonia treatment</td>
<td>Ammonia at elevated temperatures</td>
<td>Removal of lignin/decrystallizing cellulose</td>
<td>Removal of ammonia/costly</td>
</tr>
<tr>
<td>Steam explosion</td>
<td>180°C to 210°C with or without acid</td>
<td>Lignin modification and partial removal hemicellulose hydrolysis/decrystallization of cellulose</td>
<td>Partial destruction of pentoses and generation of inhibitors</td>
</tr>
<tr>
<td>Organosolv</td>
<td>Organic solvents at both high and low temperatures</td>
<td>Pure lignin obtained and used as value added product</td>
<td>Solvents inhibit enzymatic hydrolysis/costly</td>
</tr>
</tbody>
</table>

- **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**

- **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**

- 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

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
Organosolv technology

Biomass

Organosolv refining using formic/acetic acid mixture, 105°C

Organic acid recycling

Sugars + Lignins

Raw cellulose

+H₂O

+Peracids/H₂O₂

+H₂O₂/NaOH

Lignins

C₅ rich fraction

Refined cellulose

Depolymerized, chemically stable, valorizable lignin

Complex mixture, acetylation and residual acids
Functionality of wheat straw lignin extracted in organic acid media
A LITTLE ABOUT LIGNINS
Lignin: a great combustible

- 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

‘You can do anything with lignin except make money’

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
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

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

Xylitol-based polymers

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

PXC – xylitol and citric acid-based acrylate
Pentose bioconversions

- Certain bioconversions make good use of 5-carbon sugars
  - No CO₂ produced
  - Uptake of xylose and arabinose remains a challenge
  - Currently 1000 t.yr⁻¹

D-xylose → Itaconic acid + H₂ + H₂O
Theoretical yield of 0.87 g itaconic/g xylose

October 6 2015, Leaf technologies and Dutch DNA announced a R&D collaboration to produce itaconic acid
• 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 producing 160 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)

- 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

Development of Agriculture Left-Overs: Fine Organic Chemicals from Wheat Hemicellulose-Derived Pentoses

Evaluation of the transglycosylation activities of a GH 39 β-D-xylosidase for the synthesis of xylose-based glycosides
Spotlight on work in Toulouse

DEVELOPING PENTOSE-SPECIFIC TOOLS
• Many retaining GHs perform glycosylation
• The majority of GHs are active on hexoses
  – All known naturally occurring non-Leloir transglycosylases are trans-hexosidases or hexosylases
• Most GH are primarily hydrolytic enzymes
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

Molecular design of non-leloir furanose-transferring enzymes
From an α-l-arabinofuranosidase: A rationale for the engineering of evolved transglycosylases
Towards tailored polymers?

Enzyme-assisted polymerization

Selectively modified primary alcohols (e.g., esterification)

Repetitive subunit polymer
• Synthetic biology provides the means to move out of the natural framework
  – Disconnect xylose metabolism from glucose metabolism
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**

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D-glucose → D-fructose-6-phosphate → D-fructose-1,6-bisphosphate → glyceraldehyde-3-phosphate → dihydroxyacetone phosphate

\[ \text{D-xylulose} \rightarrow \text{glycolaldehyde reductase} \rightarrow \text{glycolaldehyde} \rightarrow \text{ethylene glycol} \]

\[ 0.94 \text{ mol/mol xylose} \]

\[ \text{glycolic acid} \]

\[ 0.9 \text{ mol/mol xylose} \]
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
  – Creative use of synthetic biology
  – New enzyme technologies
THANK YOU FOR LISTENING

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