Biological Conversion of Hemicelluloses Extracted From Hardwood: Enabling Co-production of Ethanol and Pulp in an Integrated Forest Bio-refinery

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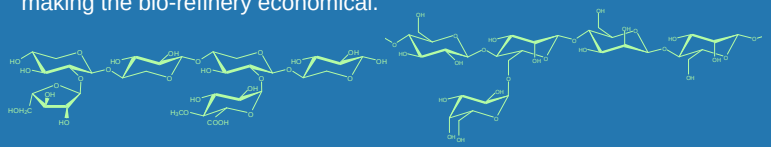
Motivation:

Lignocellulose represents a readily available renewable feedstock which may be used in the production of a variety of chemicals and fuels. An integrated bio-refinery could extract hemicellulose while preserving cellulose for pulp production. Hemicelluloses are traditionally burned in the recovery process, but do not have the high heating value of lignin, and could be converted to value added products. Fermenting the hemicellulose derived five carbon sugars is more difficult than fermenting cellulose derived glucose. Increasing the fermentation yield of hemicellulose is essential to making the bio-refinery economical.



Structure of Hemicellulose: Arabinoglucuronoxylan (left) – **Xylose** backbone with branching **Arabinose** and glucuronic acid substituents. Galactoglucomannan (right) – **glucose** and **mannose** backbone with branching **galactose** substituent.

Hemicellulose Extraction:

The hemicellulose fraction of biomass is more readily soluble because of its branched structure and lower degree of polymerization. Extraction performed in a 5L profiling digester with 3% Green liquor (Na_2CO_3 plus Na_2S expressed as Na_2O generated in pulping chemical recovery process) at 160°C for 110 minutes at high pressure resulted in the following removal

Green Liquor Extraction (% on original oven dry wood)	
Arabinan	0.1%
Galactan	0.3%
Mannan	0.2%
Glucan	0.4%
Xylan	3.4%
Lignin	1.3%
Glucuronic Acid	2.4%
Acetate	2.0%
Total Yield	10.1%

Approximately 1% of initial cellulose and 5% of the lignin is removed during extraction, partially bound to hemicelluloses. The hemicelluloses are released as oligosaccharides and then start to degrade to monosaccharides, but there is no significant furfural production. Acetyl groups bound to oligosaccharides are removed, then deacetylated to acetic acid

Hydrolysis of Extracts:

Hydrolysis to monosugars was done by acid hydrolysis with 4% sulfuric acid at 121°C for 2 hours in an autoclave. 6.8g/L of total monosugars were obtained. Future experiments will concentrate the extracts prior to hydrolysis to obtain higher monosugar concentrations.

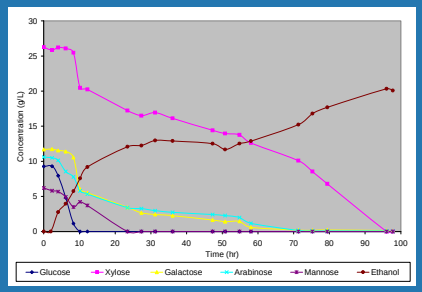
Extract Concentrations (g/L) by HPLC analysis	
Glucose	1.07
Xylose	2.96
Galactose	1.34
Arabinose	0.75
Mannose	0.70
Acetic Acid	8.78
Lactic Acid	1.32
Furfural	0.07
HMF	0.003
Total Monosugars	6.83

Experimental Set-up:

A 3L New Brunswick Scientific Bio-reactor was used for fermentation experiments. Temperature, pH, Aeration and Agitation are controlled on-line. Carbon Dioxide in the exhaust gas and Cell Mass by optical density are also recorded by on-line sensors. Product concentrations are measured by HPLC using the Bio-Rad Aminex HPX-87P and HPX-87H columns.

Fermentation Performance of *Escherichia coli* K011 for Five Sugar Mixture:

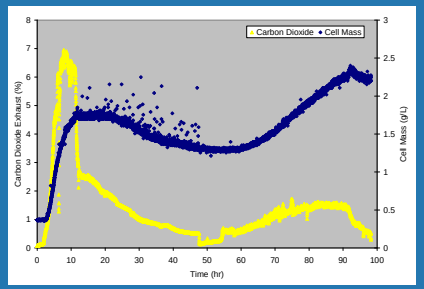
A mixture of xylose, glucose, galactose, arabinose and mannose was prepared from model sugars at ten times the concentrations measured in overlimed hemicellulose extracts. No inhibitors were added, though the extracts contain significant acetic acid and minor amounts of furfural.



Glucose was consumed preferentially within 10 hours, all sugars were consumed entirely within 96 hours. From 64g/L total monosugars, 20g/L ethanol was produced, or 62% of the theoretical maximum yield.

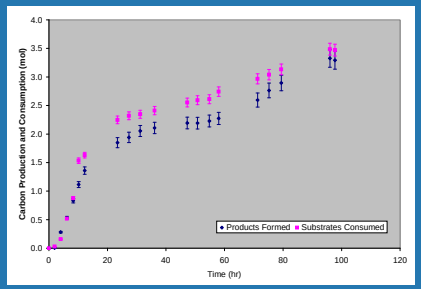
Cell Mass and Carbon Dioxide:

These monitors track alternate fates of carbon.



Material Balance:

An understanding of substrate consumption and product formation is important for fermentation optimization. The five sugar substrates are converted not only to ethanol, but to smaller quantities of acetic and lactic acid. Carbon dioxide is emitted, and the cells themselves incorporate carbon.



The mole balance is generally well satisfied, though slightly lower product formation indicates additional side products.

Benefits of Integration:

The U.S. produces 55 million tons of chemical pulp annually. In the global pulp and paper industry many competitors have lower raw material & labor costs, combined with the newest, largest machinery. Forest product prices decrease by about 1% per year while costs of production rise. North America's forest products industry needs to generate higher revenue by producing additional value-added products. Hemicellulose extraction provides additional benefits to the production of pulp such as increased pulp yield and delignification rate while reducing the recovery boiler load and lowering alkali consumption.

Conclusions:

This work has shown that the hemicellulose component of woody biomass can be pre-extracted with green liquor, while preserving the cellulose component for production of pulp. The concentrated extract liquor can be hydrolyzed to a mixture of dilute monosugars and acetic acid. Following neutralization by overliming with calcium hydroxide, the monosugars can be fermented to produce higher value chemicals. Fermentation by *E. coli* K011 of model sugars based on hemicellulose extraction liquor produced 20g/L ethanol in 96 hours.

*Special thanks to Sefik Tunc and Dr. Weiping Ban

**This material is based upon work supported by the National Science Foundation under Grant No. 0554545