Biological Conversion of Hemicellulose Extracts from Wood

Production of Fuel Ethanol by *Escherichia coli* K011

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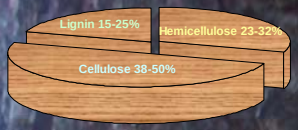
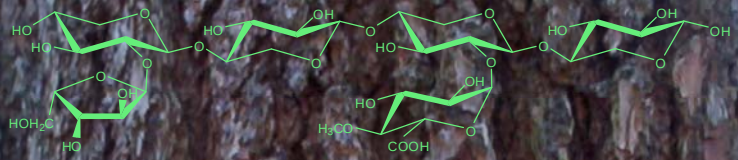
Motivation:
Lignocellulose represents a readily available renewable feedstock which may be used in the production of a variety of chemicals and fuels. An integrated bio-refinery could extract hemicellulose while preserving cellulose for pulp production. Fermenting the hemicellulose derived five carbon sugars is more difficult than fermenting cellulose derived glucose. Increasing the yield through use of hemicellulose is essential to making the bio-refinery economical.



Composition of wood
on dry, extractive free basis.

Cellulose is a polymeric chain of glucose units only

Hemicellulose is a heterogeneous polymeric chain with branching substituents



Structure of Hemicellulose (Arabinoglucuronoxylan) – **Xylose** backbone with branching **Arabinose** and glucuronic acid substituents. Other types of hemicellulose contain **Glucose, Galactose,** and **Mannose**

***Escherichia coli* K011:**

Single celled bacteria, grows at room temp. & near neutral pH.

E. coli naturally consumes many sugars to a mix of ethanol and organic acids, but was metabolically engineered to produce ethanol exclusively with genes from *Z. mobilis*. Small amounts of acetic acid, lactic acid, succinic acid, and formic acid may still be formed

3 Liter Fermentor Set-up

Thermal Mass Flow Control
Level Control
Gas Mixer
pH and DO
System Controller
Power Control
Pump Control

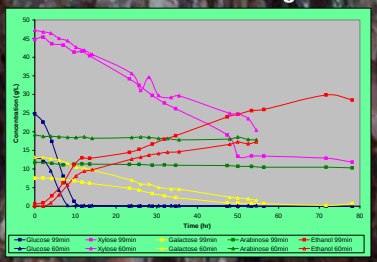
New Brunswick Scientific BioFlo-110

Fermentation Control:
pH: 6.5 by 2M NaOH
Temperature: 37°C by heat jacket / cooling coil
Aeration: 1LPM by thermal mass flow control
Agitation: 100RPM

Monitoring Cell Growth:
Optical Density at 600nm
Carbon Dioxide % in exhaust
Sugar Concentration (HPLC)
Ethanol Concentration (HPLC)

Hydrolysis to Monosugars
Before fermentation it is necessary to break the bonds linking the hemicellulose sugar units. This is done with dilute acid at 121°C. Longer reaction time, higher temperature and more acid correspond to more severe hydrolysis, which decreases monosugar recovery except for increased glucose due to cellulose degradation. Degradation products such as acetic acid, furfural, and hydroxymethyl furfural also increase. These compounds are known to inhibit the micro-organisms used in fermenting.

Fermentation of Five Sugar Mix

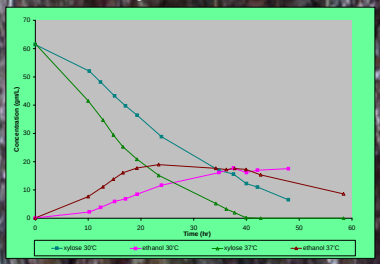


Mixtures of the five pure sugars were prepared in ratios corresponding to recovery data of hemicellulose extracts prepared in our lab, at extraction times of 60 minutes and 99 minutes.

Air flow was slightly lower for the 60min case at 0.4LPM.

In both trials glucose was consumed within the first 10 hours. Xylose remains nearly constant until this time, then ferments at a much faster rate. Galactose is entirely consumed within 60 hours. Mannose determination was not possible. Arabinose showed very little decrease in the 80 hours monitored. 30g/L ethanol was produced in the 99min trial, compared to only 17g/L in the 60min trial.

Effect of Temperature on Fermentation



Xylose fermentation at 37°C showed much faster sugar consumption and ethanol production rates that the same conditions run at 30°C.

Hemicellulose Extraction

Wood chips are treated in a Dionex ASE100 extractor with water at 150°C and 100bar. Approximately 50% of the initial hemicellulose will be recovered into solution. Hemicellulose is more readily solubilized than cellulose because of its lower degree of polymerization and the presence of side-chains.

Future work will examine fermentation of actual hardwood hemicellulose extracts produced in our lab. Response to inhibitors such as acetic acid and furfural will be evaluated. Operating conditions will be optimized and the carbon mass balance will be closed.

Conclusions: Ethanol was produced from simulated hemicellulose extracts at 85% of theoretical yield. This data provides a baseline of comparison for future work.

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