Products from Thermochemical/Biochemical Hybrid Processes

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Outline

• 1. Introduction
• 2. Enhancing the Production of Levoglucosan
• 3. Conversion of Levoglucosan into Bio-fuel precursors (Lipids, Ethanol, Butanol)
• 4. Conclusions
Introduction

*Fast Pyrolysis*

High temperature process (300 - 600 °C) in which biomass is rapidly heated in the absence of oxygen to produce high yields of oil (*up to 70 mass %*).

High yields of liquids are obtained when:

1. Very small particle (less than 2 mm) is used
2. The residence time of the pyrolysis vapors is less than 2 s to minimize secondary reactions.
Introduction

Problem 1

Existing pyrolysis technologies convert more than 50 mass % of Lignin into precursors of transportation fuels (mono-lignols and Lignin Oligomers), however only a relatively small fraction of cellulose (around 10 %) is converted into hydrolysable anhydro-sugars (levoglucosan), the rest is converted into C1-C4 molecules, gases or poorly known cross-linked oligomeric anhydrosugars with limited markets.

Problem 2

Hydrotreatment strategies are not well suited to convert cellulose products into transportation fuels and chemicals. Biological Conversion strategies for levoglucosan could offer new opportunities.
Introduction

Pyrolysis of Cellulose

Vacuum Mesh Reactor

In Collaboration with the University of Twente (Z. Wang, R. Westerhof and S. Kersten)

Low yields of Levoglucosan achieved in existing technologies are due to: (1) Pyrolysis temperature optimized to maximize oil yield (2) Catalytic effect of alkalines
Introduction

Pyrolysis of Cellulose

Atmospheric Mesh Reactor at Washington State University

Low yields of Levoglucosan achieved in existing technologies are due to: (1) Pyrolysis temperature optimized to maximize oil yield (2) Catalytic effect of alkalines
Enhancing the Production of Levoglucosan

Effect of Sulfuric Acid Addition (Py-GC/MS) (Screening)
Enhancing the Production of Levoglucosan

Effect of Sulfuric Acid

Douglas Fir

Preparing Douglas Fir/H₂SO₄ blends

Auger Pyrolysis

Fluidized Bed Pyrolysis

Bio-oil Analyses

- GC/MS, K-F Titration, Sugar Analysis, FTIR, UV-Fluorescence, TG

Lignin Oligomers Precipitation

Lignin Oligomers Characterization

- Py-GC/MS, FTIR, UV-Fluorescence, TG, ¹³C-NMR
Enhancing the Production of Levoglucosan

Auger Pyrolysis Reactor

Fluidized Bed Pyrolysis Reactor

Washington State University

Curtin University (Australia)
Enhancing the Production of Levoglucosan

**Product Yields**

The yield of products obtained with an Auger reactor and with a Fluidized bed reactor were comparable.

Bio-oil yield decreased slightly and bio-char yield increased as sulfuric acid concentration increased.
The maximum yield of these sugars was obtained at sulfuric acid concentrations of 0.05 mass % for the fluidized bed reactor and of 0.3 mass % for the auger Pyrolysis reactor.
In both reactors the **water yield increased linearly** with the concentration of sulfuric acid indicating **acceleration of dehydratation reactions**. Bio-oil viscosity decreases as sulfuric acid concentration increases.
Enhancing the Production of Levoglucosan

Yields of Mono-Lignols: GC/MS

The production of vanillin, phenol - 2 - methoxy - 4 - ( 1 -propenyl)- and eugeol was drastically reduced as the concentration of sulfuric acid increased. These compounds have methoxy groups in their structures which make them very reactive.
The oils produced in the auger reactor showed higher yields of water insoluble-CH$_2$Cl$_2$ soluble fraction (low molecular weight oligomers) but lower yields of the water-CH$_2$Cl$_2$ insoluble-methanol soluble fraction (high molecular weight oligomers). The addition of sulfuric acid reduces the yield of all lignin oligomeric fractions for both the auger and the fluidized bed pyrolysis reactor.
The yield of alkylated phenols were reduced as the acid increased. These compounds are mainly generated from the H unit in lignin or are products of secondary reactions.
The use of sulfuric acid significantly reduces the yield of phenolic compounds with methoxy group. The methoxy groups are known to be electron donors.
Analysis of Lignin Oligomers: $^{13}$C Solid State NMR

Peak assignment

<table>
<thead>
<tr>
<th>Chemical shift / ppm</th>
<th>Functional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-50</td>
<td>Aliphatic carbons</td>
</tr>
<tr>
<td>50-60</td>
<td>Methoxyl carbons</td>
</tr>
<tr>
<td>60-80</td>
<td>Aliphatic C-O carbons</td>
</tr>
<tr>
<td>100-140</td>
<td>Aromatic carbons</td>
</tr>
<tr>
<td>140-165</td>
<td>Oxygenated aromatic carbons</td>
</tr>
<tr>
<td>165-230</td>
<td>Carbonyl carbons</td>
</tr>
</tbody>
</table>

A dramatic decrease in the content of methoxy groups confirms the Py-GC/MS findings and clearly suggest that the presence of this functional group activate the ring and accelerate the formation of polyaromatic structures in the bio-char produced.
Conversion of Levoglucosan into Transportation Fuel Precursors

Process to convert Pyrolytic Sugars into Ethanol or Lipids

1. **Biomass** → **PYROLYSIS** → **Crude Bio-oil**
2. **SOLVENT EXTRACTION**
   - Aqueous Phase Rich in Sugars
3. **HYDROLYSIS OF PYROLYTIC SUGARS**
   - Using **H₂SO₄**
   - Precipitate
4. **DETOXIFICATION WITH ACTIVATED CARBON**
   - Using **Ba(OH)₂**
   - Solid BaSO₄
5. **NEUTRALIZATION**
6. **FILTRATION**
   - Detoxified Aqueous Phase
7. **FERMENTATION**
   - Ethanol
   - Lipids
8. **Organic Phase Rich in Phenols**

**Notes:**
- Process flowchart showing the conversion from biomass to transportation fuel precursors.
Conversion of Levoglucosan into Transportation Fuel Precursors

Pyrolytic Sugars Production and Hydrolysis to Produce Glucose

Cellobiosan hydrolysis scheme: Cellobiosan (1), Glucose (2), Cellobiose(3), and Levoglucosan (4)

Conversion of Levoglucosan into Transportation Fuel Precursors

Process to convert Pyrolytic Sugars into Ethanol or Lipids

- **PYROLYSIS**
  - Biomass → Crude Bio-oil

- **SOLVENT EXTRACTION**
  - Organic Phase Rich in Phenols
  - Aqueous Phase Rich in Sugars

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  - Precipitate

- **DETOXIFICATION WITH ACTIVATED CARBON**
  - Ba(OH)₂
  - Solid BaSO₄
  - Detoxified Aqueous Phase

- **NEUTRALIZATION**
  - Detoxified Aqueous Phase

- **FILTRATION**

- **FERMENTATION**
  - Ethanol
  - Lipids
In both cases, ethanol yields were very close to 0.5 g of ethanol per gram of glucose.

Toxic compounds could be the main reason for slow consumption rate of pyrolytic sugar.
Conversion of Levoglucosan into Transportation Fuel Precursors

*Cryptococcus curvatus* and *Rhodotorula glutinis* for Lipid Fermentation

*Cryptococcus curvatus* could produce up to 68% lipid mass/cell mass in 122 hr and 16 g lipid / 100 g glucose conversion in 144 hr.

*Rhodotorula glutinis* could produce up to 46% lipid mass/cell mass and 8.9 g lipid / 100 g glucose conversion in 144 hr.
Conversion of Levoglucosan into Transportation Fuel Precursors

Oleaginous Yeasts Strain Selection for Levoglucosan Fermentation

<table>
<thead>
<tr>
<th>Strains</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lipomyces starkeyi</em> ATCC12659</td>
<td>-</td>
</tr>
<tr>
<td><em>Cryptococcus curvatus</em> ATCC20509</td>
<td>+</td>
</tr>
<tr>
<td><em>Yarrowia lipolytica</em> ATCC20460</td>
<td>-</td>
</tr>
<tr>
<td><em>Rhodosporidium toruloides</em> ATCC10788</td>
<td>++</td>
</tr>
<tr>
<td><em>Rhodotorula glutinis</em> ATCC204091</td>
<td>++</td>
</tr>
</tbody>
</table>
Conversion of Levoglucosan into Transportation Fuel Precursors

Levoglucosan and glucose fermentation with oleaginous yeast *R. glutinis*

![Levoglucosan Fermentation with *R. Glutinis*](chart1)

![Glucose fermentation with *R. Glutinis*](chart2)
Conversion of Levoglucosan into Transportation Fuel Precursors

Levoglucosan and Glucose Fermentation with Oleaginous yeast *R. toruloides*

**Levoglucosan fermentation with** *R. toruloides*
- Dry biomass/ g/L
- Fatty acid in medium/g/L

**Glucose fermentation with** *R. toruloides*
- Dry biomass/ g/L
- Fatty acid in medium/g/L

Graphs showing the fermentation processes over time.
Conversion of Levoglucosan into Transportation Fuel Precursors

Levoglucosan Fermentation with fungi

Advantage of levoglucosan fermentation with fungi

| Low harvesting cost |
| Variety of substrate utilization |

Fungi Screening

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Mortierella isabellina NRRL 1757</th>
<th>Umbelopsis vinacea ATCC 20034</th>
<th>Mucor janssenii NRRL 3628</th>
<th>Cunninghamella elegans NRRL 2310</th>
<th>Aspergillus terreus NRRL 1960</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>
LG fermentation with oleaginous fungi 1960 reach 6.8 g/L of biomass, compared with 7.0 g/L for glucose fermentation. **However, lipid content was low.**
Isobutanol has been produced by *E. coli* with heterologous expression two genes of Kdc (2-ketoisovalerate decarboxylase) and Adh (aldehyde reductase) amplified from *Lactococcus lactis*. *E. coli* was obtained from *Washington University in St. Louis*.

LGK gene was synthesized based on the LGK gene from *Lipomyces starkeyi* YZ-215 in Gene wiz.
Conclusions

• Levoglucosan yields as high as 60 mass % can be obtained from cellulose. We are very hopeful similar yields could be produced from lignocellulosic materials.

• The presence of sulfuric acid mitigates the catalytic effect of alkalines enhancing the production of levoglucosan but reduces drastically the production of lignin derived precursors of transportation fuels (mono-lignols and oligomers).

• The methoxy substituted aromatic rings are more likely to be polycondensed to form extra-char in the presence of sulfuric acid.
Conclusions

• The pyrolytic sugars can be hydrolysed and fermented to produce ethanol and lipids.

• Yeasts and fungi containing Levoglucosan Kinase (LK) can directly convert Levoglucosan into Lipids.

• New genetically modified micro-organisms able to directly use levoglucosan for ethanol, lipid and butanol production are being developed at Washington State University.
AKNOWLEDGEMENT

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SUN GRANT INITIATIVE, U.S. DEPARTMENT OF TRANSPORTATION
WASHINGTON STATE UNIVERSITY AGRICULTURAL RESEARCH CENTER
QUESTIONS ?
Phenols and the carboxylic acids are the main family of toxic compounds limiting the production of ethanol and inhibiting yeast growth.

Furans are also inhibitors, but their inhibition rate is much lower.
## Fatty acid profiles of *R. glutinis* and *R. toruloides* obtained at 120 hr culture with levoglucosan (LG) and Glucose medium.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Structure</th>
<th>mass % FA LG</th>
<th>mass % FA Glucose</th>
<th>mass % FA LG</th>
<th>mass % FA Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic</td>
<td>C14:0</td>
<td>0.4</td>
<td>0.5</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Palmitic</td>
<td>C16:0</td>
<td><strong>24.3</strong></td>
<td>18.1</td>
<td><strong>25.1</strong></td>
<td>19.7</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>C16:1n7</td>
<td>0.2</td>
<td>1.2</td>
<td>0.8</td>
<td>0.9</td>
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<tr>
<td>Heptadecanoic</td>
<td>C17:0</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.4</td>
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<tr>
<td>Stearic</td>
<td>C18:0</td>
<td>10.1</td>
<td>24.4</td>
<td>9.2</td>
<td><strong>17.3</strong></td>
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<tr>
<td>Oleic</td>
<td>C18:1n9</td>
<td><strong>53.2</strong></td>
<td>44.6</td>
<td><strong>50.8</strong></td>
<td>43.5</td>
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<tr>
<td>Linoleic</td>
<td>C18:2n6</td>
<td>6.8</td>
<td>5.6</td>
<td>7.2</td>
<td>9.8</td>
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<tr>
<td>Gamma- Linolenic</td>
<td>C18:3n6</td>
<td>1</td>
<td>0.7</td>
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<td>0.6</td>
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<tr>
<td>Linolenic</td>
<td>C18:3n3</td>
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<td>0.9</td>
<td>0.7</td>
<td>0.2</td>
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<tr>
<td>Arachidic</td>
<td>C20:0</td>
<td>0.3</td>
<td>1.6</td>
<td>0.4</td>
<td>1.2</td>
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<tr>
<td>Behenic</td>
<td>C22:0</td>
<td>0.7</td>
<td>0.6</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Lignoceric</td>
<td>C24:0</td>
<td>2.4</td>
<td>1.7</td>
<td>3.5</td>
<td>4.7</td>
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</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>mass % in dry biomass</th>
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<tr>
<td>Saturated</td>
<td>39.1</td>
</tr>
<tr>
<td>MUFA</td>
<td><strong>53.4</strong></td>
</tr>
<tr>
<td>PUFA</td>
<td>7.2</td>
</tr>
<tr>
<td>Omega-3</td>
<td>0.3</td>
</tr>
<tr>
<td>Omega-6</td>
<td>6.9</td>
</tr>
<tr>
<td>Identified</td>
<td>99.7</td>
</tr>
<tr>
<td>Unknown</td>
<td>0.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total Fat</th>
<th>mass % in dry biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. glutinis</em></td>
<td>42</td>
</tr>
<tr>
<td><em>R. toruloides</em></td>
<td>45</td>
</tr>
</tbody>
</table>

**Conversion of Levoglucosan into Transportation Fuel Precursors**