FERMENTATIVE UPGRADING OF A BIOMASS PYROLYSIS BYPRODUCT

Karen Schwab\textsuperscript{1,2}, Martin Siemann-Herzberg\textsuperscript{2} and Lars Rehmann\textsuperscript{1}

\textsuperscript{1} Department of Chemical and Biochemical Engineering, University of Western Ontario, Canada
\textsuperscript{2} Institute of Biochemical Engineering, University Stuttgart, Germany
The University of Western Ontario

- Founded in 1878
- 1,200 faculty members
- 30,000 students
- Research intensive university (>200Mio/year external research funding)
- Highest entrance average of undergraduate students in Faculty of Engineering
Visit us in October

http://www.csche2011.ca/
Research Focus in my Lab

**Pyrolysis Oil**
- Aqueous fraction
- High content of carbohydrates
- “Low toxicity”

**Fermentation Process**
- Reactor Design
- Choice of biocatalyst
- Engineering of biocatalyst

**In-situ Product Recovery**
- Biphasic systems
- Polymer
- Ionic liquid
- Membranes
- Pervaporation
- Cascade reactions

**Bioconversion**
- Butanol (commodity chemical/fuel)
- Ethanol (fuel)
- Polymer/pharma precursors...

**Pretreatment of Feedstock**
- Fractionation in ionic liquids
- Enzymatic hydrolysis in ILs
- High-throughput screening

**Environmental Applications**
- Soil and water remediation
- Wastewater treatment
- Toxicity tests

**Polymer Design**
- Fundamental study of polymer/solute interaction
- Design of smart polymers (see drug delivery)

**Ionic Liquids**
- Environmental impact
- Biodegradability
- Design of functional ionic liquids

**Environmental Applications**
- Soil and water remediation
- Wastewater treatment
- Toxicity tests

Prospective Graduate Students
Open M.E.Sc. and Ph.D. projects
Contact rehmann@eng.uwo.ca

www.eng.uwo.ca
Preliminary Objectives

1. Use aqueous phase of pyrolysis oil as fermentation feedstock

2. Convert carbohydrates to ethanol

3. Profit
Preliminary Results

- Corncob pyrolysis oil aqueous fraction as feedstock for bio-ethanol production
- Two biocatalysts
  - *Saccharomyces cerevisiae*
  - *Zymomonas mobilis*

Addition of 1% aqueous fractions stops all cell activity
Revised Objectives

1. Evaluation of bio-oil aqueous phase as feedstock for microbial ethanol production

2. Identification and removal of potential inhibitory compounds

3. Definition of a synthetic bio-oil aqueous phase for fundamental studies
Aqueous Phase...

- Composition varies based on operating conditions and feedstock
- Proposed Synthetic Aqueous Phase (SAP):

<table>
<thead>
<tr>
<th>Substance</th>
<th>C. in bio-oil[^1] (%w/w)</th>
<th>P_{o/w}</th>
<th>C. in SAP (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-cresol</td>
<td>0.02 – 0.04[^b]</td>
<td>87.1</td>
<td>0.149</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.1-2.55[^a]</td>
<td>40.74</td>
<td>0.069</td>
</tr>
<tr>
<td>Guaiacol</td>
<td>0.05-0.31[^a]</td>
<td>17.78</td>
<td>0.03</td>
</tr>
<tr>
<td>2,6-DMP</td>
<td>0.04-0.34[^a]</td>
<td>18.2</td>
<td>0.031</td>
</tr>
<tr>
<td>Pyrocatechol</td>
<td>0.08-0.48[^a]</td>
<td>12.59</td>
<td>0.02</td>
</tr>
<tr>
<td>Furfural</td>
<td>1.58-2.52[^a]</td>
<td>1.622</td>
<td>2.0</td>
</tr>
<tr>
<td>Furfuryl alco.</td>
<td>n.s.</td>
<td>0.977</td>
<td>1.7</td>
</tr>
<tr>
<td>Formic acid</td>
<td>n.s.</td>
<td>0.537</td>
<td>1.5</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>13.84-18.57[^a]</td>
<td>0.513</td>
<td>1.5</td>
</tr>
<tr>
<td>Glucose</td>
<td>n.s.</td>
<td>0.0032</td>
<td>6.0</td>
</tr>
<tr>
<td>Galactose</td>
<td>n.s.</td>
<td>0.0032</td>
<td>0.8</td>
</tr>
<tr>
<td>Xylose</td>
<td>n.s.</td>
<td>0.0074</td>
<td>1.2</td>
</tr>
</tbody>
</table>
Testing of Microbial Activity

High Throughput Experiment:

Stock culture:
- Exponential phase

Sample preparation:
- Centrifugation

Batch-fermentations:
- Micro titer plate
- 8 experiments
- Reaction volume: 200µl
- Addition of aq. phase
- Plate Reader

Further Analyses of the samples:
- Gas Chromatograph
- HPLC

Increased efficiency because of small volumes
**Furfural**

*Saccharomyces cerevisiae*

*Zymomonas mobilis*

---

www.eng.uwo.ca
Furfuryl Alcohol

**Saccharomyces cerevisiae**

- Standard
- 4.0 g/kg Furfurylalkohol (FA)
- 6.0 g/kg FA
- 8.0 g/kg FA
- 10 g/kg FA
- 12 g/kg FA
- 14 g/kg FA

**Zymomonas mobilis**

- Standard
- 1.0 g/kg Furfurylalkohol (FA)
- 2.0 g/kg FA
- 4.0 g/kg FA

Cell dry weight [g kg⁻¹]

Time [min]

**Growth rate μ [h⁻¹]**

- **Furfuryl alcohol concentration [g kg⁻¹]**
- 0.00  0.05  0.10  0.15  0.20  0.25  0.30  0.35  0.40
- 0    4    8    12   14

- **Furfuryl alcohol concentration [g kg⁻¹]**
- 0.00  0.05  0.10  0.15  0.20  0.25  0.30  0.35  0.40
- 0    1    2    3    4    5    6
Phenols

**Saccharomyces cerevisiae**

- Standard
- 1.0 g/kg Phenolmix (PM)
- 1.2 g/kg Phenolmix
- 1.4 g/kg Phenolmix
- 1.6 g/kg Phenolmix

**Zymomonas mobilis**

- Standard
- 1.0 g/kg PM
- 1.2 g/kg PM
- 1.8 g/kg PM

**Growth rate μ [h⁻¹]**

- **Saccharomyces cerevisiae**
- **Zymomonas mobilis**

Phenol concentration [g kg⁻¹]
Detoxification

RECYCLING MATERIALIEN!

- Polyamide
- Paper towel
- Cotton balls
- Cotton cloth
- Switch grass
- Styrene butadiene/ethylene
- Polyethylene terephthalate (PET)
- XAD-4
- Nylon
Phenol adsorption isotherms for the textile recycle polymers nylon (■), polyamide (▲), PET (●), cotton balls (△) and cotton cloth (〇) (left) and XAD4 (■), switchgrass (▲), paper towel (●) (right).
Detoxification

Absorption spectra of SAP (left) and of corncob bio-oil aqueous phase after multiple purification steps using nylon
Bioconversion

*S. cerevisiae* microscale batch fermentations. 50% (v/v) (□), 40% (v/v) (○) and 30% (v/v) dilutions (△) of corncob bio-oil aqueous phase (6 purification steps, nylon) (right). No carbohydrates added.
Conclusions

- Bio-oil aqueous phase needs to be treated prior to bio-conversion.

- Defined synthetic composition can be used to study effects of individual inhibitors.

- Recycled polymers can be used as sorption materials for inhibitor removal.

- Yeast cells can convert purified aqueous phase.
Ongoing and Future Work

- Development of continuous sorption column
- Recycling of sorption material via microbial degradation on inhibitors
- Scale-up of process to bench-scale
- Quantification of ethanol production
- Evaluation of bio-oil as substrate for butanol fermentation