Pretreatment & Enzymatic hydrolysis for production of substituted fuels from renewable biomass

Venkata Prabhakar Soudham
Prof. Jyri-Pekka Mikkola
Industrial collaboration: Processum Biorefinery Initiative, MoRe Research and SEKAB E-Technology (Örnsköldvik)
The benefit of the present oil price hikes could be to focus attention on the possibility of a world less dependent on oil.

“Oil Will Be A Past Relic When Today’s Babies Hit Fifty”.

May 1987 – November 2011 monthly average Brent spot prices
Conversion to November 2011 dollars uses US CPI for All Urban Consumers (CPI-U)
Sources: Energy Information Administration and Bureau of Labor Statistics

Source: http://websitebuilding.biz/business/oil-unsustainable-price

Source: www.conservativedailynews.com
Biofuels: a barrier for “sustainable fuel”

Ethanol and Butanol contain oxygen and can both be used as oxygenates for gasoline.

<table>
<thead>
<tr>
<th></th>
<th>Gasoline</th>
<th>Ethanol</th>
<th>Butanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octane Number</td>
<td>86 – 94</td>
<td>112.5 – 114</td>
<td>87</td>
</tr>
<tr>
<td>LHV (MJ/Lit)</td>
<td>31.2 – 32.4</td>
<td>21.1 – 21.3</td>
<td>27.8</td>
</tr>
<tr>
<td>Heat of vaporization (MJ/kg)</td>
<td>0.36</td>
<td>0.92</td>
<td>0.43</td>
</tr>
<tr>
<td>Energy density (MJ/L)</td>
<td>32</td>
<td>19.6</td>
<td>29</td>
</tr>
<tr>
<td>Flash point (°C)</td>
<td>-</td>
<td>15</td>
<td>37</td>
</tr>
<tr>
<td>Solubility in water</td>
<td>&lt;0.1</td>
<td>Fully miscible</td>
<td>7</td>
</tr>
<tr>
<td>Boiling point</td>
<td>-</td>
<td>78°C</td>
<td>118°C</td>
</tr>
<tr>
<td>Density (kg/L)</td>
<td>0.74</td>
<td>0.79</td>
<td>0.81</td>
</tr>
<tr>
<td>Vapor Pressure (Bar)</td>
<td>0.480 – 1.034</td>
<td>0.159</td>
<td>0.023</td>
</tr>
</tbody>
</table>

➢ Can be blended with gasoline (e.g E5, E10, E25, E85..)

Butanol is better biofuel than ethanol

➢ More compatible with oil infrastructure
➢ Existing pipelines and filling stations can be used
➢ Can be blended with gasoline and with diesel

“Major oil companies show more interest in butanol than ethanol.”
Biofuels production

First generation

\[ \text{C}_6\text{H}_{12}\text{O}_6 \xrightarrow{\text{Microorganisms}} \text{C}_4\text{H}_9\text{OH} + 2\text{CO}_2 + \text{H}_2\text{O} \]

Sugars (e.g. glucose) (Butanol)

Ethanol from Fermentation:

Sugar(s) \xrightarrow{\text{Microorganisms}} \text{Ethanol + By-products}

\[(\text{C}_6\text{H}_{10}\text{O}_5)_n + n\text{H}_2\text{O} \rightarrow n\text{C}_6\text{H}_{12}\text{O}_6 \text{ (glucose)} \]

\[\text{C}_6\text{H}_{12}\text{O}_6 \xrightarrow{\text{yeast}} 2\text{CH}_3\text{CH}_2\text{OH} + 2\text{CO}_2 + \text{heat} \text{ (ethanol)}\]

"...large increases in biofuels production is the main reason behind the steep rise in global food prices" - World Bank policy research working paper July 2008
Lignocellulosic materials

- Abundant
- Inexpensive
- Sustainable and potential feed stocks

Forest residues
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Source: www.bioimprove.se
Chemical composition of Lignocellulose

- **Lignin**: (10-35 %)
- **Extractives**: (<10 %)
- **Hemicellulose**: (20-35 %)
- **Cellulose**: (20-50 %)
Monomer sugars

Source: http://www.catchbio.com/results/catalytic_hydrolysis_of_cellulose
Cellulosic fuels can achieve much greater energy and GHG benefits
## Pretreatment

### Goals:

- Opening crystalline structure of Lignocellulose
- Hydrolysis of hemicelluloses
- Enhance the enzymatic hydrolysis

### Pretreatment Methods

- Mechanical Pretreatment.
- Heat treatment
- Microwave irradiation
- Steam explosion.
- Liquid hot water (LHW).
- Ammonia fiber explosion (AFEX).
- CO2 explosion.
- Ozonolysis.
- Dilute-acid hydrolysis.
- Concentrated-acid hydrolysis.
- Alkaline hydrolysis.
- Oxidative delignification.
- Wet oxidation.
- Organosolv process.
- Dissolution in IL’s
- Fungal pretreatment.

### IL’s:

Novel alternative green solvents for the pretreatment of lignocelluloses.
Hydrolysis

Splitting of cellulose to glucose by

- **Concentrated acid hydrolysis**
  at room Temp. by e.g.
  » 72% H₂SO₄
  » 42% HCl

- **Dilute acid hydrolysis**
  » e.g. 0.5% H₂SO₄ at 200-220ºC
    (ca 20-25 bar), 5-10 min

- **Cellulases enzymes**
  at ca 45ºC and 24-72 hours

Source: Arantes et al., 2010
Toxic compounds from Pretreatment

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Wood
- Ash <1%
- Extractives <10%
- Lignin 20-32%
- Hemicellulose 20-30%
- Cellulose 40-45%

Fermentation inhibitors
- Phenolic compounds
- HMF
- Furfural
- Formic acid

Hexose sugars
- Glucose
- Mannose
- Galactose
- Rhamnose

Pentose sugars
- Xylose
- Arabinose

Uronic acids
- Acetic acid
- Levulinic acid

(Source: Alriksson B., 2006)
Work flow

- Identifying biomass with good bio-processing properties
- Evaluating the existing and new solvents for pretreatment of biomass. 
  \((soudham \text{ et al.}, 2011a) \ (soudham \text{ et al.}, 2013)\)
- Investigations to improve hydrolysis efficiency. 
  \((soudham \text{ et al.}, 2011b)\).
Identifying biomass
screening of transgenic aspens

Strategy for pretreatment and saccharification

- Harvesting, cutting, lyophilization, milling, sieving
- Acid pretreatment targeting hemicellulose
- Steam pretreatment
- No pretreatment
- IL pretreatment
- Pretreatment targeting Lignin
- Steam pretreatment
- Separation of liquid and solid fraction
- Wash of solid fraction
- Enzymatic hydrolysis
- Rapid analysis of glucose in liquid fraction
- Detailed analysis of liquid fractions
- Analysis of solid fractions
- Analysis of wood composition

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www.bioimprove.se
No pretreatment

<table>
<thead>
<tr>
<th>Total reducing sugars (g/L)</th>
<th>Line &quot;X&quot;</th>
<th>Line &quot;Y&quot;</th>
<th>Line &quot;Z&quot;</th>
<th>Wild type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.07</td>
<td>2.92</td>
<td>2.79</td>
<td>2.81</td>
</tr>
</tbody>
</table>

T-Test: P Critical = 0.05

Line "X" 0.52
Line "Y" 0.76
Line "Z" 0.96

Steam pretreatment with acid catalyst

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<th>Line &quot;X&quot;</th>
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<th>Line &quot;Z&quot;</th>
<th>Wild type</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>7.87</td>
<td>6.93</td>
<td>6.64</td>
<td>5.91</td>
</tr>
</tbody>
</table>

28 %
17 %
12 %

T-Test: P Critical = 0.05

Line "X" <0.01
Line "Y" <0.01
Line "Z" <0.01

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Delignification strategy:
Steam pretreatment with organic solvents
- Acetosolve delignification (ASD) (Acetic acid, HCl)
- Ethanol

Dissolution in green solvents:
- IL’s such as 
  - [emim][OAc]
  - [emim][Me2PO4] (Not studied)

Volumetric glucose production rate (GPR) (g of glucose per L and h) after enzymatic hydrolysis

GPR compared to wt (%)

Time (h)

A  Wild type  B

GPR compared to wt (%)

Time (h)

Volumetric glucose production rate (GPR) (g of glucose per L and h) after enzymatic hydrolysis

Glucose (mmol/L)

Hydrolysis time

24 h  70 h

Solids from ASD  Milled Aspen wood
Fractionation of Marabou wood

Marabou Wood

Acetosolv (50, 70, 90 %) delignification
NBT, 121°C (1 h)

Solid stream

Liquid stream

Lignin precipitation & Filtration

Lignin

Liquor

(Dilute acid pre-hydrolysis)

Dilute acid pre-hydrolysis

Filtration

Solid stream

Liquid stream

Acetosolv delignification

Filtration

Liquid stream

Solid stream

Lignin precipitation & Filtration

Lignin

Liquor

(liquin recovery)

(solvent recovery)

Cellulose

Hemicellulose

Lignin

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Lignin removal and recovery

Chemical composition of Marabou wood

- Cellulose: 40%
- Hemicelluloses: 32%
- Lignin: 4%
- Extractives: 22%
- Ash: 1%
- Unknown: 4%

Lignin removal (%)

- AS-121C
- AS-NBT
- DAPH/AS-121C
- DAPH/AS-NBT

Recovered lignin

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Enzymatic hydrolysis of softwood and agricultural residues after treatment with ionic liquid (1-allyl-3-methylimidazolium formate).

Thermochemical pretreatment of sugarcane bagasse and Norwegian spruce was performed in the Örnsköldsvik demo plant in Sweden (<www.sekab.com>).

Solvation of cellulosic material in ionic liquid. The figure shows 5% (w/w) cellulose dissolved in [AMIM][Fo].
Enzymatic hydrolysis of Norway spruce and sugarcane bagasse after treatment with Ionic Liquid

Monomer sugars obtained after enzymatic hydrolysis (72 h at 45 °C) of native (N) and thermo – chemically pretreated (TCP) spruce and sugarcane bagasse. The cellulosic substrates were treated with [AMIM]Fo ionic liquid (IL) or deionized water (DW).
Investigations to improve the enzymatic hydrolysis of toxic lignocellulose hydrolyzates

- In-situ detoxification of toxic lignocellulose hydrolyzates with the addition of a reducing agent sodium sulfite, dithionite, or dithiothreitol. (Soudham et al., 2011b)

- Chemical conditioning of inhibitory lignocellulose hydrolyzates with the solvents “X” or “Y”.
Inhibition of enzymatic hydrolysis by pretreatment liquid

Composition of total inhibition Caused by the (A) PL, (B) sugars and other compounds in PL, (C) glucose, rest of the sugars and other compounds in PL, (D) total sugars and 15mM Dithionite in CB, and Composition of inhibition after conditioning PL with (E) 15mM DTT (F) 15mM Sodium sulfite, (G) 15mM Dithionite.

*Inhibition was calculated based on glucose production during the 96 h enzymatic hydrolysis

PL: Pretreatment Liquid       CB: Aqueous citrate buffer
Reducing agents improve enzymatic hydrolysis of cellulosic substrates

% increase in glucose production after 120 h enzymatic hydrolysis compared to the unconditioned references

-60
-40
-20
0
20
40
60

DTT Sodium sulfite Sodium dithionite PL-1 PL-2
DTT Sodium sulfite Sodium dithionite Citrate buffer

PL-1: Pretreatment liquid of slurry from Norway spruce impregnated with sulfur dioxide
PL-2: Liquid fraction from first step of a two-step dilute sulfuric acid hydrolysate of Norwegian spruce
Conditioning of inhibitory lignocellulose hydrolyzate with chemical solvents was improved saccharification efficiency

Glucose production from 72 h enzymatic hydrolysis of chemically conditioned and unconditioned toxic lignocellulose hydrolysate.

GPC analyses of the conditioned and unconditioned pretreatment liquids of lignocellulose hydrolysate.
Comparison of classical thermo-chemical pretreatment (TCP) techniques (pilot scale) with the catalysts “A” and “B”

Ethanol from PL’s

Total Phenols
PL – A: 2,2 (g/l); PL – B: 5,4 (g/l)

Slurry of ”A”  suspended solids (16.9%)  pH: 1.6–1.7

*PL – Pretreatment liquid

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Conclusion

➢ About 210 transgenic aspens were screened with a battery of pretreatment techniques.

➢ Acetosolv delignification efficiently removed >90% of lignin from forestry wood. But, the enzymatic hydrolysis of acetosolv pulps was very poor.

➢ Dissolution of both forestry wood and agricultural residues in IL (1-allyl-3-methylimidazolium formate) was very effective even under very mild conditions.
➢ IL’s as solvents for screening of transgenic plants could be a useful novel approach.

➢ Chemical treatment of toxic lignocellulose hydrolysates improved the hydrolysis efficiency up to 57%.

➢ Furthermore, not all, only few, specific phenolic compounds could be inhibitory in the bioconversion of biomass to biofuels.
Acknowledgements

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Thank you

"...Any type of bio-fuel helps substantially reduce fossil energy and petroleum usage, and there by also reduces GHG emissions".