# Applications of Molecular Biotechnology:
## Ethanol Production from Cellulosic Biomass

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***CM4710 Biochemical Processes***
***November 30, 2007***

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## Presentation Overview

- Ethanol from Lignocellulosic Biomass and its Potential to Displace Petroleum in the USA
- Research Needs in Forest Resources, Bioconversion Processing, Engines, and Decision Analysis
- Dilute Acid Pretreatment of Tree Species from the Upper Midwest Region
- Enzymatic Hydrolysis of Pretreated Woody Biomass
- Genetic Engineering of E. coli for ethanol production from woody biomass

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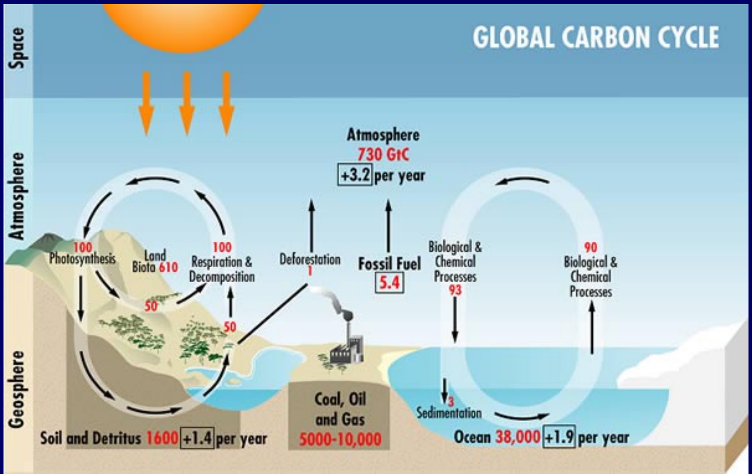
# Managing the Carbon Cycle:

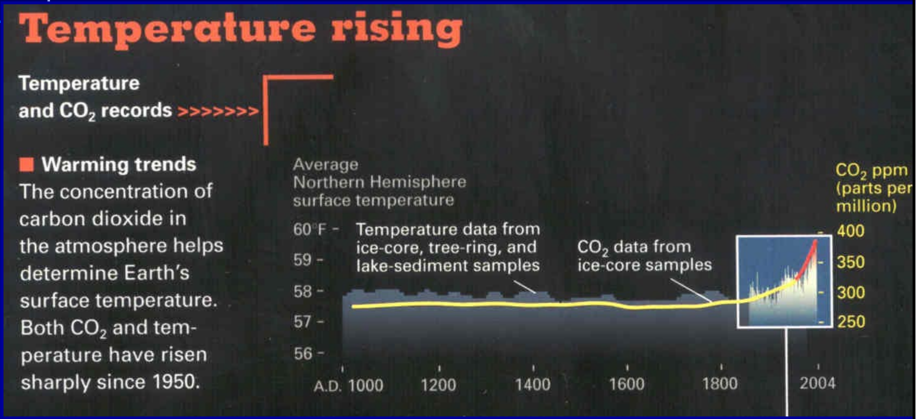
## *A Sustainable Energy Challenge*

*From http://www.bom.gov.au/info/climate/change/gallery/index.shtml*

***Combustion of Fossil Fuels acts as a Carbon Pump***



# $CO_2$ and Temperature in the Northern Hemisphere are Rising

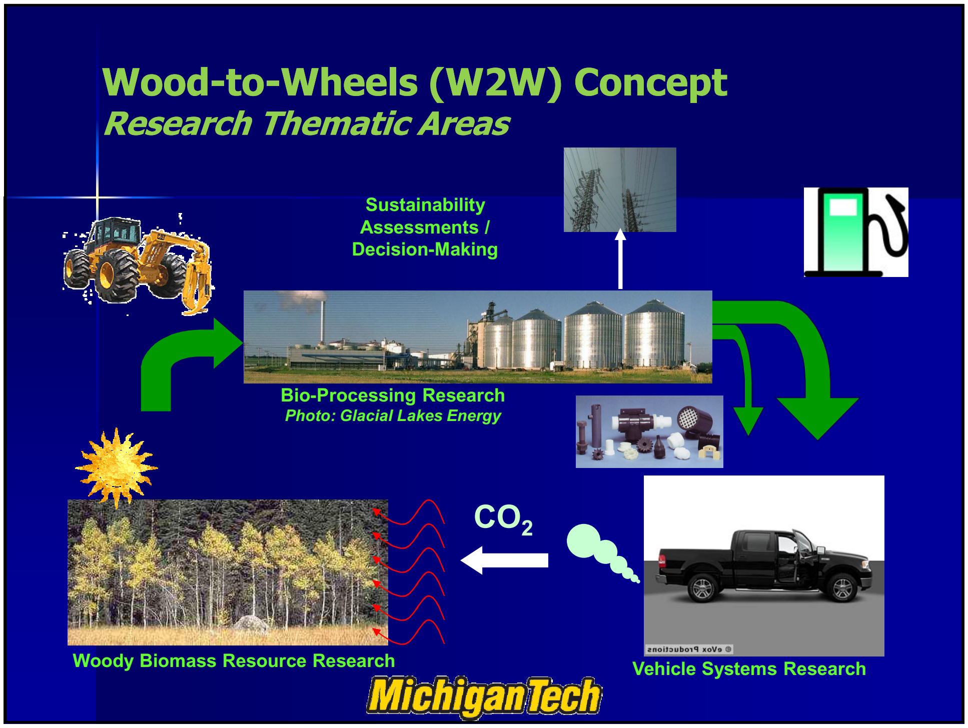


**Temperature rising**

**Temperature and $CO_2$ records >>>>>>>**

**Warming trends**
The concentration of carbon dioxide in the atmosphere helps determine Earth's surface temperature. Both $CO_2$ and temperature have risen sharply since 1950.

*National Geographic, September 2004, pg 20, National Geographic Society, Washington, D.C.*



# Wood-to-Wheels (W2W) Concept

*Research Thematic Areas*

Sustainability Assessments / Decision-Making

Bio-Processing Research

*Photo: Glacial Lakes Energy*

Woody Biomass Resource Research

Vehicle Systems Research

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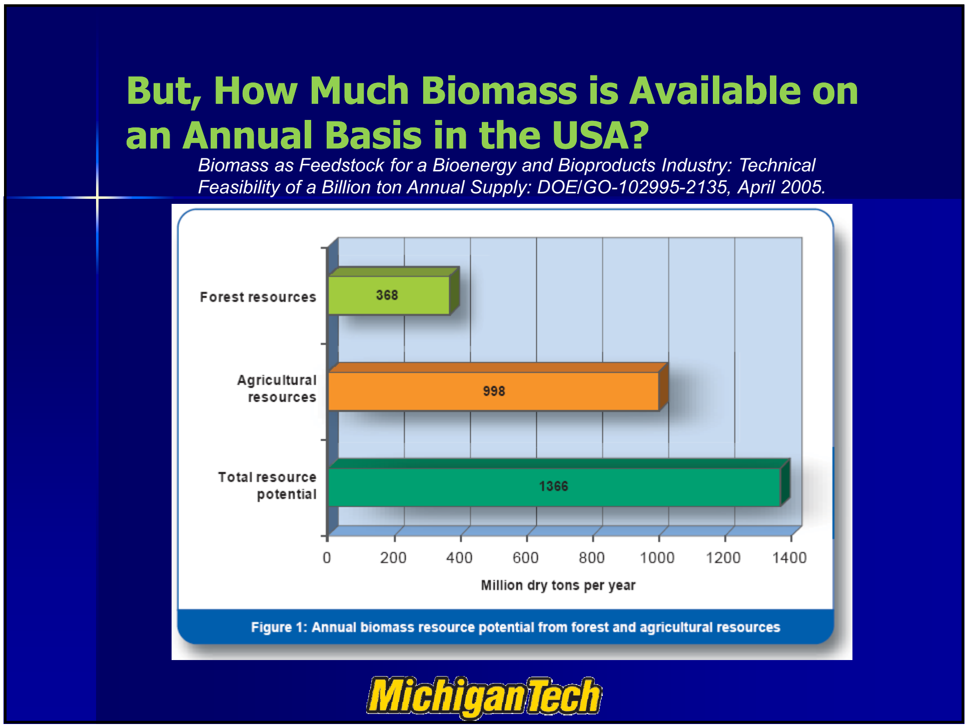

# But, How Much Biomass is Available on an Annual Basis in the USA?

*Biomass as Feedstock for a Bioenergy and Bioproducts Industry: Technical Feasibility of a Billion ton Annual Supply: DOE/GO-102995-2135, April 2005.*

Figure 1: Annual biomass resource potential from forest and agricultural resources

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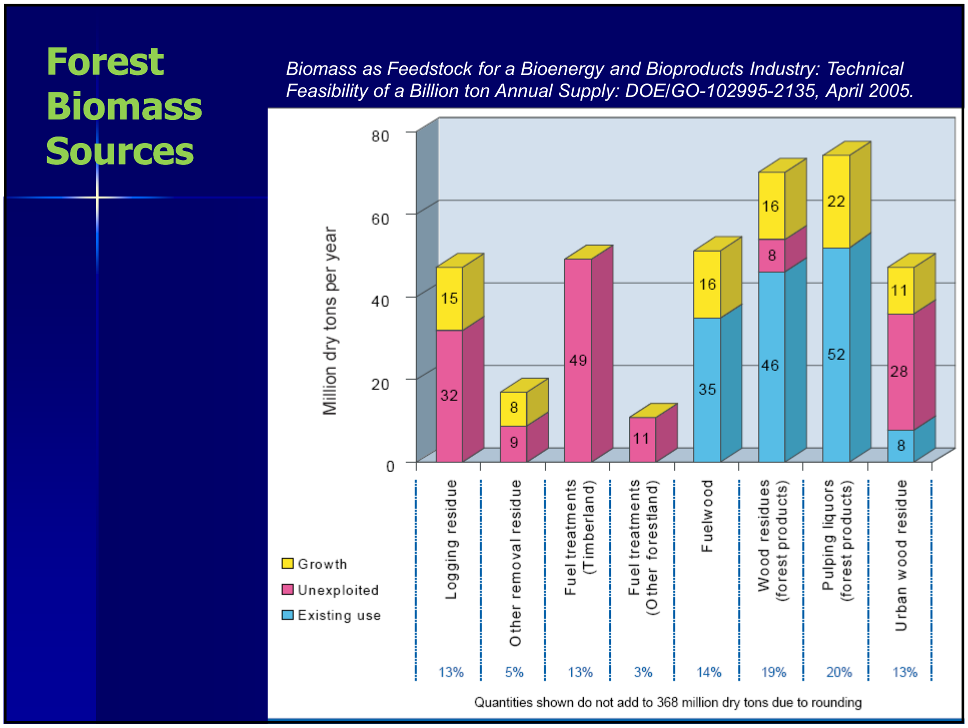
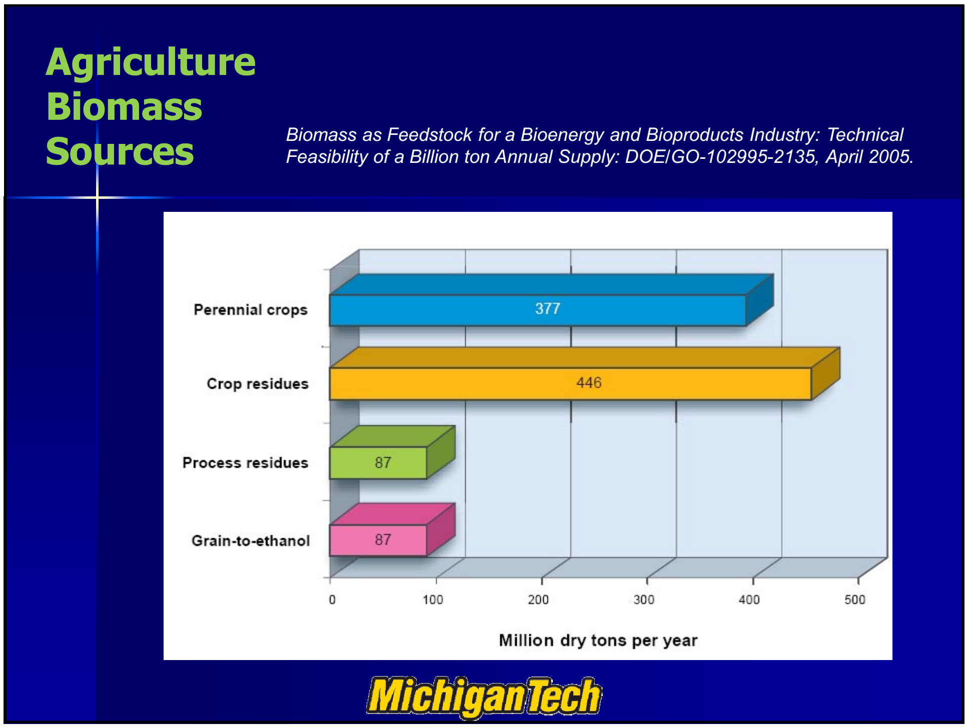


# Forest Biomass Sources

*Biomass as Feedstock for a Bioenergy and Bioproducts Industry: Technical Feasibility of a Billion ton Annual Supply: DOE/GO-102995-2135, April 2005.*

Quantities shown do not add to 368 million dry tons due to rounding

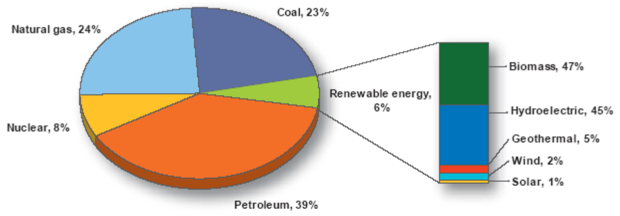


# Agriculture Biomass Sources

*Biomass as Feedstock for a Bioenergy and Bioproducts Industry: Technical Feasibility of a Billion ton Annual Supply: DOE/GO-102995-2135, April 2005.*

## How Much Petroleum is Used ….

*Biomass as Feedstock for a Bioenergy and Bioproducts Industry: Technical Feasibility of a Billion ton Annual Supply: DOE/GO-102995-2135, April 2005.*



| Biomass Consumption | Million dry tons/year |
|---|---|
| Forest products industry | |
| Wood residues | 44 |
| Pulping liquors | 52 |
| Urban wood and food & other process residues | 35 |
| Fuelwood (residential/commercial & electric utilities) | 35 |
| Biofuels | 18 |
| Bioproducts | 6 |
| Total | 190 |

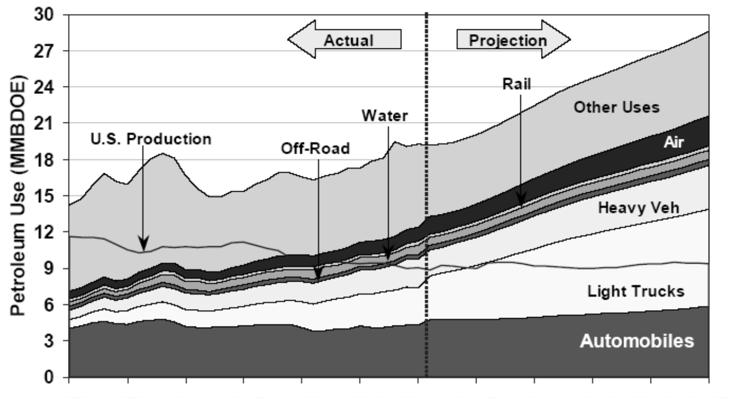
- Forestlands and agricultural lands contribute 190 million dry tons of biomass - 3% of America's current energy consumption.

Source: EIA, 2004a & b

**Figure 2: Summary of biomass resource consumption**

## .. and for What Purpose?

*Wang, Michael; Center for Transportation Research, Argonne National Laboratory*



Data sources: ORNL (2003), the Transportation Energy Databook;
EIA (2003), Annual Energy Outlook; Annual Energy Review

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## How Much Gasoline Could be Replaced with Ethanol From 1B tons Lignocellulose?

**….displace 37.5-75% of current U.S. gasoline demand**

{ 1B tons biomass x 70-100 gal Ethanol/ton biomass x

.75 gal gasoline/gal Ethanol x 1-2 (efficiency of automobiles)} /

140B gal gasoline demand = 37.5-75%

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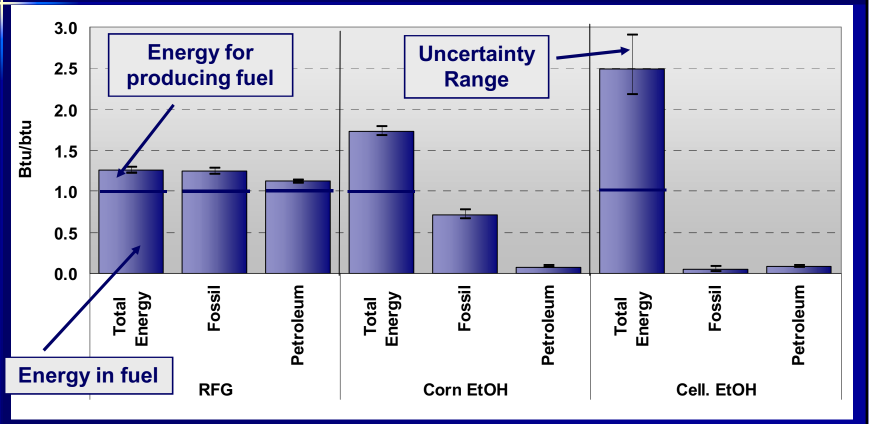
## Are There Energy Benefits of Fuel Ethanol? Fossil Energy and Petroleum Use

*Wang, Michael; Center for Transportation Research, Argonne National Laboratory*



**Energy Use for Each Btu of Fuel Used**

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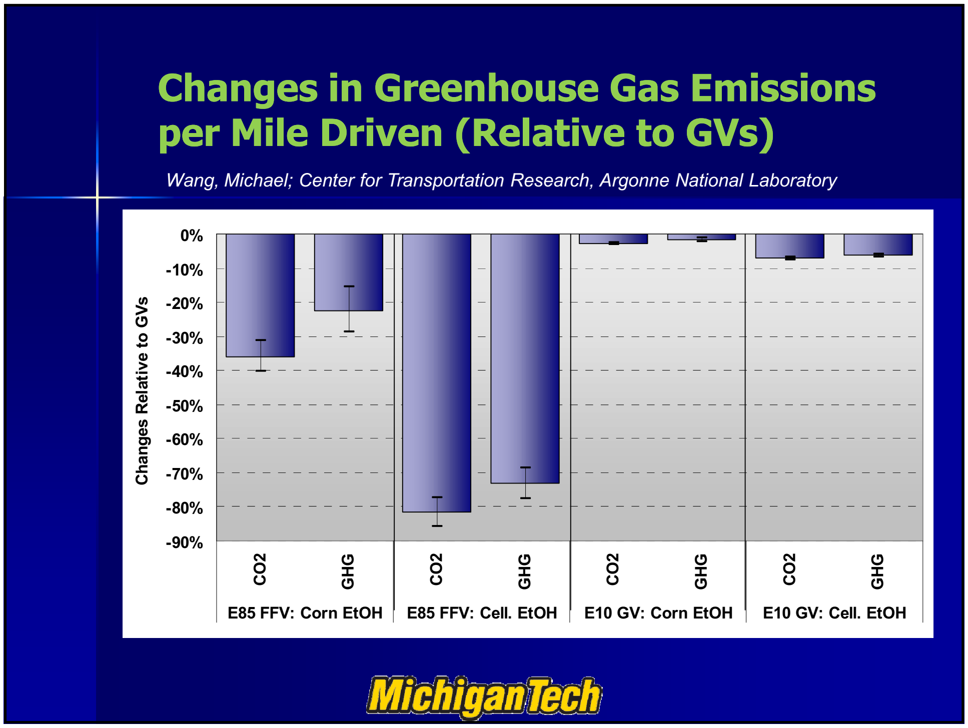

## Changes in Greenhouse Gas Emissions per Mile Driven (Relative to GVs)

*Wang, Michael; Center for Transportation Research, Argonne National Laboratory*

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**Forest Resources**

Biotechnology/Genetic engineering

Forest policy and management

Carbon cycling

**Bio-processing**

Enzyme improvement

Pilot plant operations

Metabolic engineering

## Michigan Tech's Qualifications

**Vehicle/Engines**

Engine research

Engine tests w/emissions

Hybrid vehicle design

Vehicle dynamometer

**Assessment/Decisions**

Technology evaluation

Logistics and facilities

Life-cycle, environmental, and policy assessments

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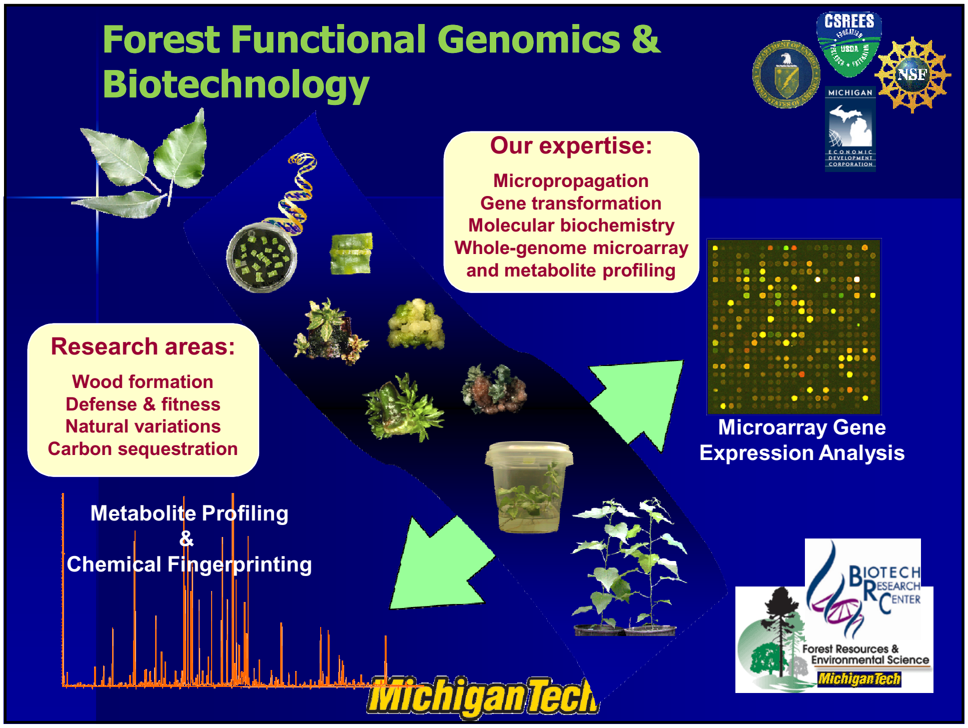


## Forest Functional Genomics & Biotechnology

**Our expertise:**

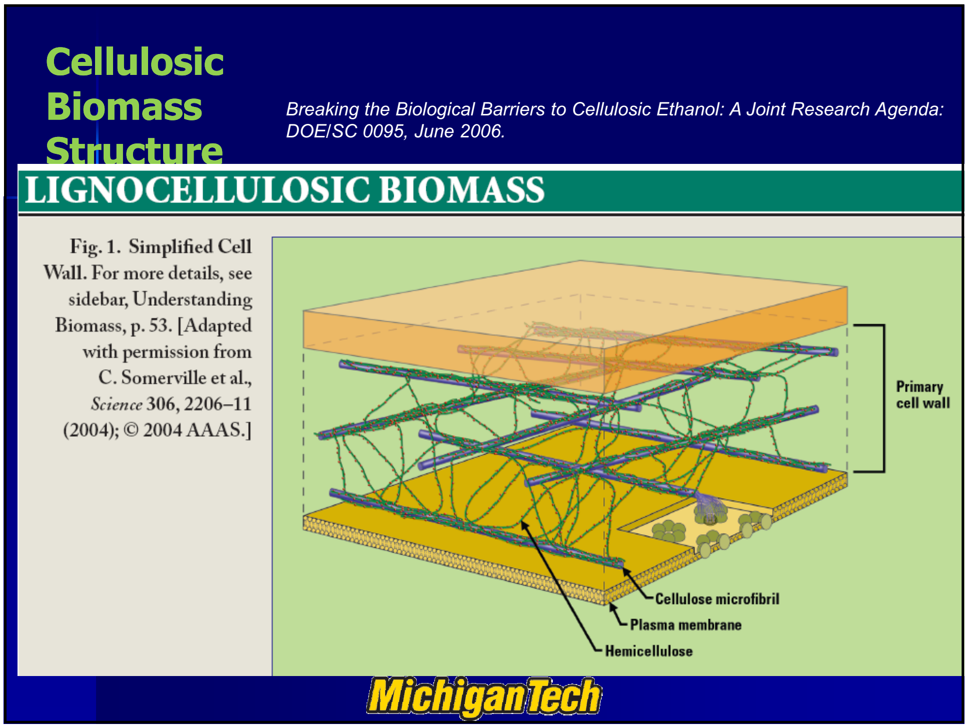
Micropropagation
Gene transformation
Molecular biochemistry
Whole-genome microarray and metabolite profiling

**Research areas:**

Wood formation
Defense & fitness
Natural variations
Carbon sequestration

Microarray Gene Expression Analysis

Metabolite Profiling & Chemical Fingerprinting



## Cellulosic Biomass Structure

*Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda: DOE/SC 0095, June 2006.*

### LIGNOCELLULOSIC BIOMASS

Fig. 1. Simplified Cell Wall. For more details, see sidebar, Understanding Biomass, p. 53. [Adapted with permission from C. Somerville et al., *Science* 306, 2206–11 (2004); © 2004 AAAS.]

## Composition of Woody Biomass

1. Cellulose
2. Hemicellulose
3. Lignin

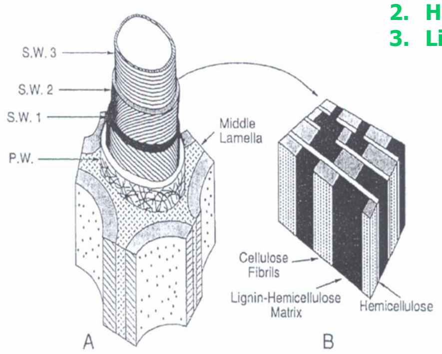
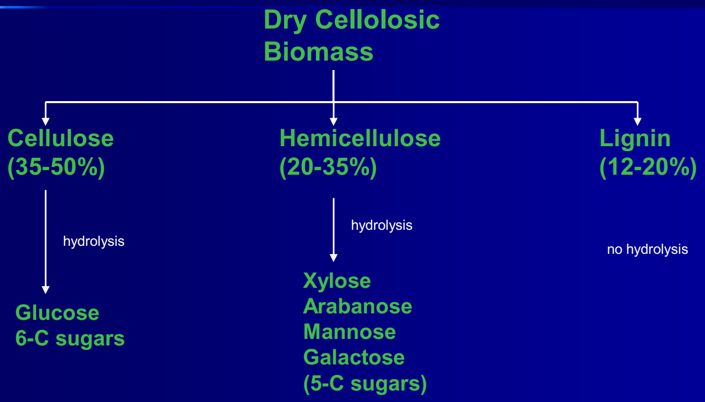


Fig. 2. (A) Cutaway view showing the organization of the cell wall layers composing woody fibres. (B) Probable relationship of lignin and hemicellulose to the cellulose microfibrils in the secondary walls. The diameter of each cell is approximately 25 μm. P.W., primary cell wall; S.W. 1–S.W. 3, secondary cell walls. Redrawn from [250].

Beguin, P., J.P. Aubert. 1994. "The biological Degradation of Cellulose". FEMS Microbiology Reviews. 13:25-58

## Composition of Dry Cellulosic Biomass



Dry Cellolosic Biomass

Cellulose (35-50%)
hydrolysis
Glucose
6-C sugars

Hemicellulose (20-35%)
hydrolysis
Xylose
Arabanose
Mannose
Galactose
(5-C sugars)

Lignin (12-20%)
no hydrolysis

# Bio-processing Initiatives:

### *Thermochemical Conversions*

- Optimize biomass-to-sugar reactions
- Reduce byproduct reactions
- Evaluate timber species' mixtures
- Increase biodiesel yields

### *Integrated Bioprocess Facility*

- Integrate fermentation and purification to increase fuel yields
- Test monitoring devices and process control schemes
- Minimize energy consumption and waste generation

### *Biochemical Conversions*

- Develop/test high-activity cellulases for tree species mixtures
- Optimize cellulose hydrolysis using peptidomimetics
- Improve fermentations for high yields of ethanol / other bio-based materials
- Use metabolic flux analysis to guide strain improvement

### *Product Purification*

- Boost yields by coupling membrane separation with fermentation
- Conserve water by recovering and recycling reactants

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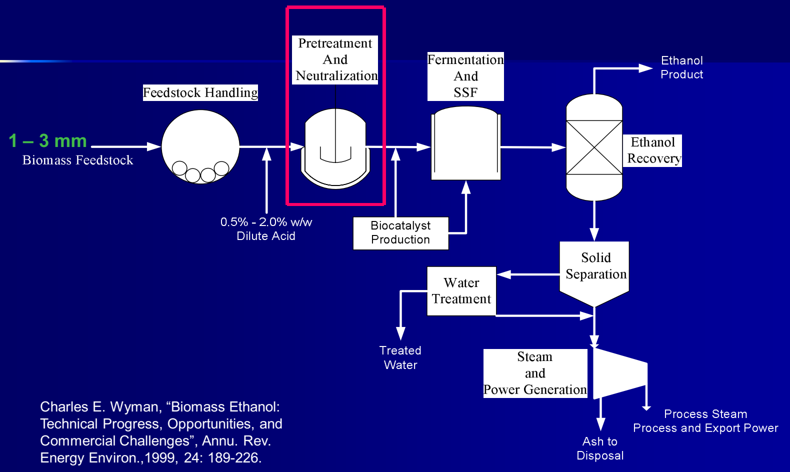
# NREL Process to Convert Lignocellulosic Biomass to Ethanol



Charles E. Wyman, "Biomass Ethanol: Technical Progress, Opportunities, and Commercial Challenges", Annu. Rev. Energy Environ.,1999, 24: 189-226.

Saccharification is enzymatic hydrolysis of pretreated cellulose yielding Glucose using cellulase from *Trichoderma reesei*

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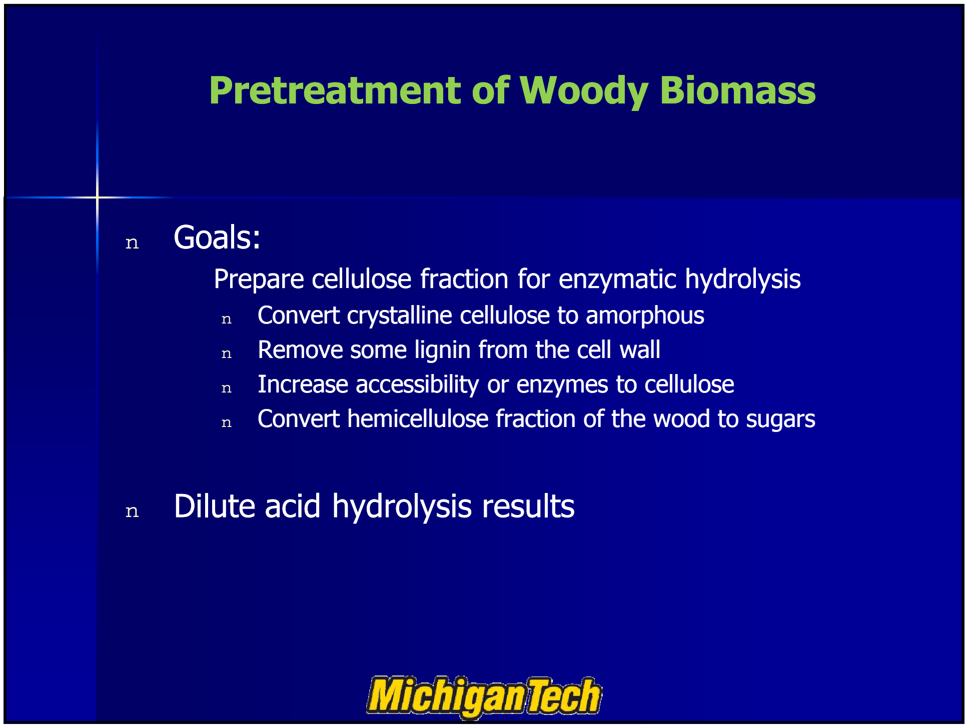

## Pretreatment of Woody Biomass

- Goals:

  Prepare cellulose fraction for enzymatic hydrolysis
  - Convert crystalline cellulose to amorphous
  - Remove some lignin from the cell wall
  - Increase accessibility or enzymes to cellulose
  - Convert hemicellulose fraction of the wood to sugars

- Dilute acid hydrolysis results

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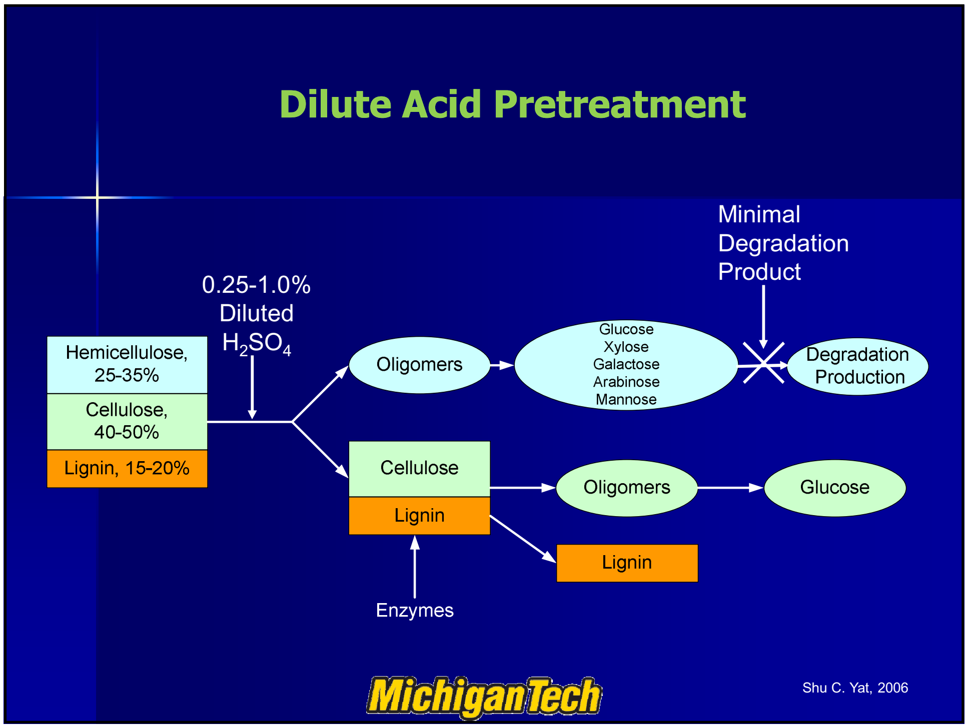

## Dilute Acid Pretreatment

Hemicellulose, 25-35%

Cellulose, 40-50%

Lignin, 15-20%

0.25-1.0% Diluted $H_2SO_4$

Oligomers

Glucose
Xylose
Galactose
Arabinose
Mannose

Minimal Degradation Product

Degradation Production

Cellulose

Lignin

Oligomers

Glucose

Lignin

Enzymes

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Shu C. Yat, 2006

## Goals of Pretreatment Research

- Investigate Mixture Effects
  - Mixtures of timber species plus switchgrass
  - Hypothesis: no synergistic or antagonistic effects due to use of mixtures
- Model pretreatment reactions
  - Develop kinetic parameters from single species expts.
  - Predict monomeric sugar concentrations for single species and mixtures and compare with experimental yields
- Small scale pretreatments using "mini" reactor system
  - Analyze small scale (< 1/100x) samples of biomass

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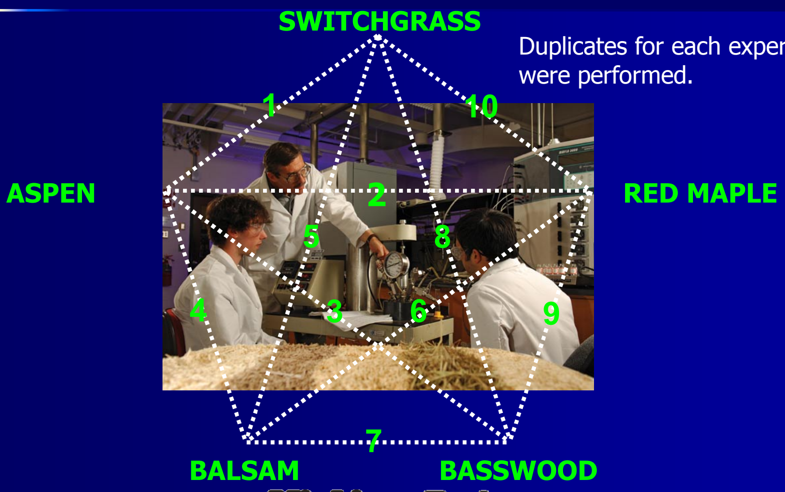
## Experimental Strategy for Pretreatment of 50:50 Biomass Mixtures



Duplicates for each experiment were performed.

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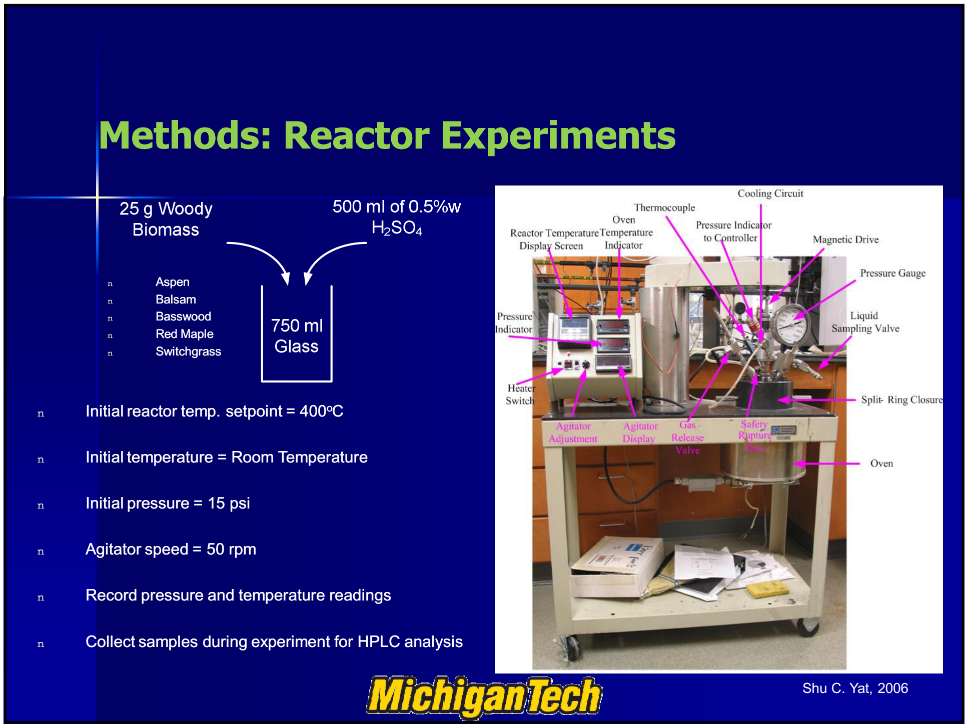

## Methods: Reactor Experiments

25 g Woody Biomass

500 ml of 0.5%w $H_2SO_4$

750 ml Glass

- Aspen
- Balsam
- Basswood
- Red Maple
- Switchgrass

- Initial reactor temp. setpoint = 400ºC
- Initial temperature = Room Temperature
- Initial pressure = 15 psi
- Agitator speed = 50 rpm
- Record pressure and temperature readings
- Collect samples during experiment for HPLC analysis

Shu C. Yat, 2006

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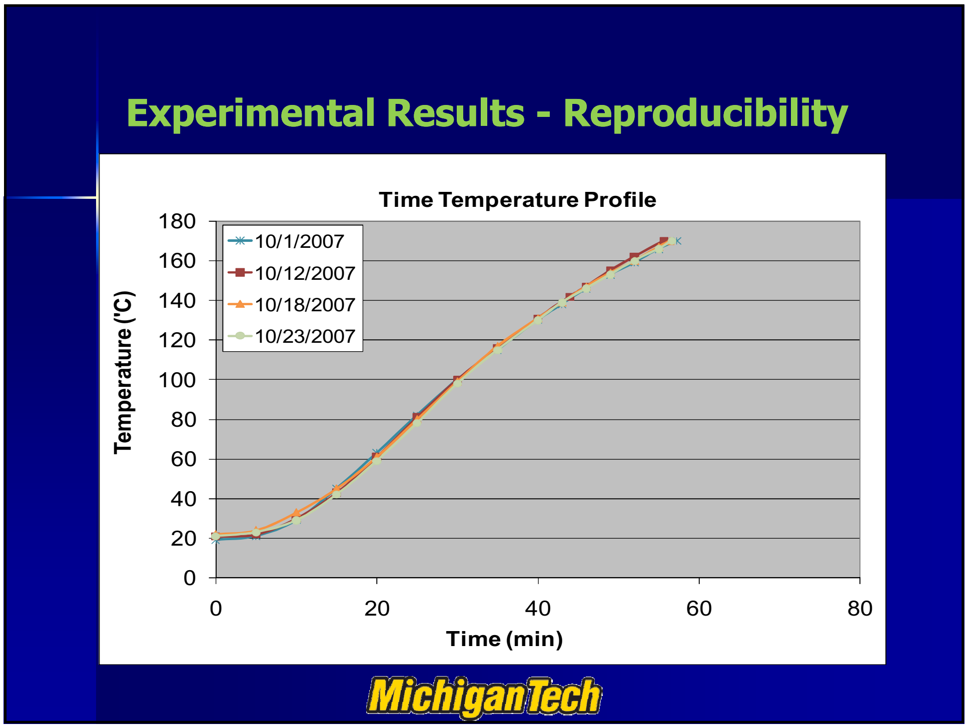

## Experimental Results - Reproducibility

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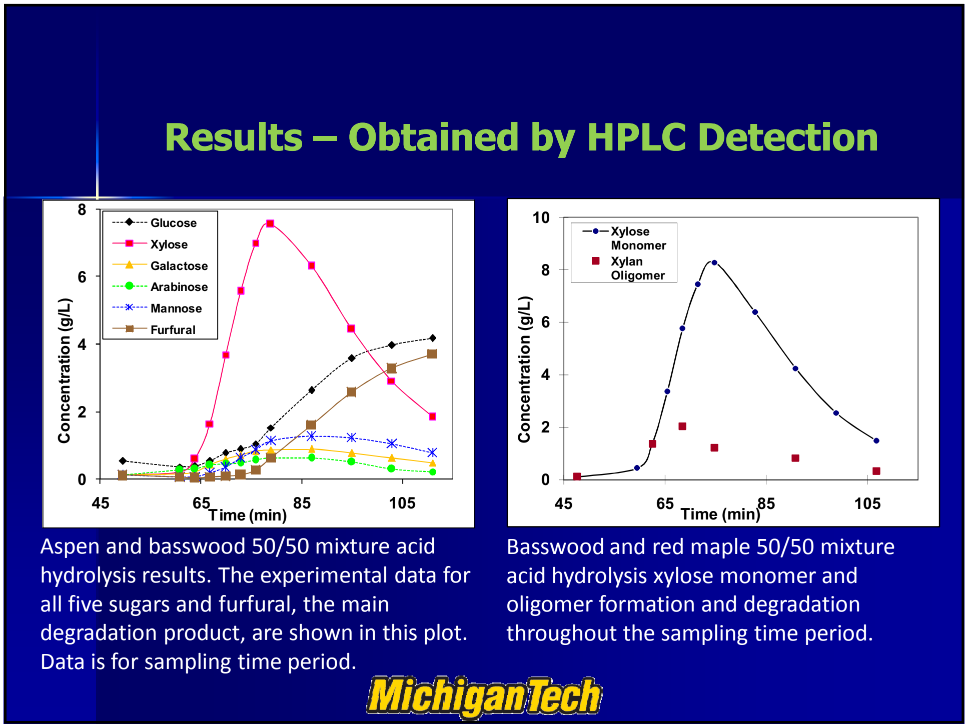
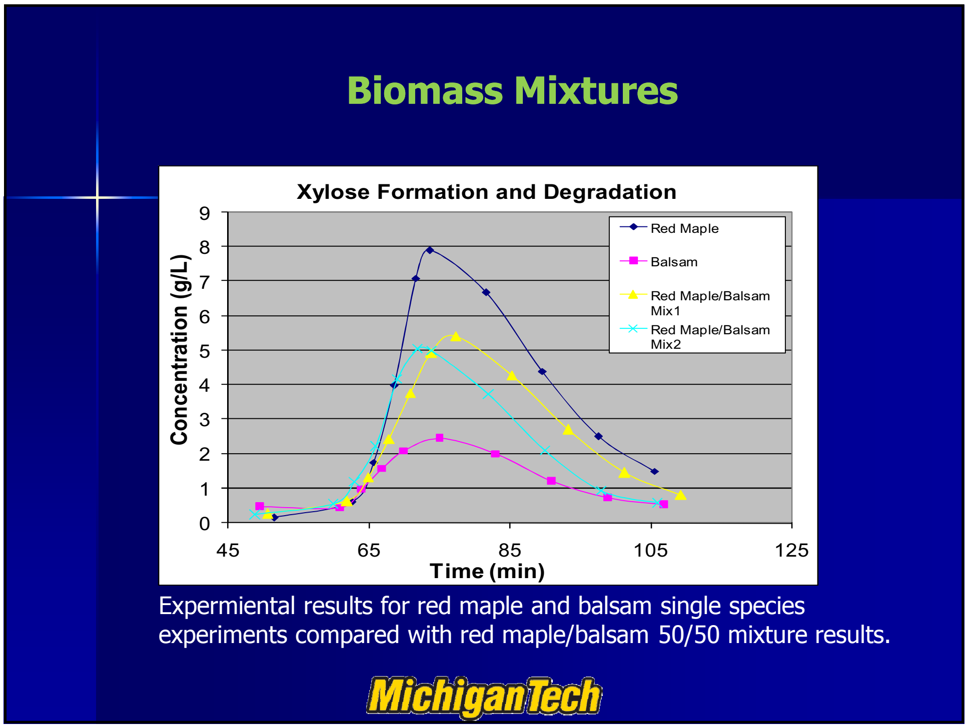


## Results – Obtained by HPLC Detection

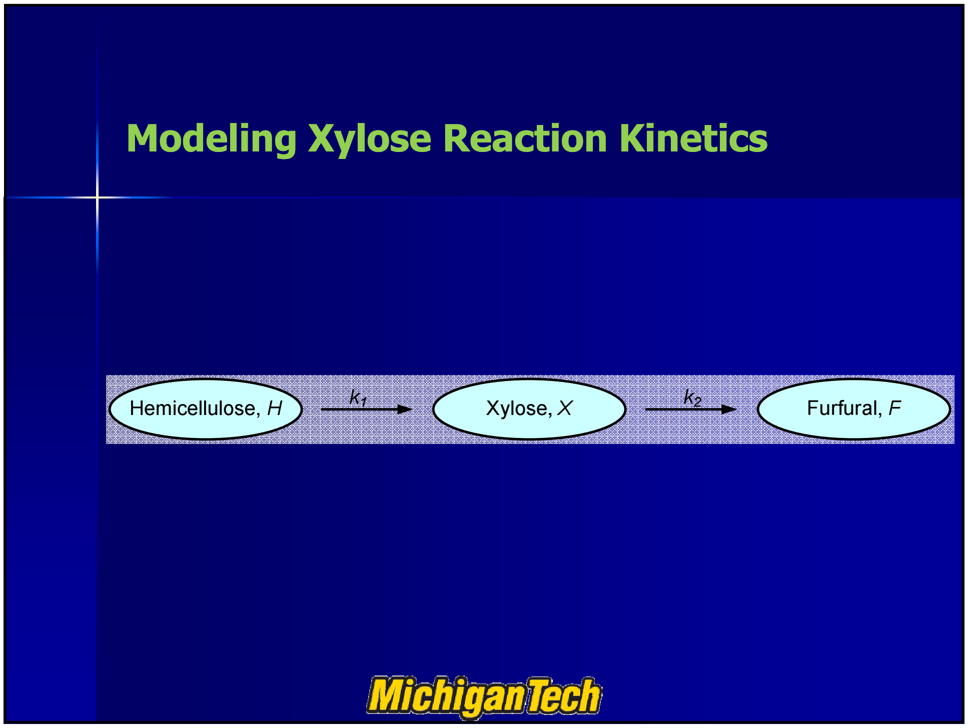
Aspen and basswood 50/50 mixture acid hydrolysis results. The experimental data for all five sugars and furfural, the main degradation product, are shown in this plot. Data is for sampling time period.

Basswood and red maple 50/50 mixture acid hydrolysis xylose monomer and oligomer formation and degradation throughout the sampling time period.



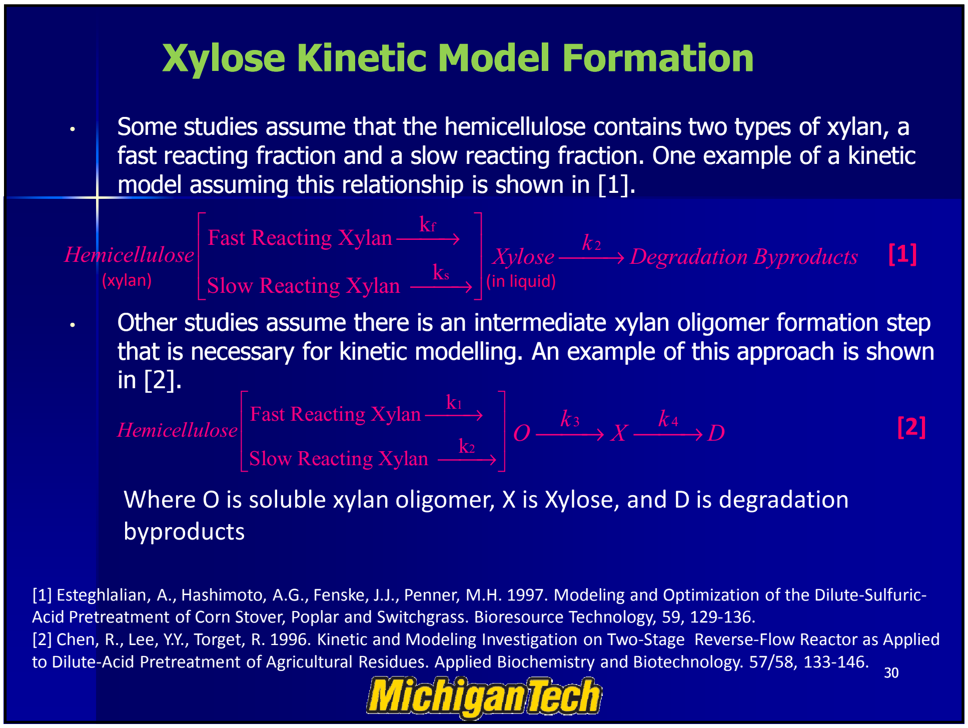
## Biomass Mixtures

Expermiental results for red maple and balsam single species experiments compared with red maple/balsam 50/50 mixture results.



## Modeling Xylose Reaction Kinetics

$$\text{Hemicellulose, } H \xrightarrow{k_1} \text{Xylose, } X \xrightarrow{k_2} \text{Furfural, } F$$



## Xylose Kinetic Model Formation

- Some studies assume that the hemicellulose contains two types of xylan, a fast reacting fraction and a slow reacting fraction. One example of a kinetic model assuming this relationship is shown in [1].

$$\underset{\text{(xylan)}}{\text{Hemicellulose}}\left[\begin{array}{l}\text{Fast Reacting Xylan}\xrightarrow{k_f}\\ \text{Slow Reacting Xylan}\xrightarrow{k_s}\end{array}\right]\underset{\text{(in liquid)}}{\text{Xylose}}\xrightarrow{k_2}\text{Degradation Byproducts} \quad [1]$$

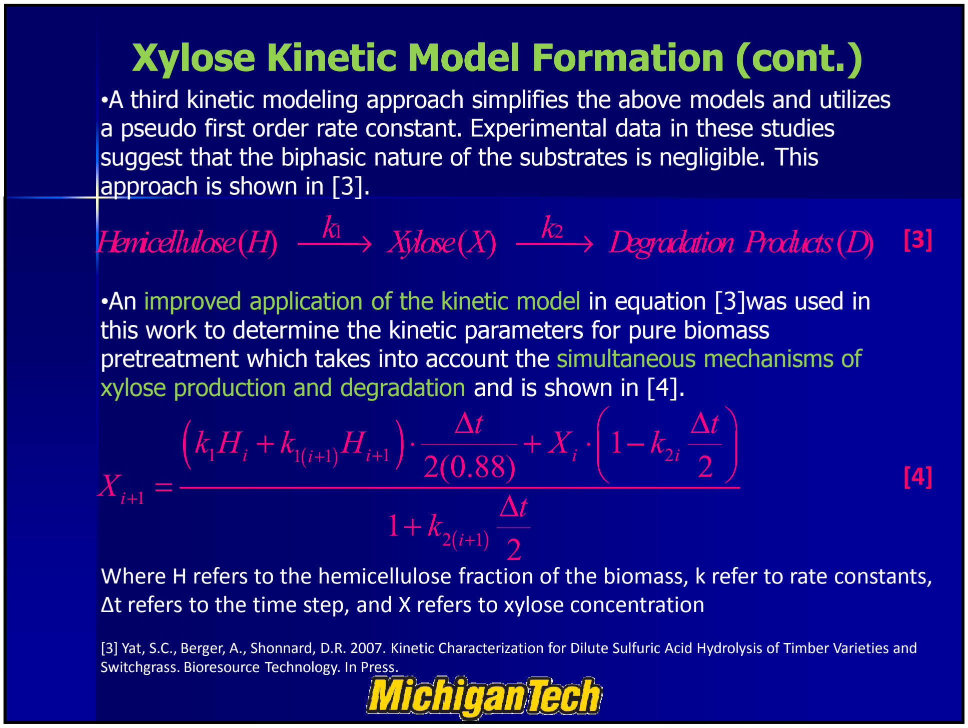
- Other studies assume there is an intermediate xylan oligomer formation step that is necessary for kinetic modelling. An example of this approach is shown in [2].

$$\text{Hemicellulose}\left[\begin{array}{l}\text{Fast Reacting Xylan}\xrightarrow{k_1}\\ \text{Slow Reacting Xylan}\xrightarrow{k_2}\end{array}\right]O\xrightarrow{k_3}X\xrightarrow{k_4}D \quad [2]$$

Where O is soluble xylan oligomer, X is Xylose, and D is degradation byproducts

[1] Esteghlalian, A., Hashimoto, A.G., Fenske, J.J., Penner, M.H. 1997. Modeling and Optimization of the Dilute-Sulfuric-Acid Pretreatment of Corn Stover, Poplar and Switchgrass. Bioresource Technology, 59, 129-136.

[2] Chen, R., Lee, Y.Y., Torget, R. 1996. Kinetic and Modeling Investigation on Two-Stage Reverse-Flow Reactor as Applied to Dilute-Acid Pretreatment of Agricultural Residues. Applied Biochemistry and Biotechnology. 57/58, 133-146.



## Xylose Kinetic Model Formation (cont.)

- A third kinetic modeling approach simplifies the above models and utilizes a pseudo first order rate constant. Experimental data in these studies suggest that the biphasic nature of the substrates is negligible. This approach is shown in [3].

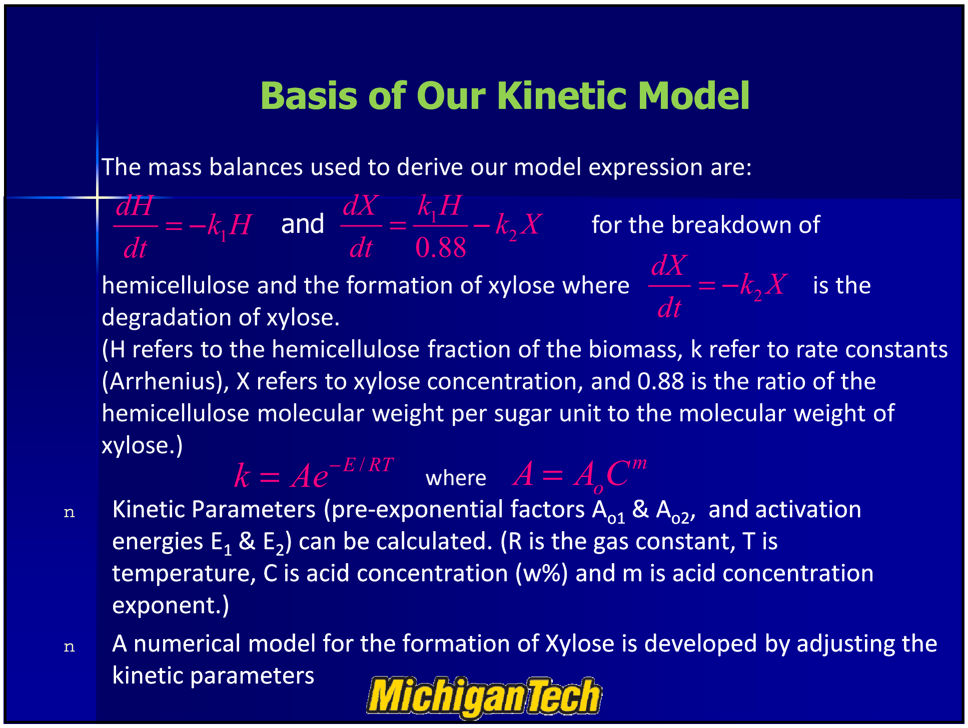
$$\text{Hemicellulose}(H) \xrightarrow{k_1} \text{Xylose}(X) \xrightarrow{k_2} \text{Degradation Products}(D) \qquad [3]$$

- An improved application of the kinetic model in equation [3]was used in this work to determine the kinetic parameters for pure biomass pretreatment which takes into account the simultaneous mechanisms of xylose production and degradation and is shown in [4].

$$X_{i+1} = \frac{\left(k_1 H_i + k_{1(i+1)} H_{i+1}\right) \cdot \frac{\Delta t}{2(0.88)} + X_i \cdot \left(1 - k_{2i}\frac{\Delta t}{2}\right)}{1 + k_{2(i+1)}\frac{\Delta t}{2}} \qquad [4]$$

Where H refers to the hemicellulose fraction of the biomass, k refer to rate constants, Δt refers to the time step, and X refers to xylose concentration

[3] Yat, S.C., Berger, A., Shonnard, D.R. 2007. Kinetic Characterization for Dilute Sulfuric Acid Hydrolysis of Timber Varieties and Switchgrass. Bioresource Technology. In Press.



## Basis of Our Kinetic Model

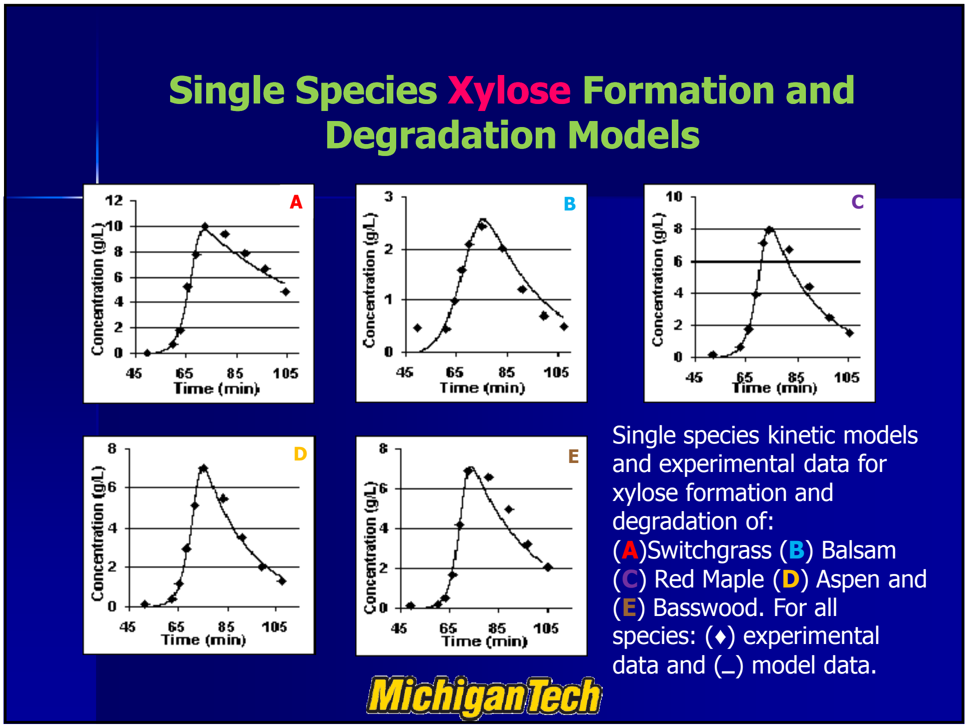
The mass balances used to derive our model expression are:

$\frac{dH}{dt} = -k_1 H$ and $\frac{dX}{dt} = \frac{k_1 H}{0.88} - k_2 X$ for the breakdown of hemicellulose and the formation of xylose where $\frac{dX}{dt} = -k_2 X$ is the degradation of xylose.

(H refers to the hemicellulose fraction of the biomass, k refer to rate constants (Arrhenius), X refers to xylose concentration, and 0.88 is the ratio of the hemicellulose molecular weight per sugar unit to the molecular weight of xylose.)

$$k = Ae^{-E/RT} \quad \text{where} \quad A = A_o C^m$$

- Kinetic Parameters (pre-exponential factors $A_{o1}$ & $A_{o2}$, and activation energies $E_1$ & $E_2$) can be calculated. (R is the gas constant, T is temperature, C is acid concentration (w%) and m is acid concentration exponent.)
- A numerical model for the formation of Xylose is developed by adjusting the kinetic parameters



## Single Species Xylose Formation and Degradation Models

Single species kinetic models and experimental data for xylose formation and degradation of: (A)Switchgrass (B) Balsam (C) Red Maple (D) Aspen and (E) Basswood. For all species: (♦) experimental data and (_) model data.

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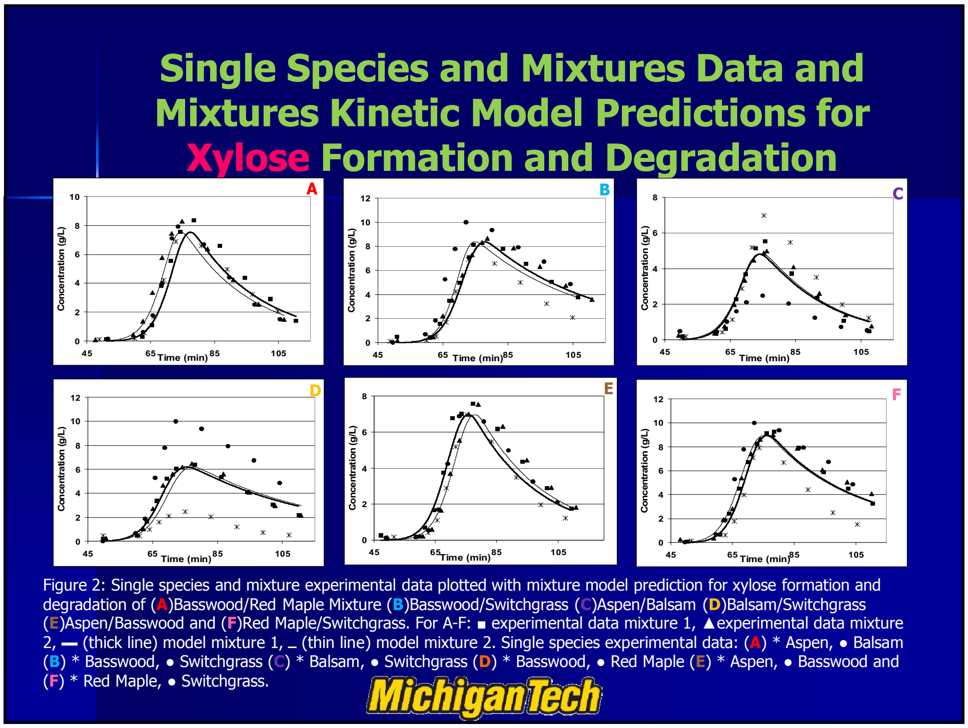

## Single Species and Mixtures Data and Mixtures Kinetic Model Predictions for Xylose Formation and Degradation

Figure 2: Single species and mixture experimental data plotted with mixture model prediction for xylose formation and degradation of (A)Basswood/Red Maple Mixture (B)Basswood/Switchgrass (C)Aspen/Balsam (D)Balsam/Switchgrass (E)Aspen/Basswood and (F)Red Maple/Switchgrass. For A-F: ■ experimental data mixture 1, ▲experimental data mixture 2, — (thick line) model mixture 1, _ (thin line) model mixture 2. Single species experimental data: (A) * Aspen, ● Balsam (B) * Basswood, ● Switchgrass (C) * Balsam, ● Switchgrass (D) * Basswood, ● Red Maple (E) * Aspen, ● Basswood and (F) * Red Maple, ● Switchgrass.

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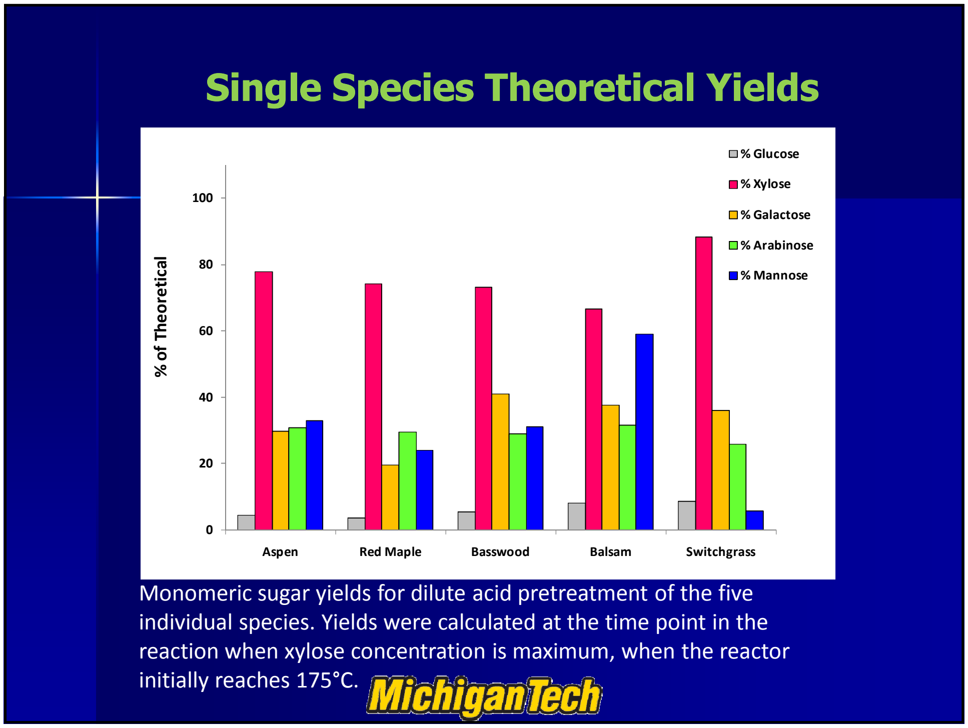

## Single Species Theoretical Yields

Monomeric sugar yields for dilute acid pretreatment of the five individual species. Yields were calculated at the time point in the reaction when xylose concentration is maximum, when the reactor initially reaches 175°C.

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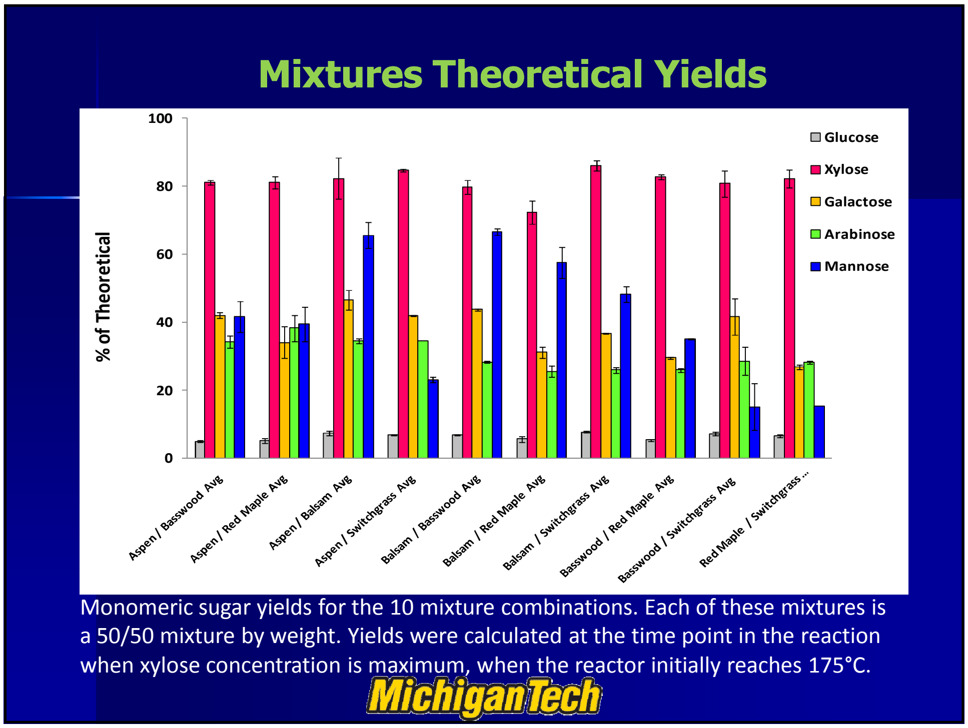

## Mixtures Theoretical Yields

Monomeric sugar yields for the 10 mixture combinations. Each of these mixtures is a 50/50 mixture by weight. Yields were calculated at the time point in the reaction when xylose concentration is maximum, when the reactor initially reaches 175°C.

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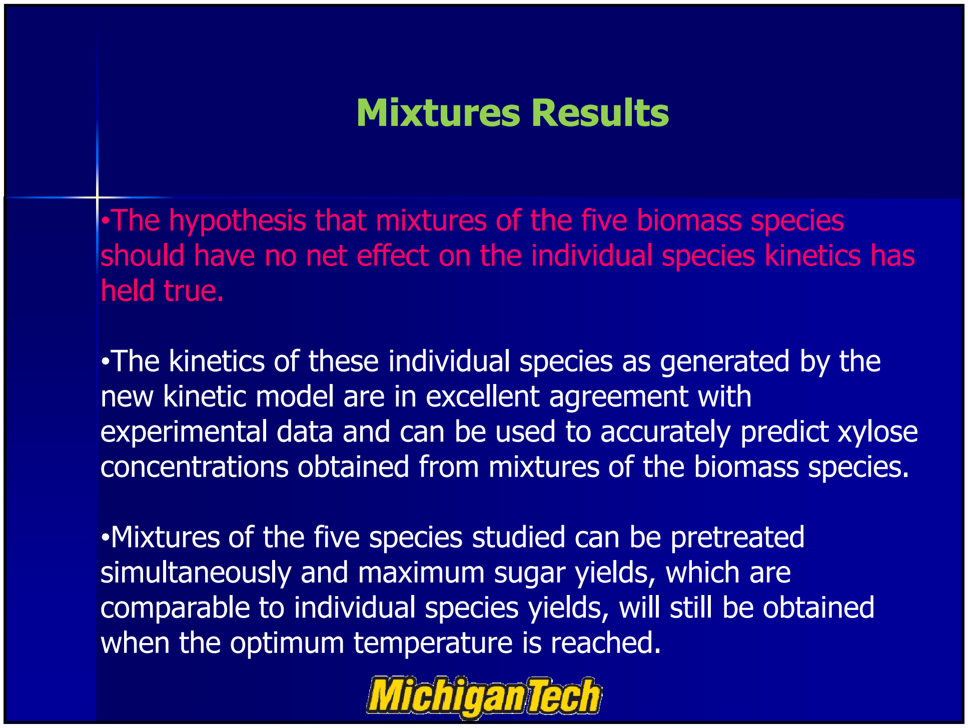

## Mixtures Results

- The hypothesis that mixtures of the five biomass species should have no net effect on the individual species kinetics has held true.

- The kinetics of these individual species as generated by the new kinetic model are in excellent agreement with experimental data and can be used to accurately predict xylose concentrations obtained from mixtures of the biomass species.

- Mixtures of the five species studied can be pretreated simultaneously and maximum sugar yields, which are comparable to individual species yields, will still be obtained when the optimum temperature is reached.

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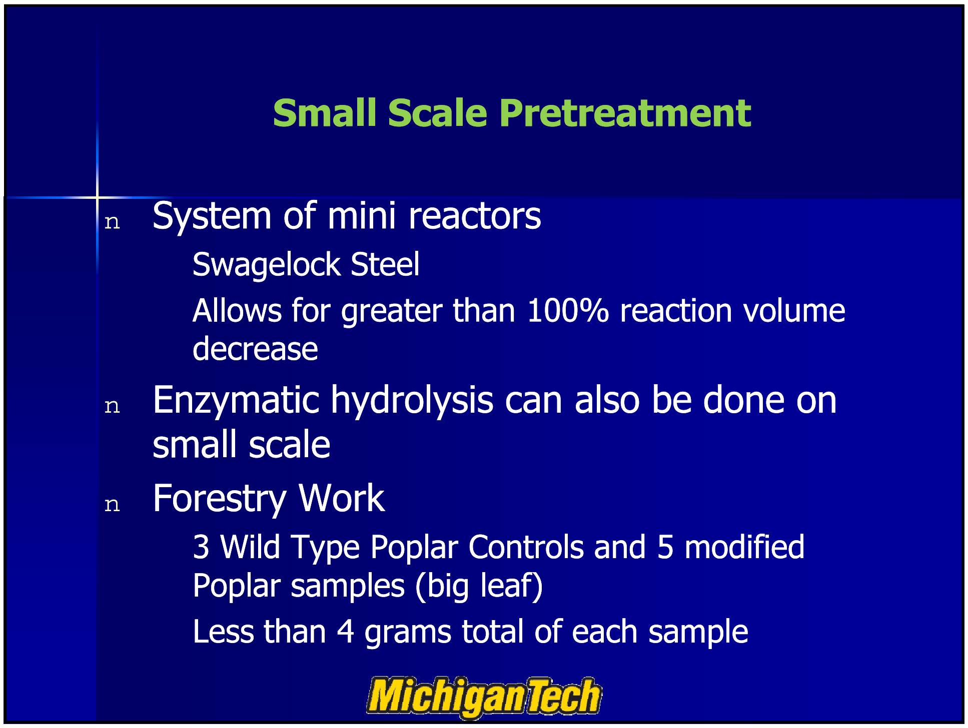

## Small Scale Pretreatment

- System of mini reactors
  - Swagelock Steel
  - Allows for greater than 100% reaction volume decrease
- Enzymatic hydrolysis can also be done on small scale
- Forestry Work
  - 3 Wild Type Poplar Controls and 5 modified Poplar samples (big leaf)
  - Less than 4 grams total of each sample

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## Similarities and Differences in Reaction Setup

- Setup still allows for temperature control
- Only one sugar sample
- Normal heat up with rapid cooling
- Identified 170°C as optimum temperature
- Not enough liquid volume to study oligomers

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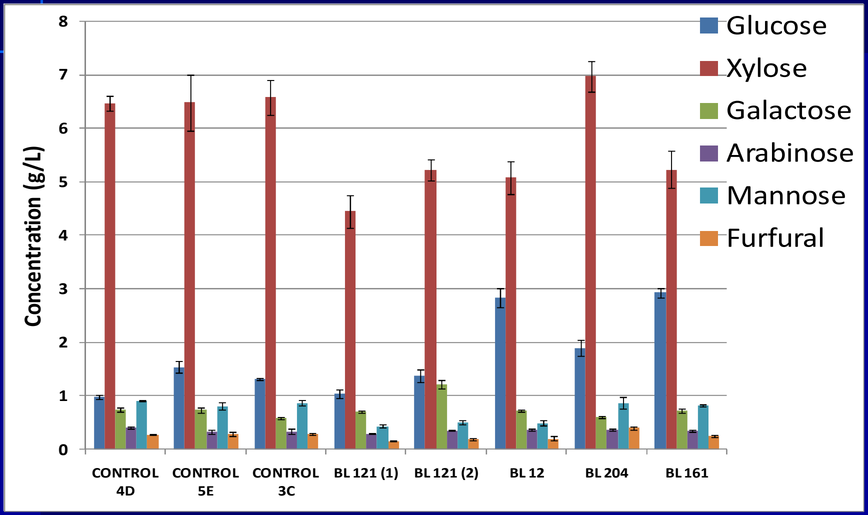
## Forestry Work Results



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## Future and Ongoing Work

- Future work needs to be done to further study the oligomeric trends as well as the feasibility of converting the oligomers into monomers.
- Modeling Alternative Conditions of Reactor Operation - Different reactor configurations will be investigated in order to optimize process by maximizing products and minimizing byproducts
  - CSTR: *Continuous Stirred Tank Reactor*
  - PFR: *Plug Flow Reactor*
- Small scale enzymatic hydrolysis of hybrid poplar

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## Future and Ongoing Work (cont.)

- Heterologous Expression and Mutagenesis of Cellulose Hydrolases for Improved Performance
- Characterization of Improved Cellulase Enzymes for Cellulose Hydrolysis
- Life cycle assessment
- Advanced imaging technology, including Scanning Electron Microscopy (SEM) and optical microscopy, of untreated, pretreated, and enzymatically hydrolyzed biomass samples to view structural changes with fluorescent tags

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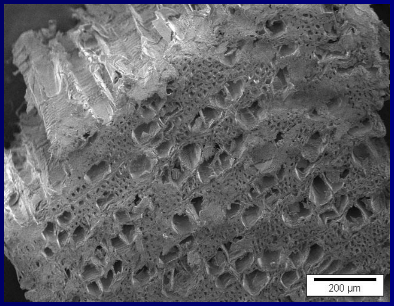
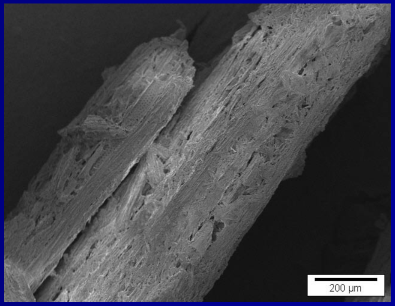
## Scanning Electron Microscopy



SEM Image of Aspen
prior to pretreatment



SEM Image of Aspen
after pretreatment

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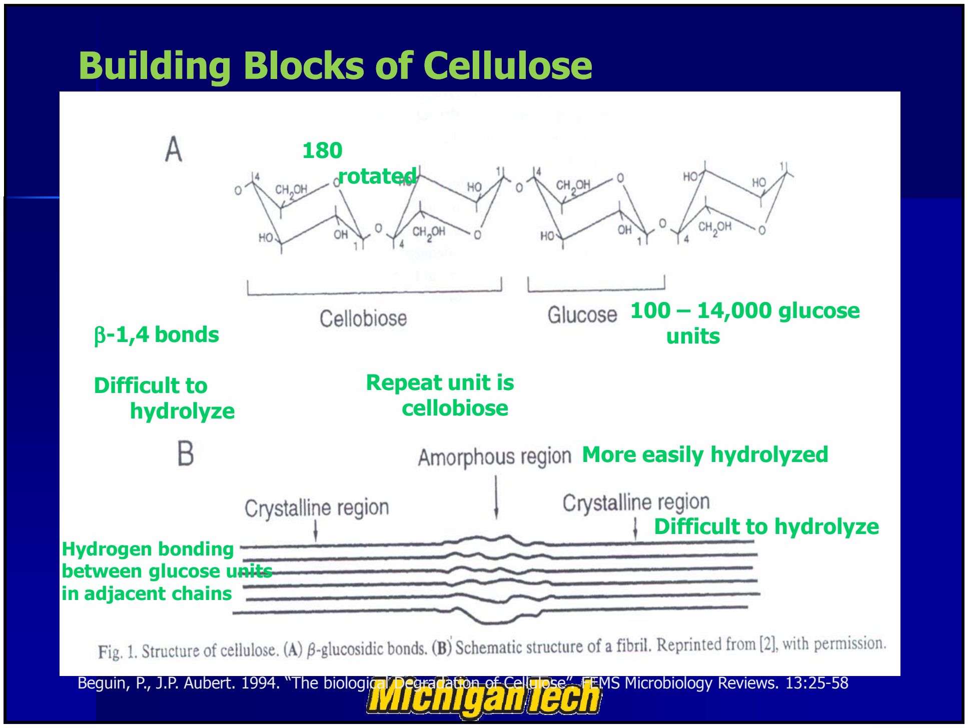
## Enzymatic Hydrolysis of Cellulose to Yield Glucose for Fermentation

- Introduction
- Technological issues



## Building Blocks of Cellulose

100 – 14,000 glucose units

β-1,4 bonds

Difficult to hydrolyze

Repeat unit is cellobiose

More easily hydrolyzed

Difficult to hydrolyze

Hydrogen bonding between glucose units in adjacent chains

Fig. 1. Structure of cellulose. (A) β-glucosidic bonds. (B) Schematic structure of a fibril. Reprinted from [2], with permission.

Beguin, P., J.P. Aubert. 1994. "The biological Degradation of Cellulose". FEMS Microbiology Reviews. 13:25-58

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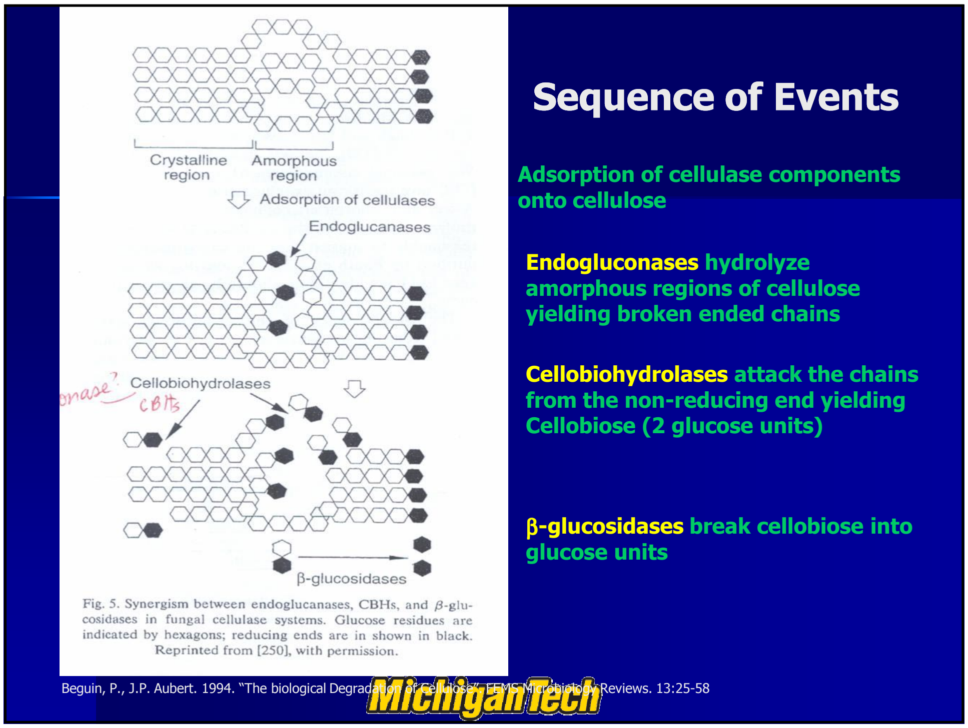

## Sequence of Events

Adsorption of cellulase components onto cellulose

**Endogluconases** hydrolyze amorphous regions of cellulose yielding broken ended chains

**Cellobiohydrolases** attack the chains from the non-reducing end yielding Cellobiose (2 glucose units)

**β-glucosidases** break cellobiose into glucose units

Fig. 5. Synergism between endoglucanases, CBHs, and β-glucosidases in fungal cellulase systems. Glucose residues are indicated by hexagons; reducing ends are in shown in black. Reprinted from [250], with permission.

Beguin, P., J.P. Aubert. 1994. "The biological Degradation of Cellulose". FEMS Microbiology Reviews. 13:25-58

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## Components of Cellulases

**Table 1**
**Some properties of the cellulases from *Trichoderma reesei***

| Name | Designation | Molecular weight (kDa) | Isoelectric point (pI) | Position of CBD |
|---|---|---|---|---|
| CBH I | Cel7A | 57 | 3.9 | C |
| CBH II | Cel6A | 53 | 5.9 | N |
| EG I | Cel7B | 55 | 4.5 | C |
| EG II | Cel5A | 50 | 5.5 | N |
| EG III | Cel12A | 25 | 7.5 | lack of CBD |
| EG V | Cel45A | 36 | 2.9 | C |

- CBH – Cellobiohydrolases: I attacks the non-reducing ends, II attacks the the reducing ends of chains
- EG – endoglucanases: hydrolyze amorphous regions
- β-glucosidases: split cellobiose to glucose
- CBD – Cellulose-binding Domain

Valjamae, P. "The Kinetics of Cellulose Enzymatic Hydrolysis – Implications of the Synergism Between Enzymes". ACTA Universitatis Upsaliensis. Uppsala. 2002

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## Cellobiohydrolases

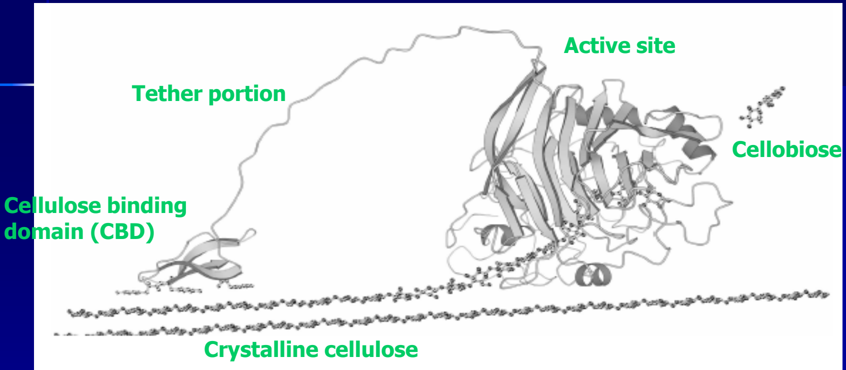

**Figure 2. A hypothetical model of CBH I acting on the crystalline cellulose** The picture was done with MolScript and kindly provided by Dr. Jerry Ståhlberg with permission from Dr. Christina Divne (© Christina Divne 1998).

Valjamae, P. "The Kinetics of Cellulose Enzymatic Hydrolysis – Implications of the Synergism Between Enzymes". ACTA Universitatis Upsaliensis. Uppsala. Sweden. 2002

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## Observed rate of Cellulose Hydrolysis

Rate decreases over time

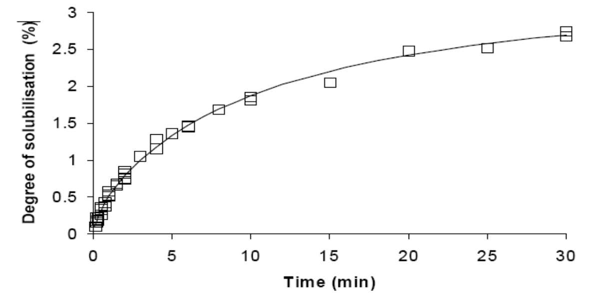

**Figure 3. Typical pattern of the degree of solubilization as a function of time in enzymatic hydrolysis of cellulose** Bacterial cellulose (0.5 mg/mL) was incubated with 0.5 µM CBH I in 0.05M NaAc buffer, pH 5.0 at 25° C. Solid line is according to Eq. 1.

Hypotheses

1. Consumption of easily hydrolysable components
2. Inhibition by reaction product cellobiose
3. Inactivation of cellulase

Valjamae, P. "The Kinetics of Cellulose Enzymatic Hydrolysis – Implications of the Synergism Between Enzymes". ACTA Universitatis Upsaliensis. Uppsala. Sweden. 2002

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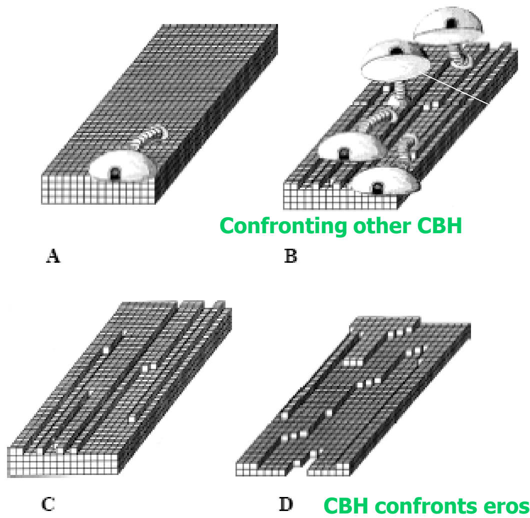
## Surface Erosion Model



Cellobiohydrolase (CBH)

Confronting other CBH

CBH confronts erosion features

Figure 4. Pictures of typical modeled cellulose surface erosion patterns A) At the beginning of the adsorption of cellobiohydrolase B) after limited hydrolysis by a processive cellobiohydrolase, adsorption equilibrium has been reached C) the same as in B but enzymes are not shown C) after extended hydrolysis by a processive cellobiohydrolase, enzymes are not shown.

1. Processivity of the CBH is hindered by the surface erosion pattern due to the strong binding of the CBD.
2. Mechanisms 2 and 3 from the previous slide are not important, as shown in the referenced work below.

Valjamae, P. "The Kinetics of Cellulose Enzymatic Hydrolysis – Implications of the Synergism Between Enzymes". ACTA Universitatis Upsaliensis. Uppsala. Sweden. 2002

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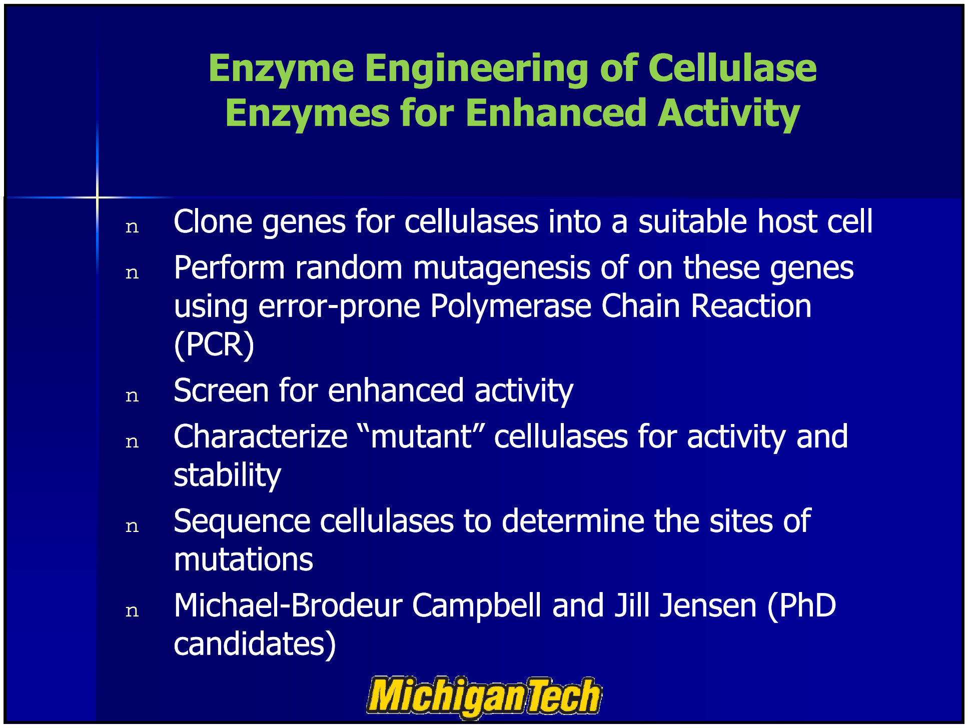

## Enzyme Engineering of Cellulase Enzymes for Enhanced Activity

- Clone genes for cellulases into a suitable host cell
- Perform random mutagenesis of on these genes using error-prone Polymerase Chain Reaction (PCR)
- Screen for enhanced activity
- Characterize "mutant" cellulases for activity and stability
- Sequence cellulases to determine the sites of mutations
- Michael-Brodeur Campbell and Jill Jensen (PhD candidates)

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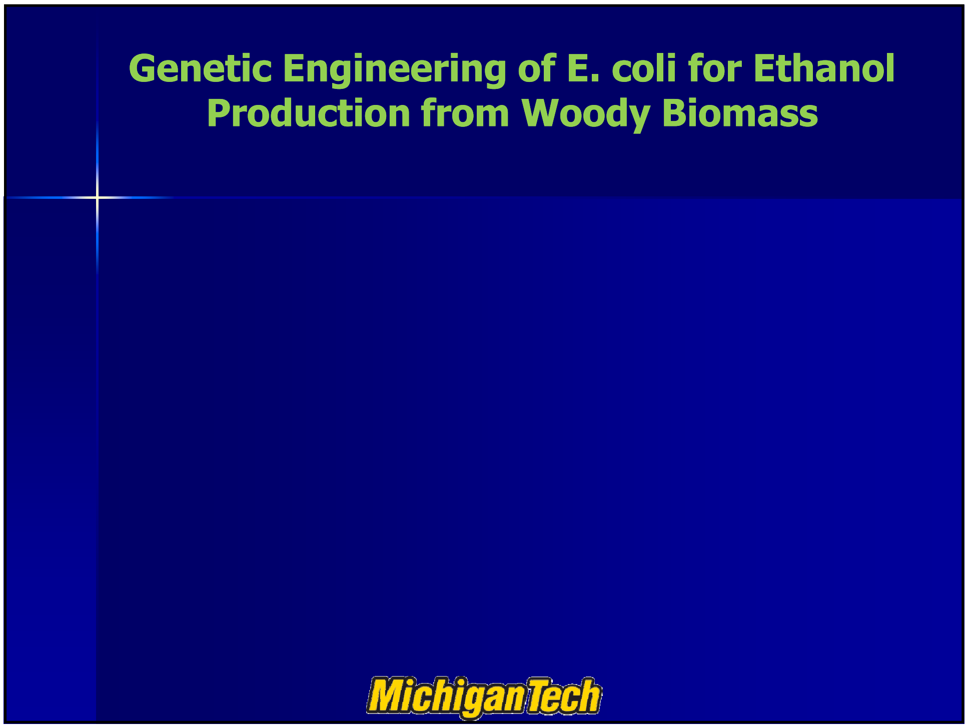

## Genetic Engineering of E. coli for Ethanol Production from Woody Biomass

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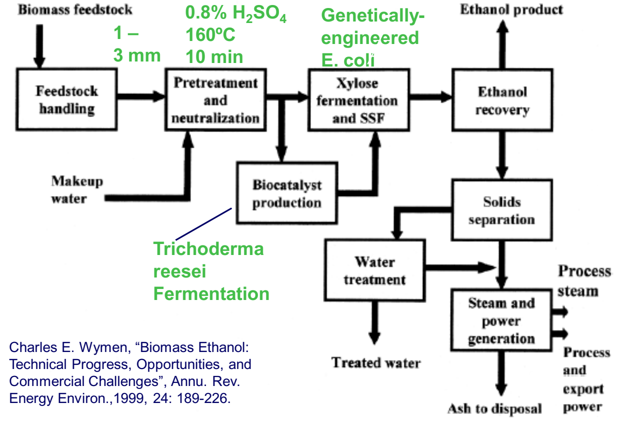
## Process to Convert Cellulosic Biomass to Ethanol



1 – 3 mm

0.8% $H_2SO_4$
160°C
10 min

Genetically-engineered E. coli

Trichoderma reesei Fermentation

Charles E. Wymen, "Biomass Ethanol: Technical Progress, Opportunities, and Commercial Challenges", Annu. Rev. Energy Environ.,1999, 24: 189-226.

**Figure 1** Block flow diagram for conversion of biomass to ethanol by the NREL process configuration. SSF, Simultaneous saccharification and fermentation.

Saccharification is enzymatic hydrolysis of pretreated cellulose yielding Glucose using cellulase from Trichoderma reesei

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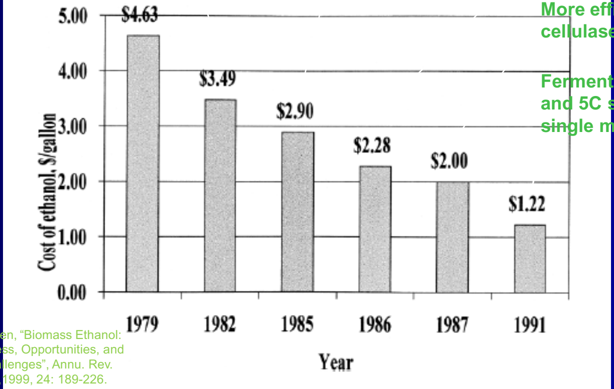
## History of Costs for Ethanol Production

Sequential enzymatic hydrolysis then fermentation

Improved fungal strain for cellulase production

Improved cellulase (150L) produced by Genencore

Simultaneous Saccharification-Fermentation process



More efficient cellulase

Fermentation of 6C and 5C sugars using a single microorganism

Charles E. Wymen, "Biomass Ethanol: Technical Progress, Opportunities, and Commercial Challenges", Annu. Rev. Energy Environ., 1999, 24: 189-226.

**Figure 2** Progress in reducing the cost of producing ethanol from biomass based on enzymatic cellulose hydrolysis technology, as shown in 1990 dollars.

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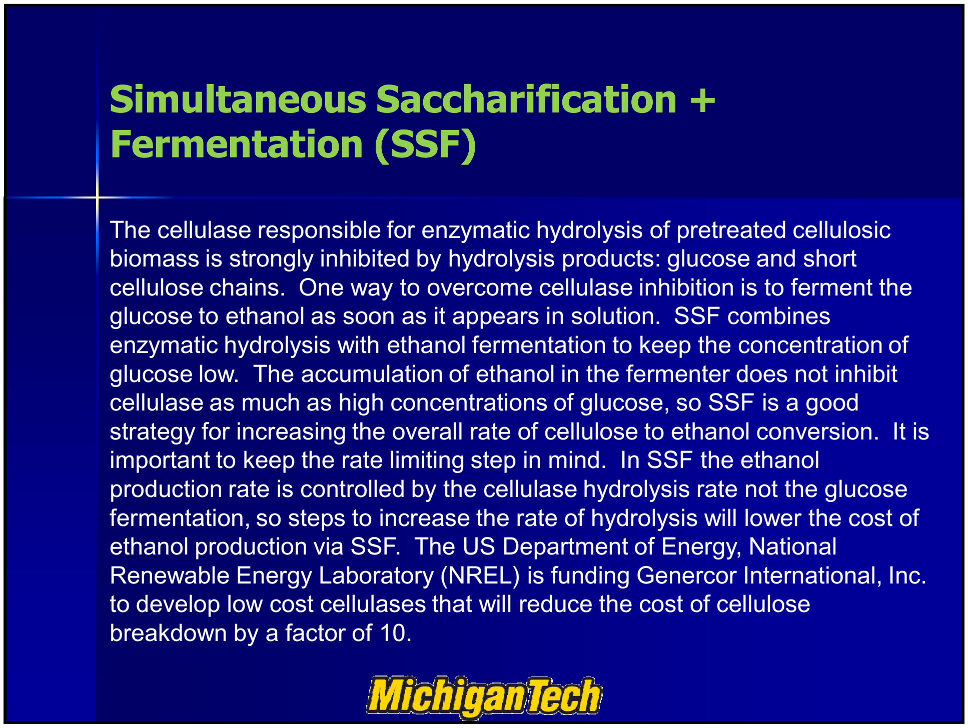

## Simultaneous Saccharification + Fermentation (SSF)

The cellulase responsible for enzymatic hydrolysis of pretreated cellulosic biomass is strongly inhibited by hydrolysis products: glucose and short cellulose chains. One way to overcome cellulase inhibition is to ferment the glucose to ethanol as soon as it appears in solution. SSF combines enzymatic hydrolysis with ethanol fermentation to keep the concentration of glucose low. The accumulation of ethanol in the fermenter does not inhibit cellulase as much as high concentrations of glucose, so SSF is a good strategy for increasing the overall rate of cellulose to ethanol conversion. It is important to keep the rate limiting step in mind. In SSF the ethanol production rate is controlled by the cellulase hydrolysis rate not the glucose fermentation, so steps to increase the rate of hydrolysis will lower the cost of ethanol production via SSF. The US Department of Energy, National Renewable Energy Laboratory (NREL) is funding Genercor International, Inc. to develop low cost cellulases that will reduce the cost of cellulose breakdown by a factor of 10.

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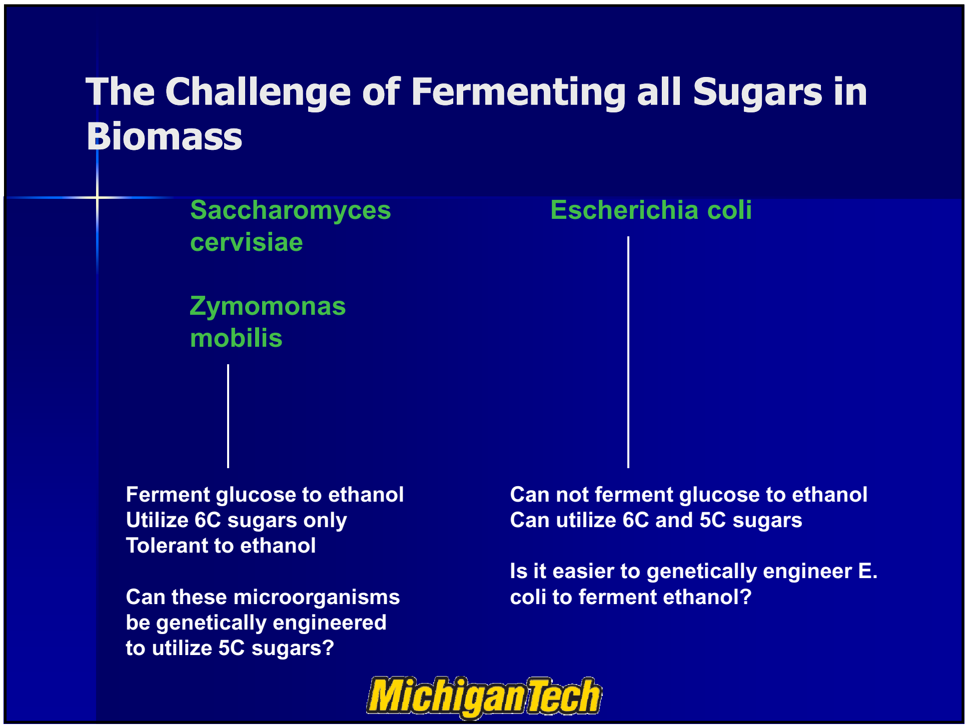

## The Challenge of Fermenting all Sugars in Biomass

Saccharomyces cervisiae

Zymomonas mobilis

**Ferment glucose to ethanol**
**Utilize 6C sugars only**
**Tolerant to ethanol**

**Can these microorganisms be genetically engineered to utilize 5C sugars?**

Escherichia coli

**Can not ferment glucose to ethanol**
**Can utilize 6C and 5C sugars**

**Is it easier to genetically engineer E. coli to ferment ethanol?**

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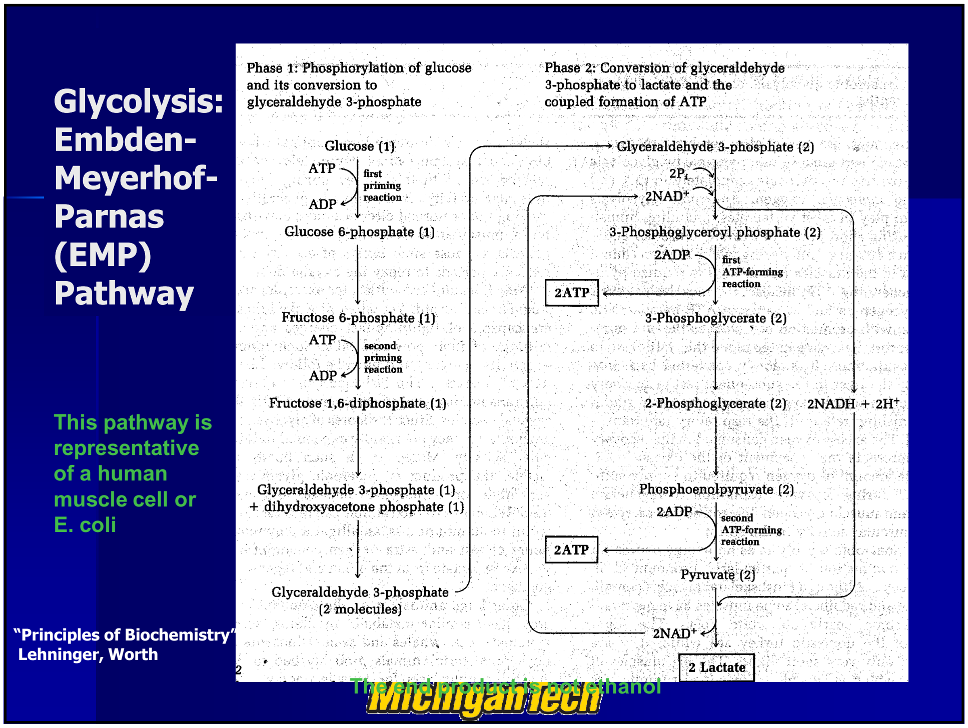

# Glycolysis: Embden-Meyerhof-Parnas (EMP) Pathway

This pathway is representative of a human muscle cell or E. coli

Phase 1: Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate

Glucose (1)
ATP → ADP: first priming reaction
Glucose 6-phosphate (1)
Fructose 6-phosphate (1)
ATP → ADP: second priming reaction
Fructose 1,6-diphosphate (1)
Glyceraldehyde 3-phosphate (1) + dihydroxyacetone phosphate (1)
Glyceraldehyde 3-phosphate (2 molecules)

Phase 2: Conversion of glyceraldehyde 3-phosphate to lactate and the coupled formation of ATP

Glyceraldehyde 3-phosphate (2)
$2P_i$
$2NAD^+$
3-Phosphoglyceroyl phosphate (2)
2ADP → **2ATP**: first ATP-forming reaction
3-Phosphoglycerate (2)
2-Phosphoglycerate (2)
$2NADH + 2H^+$
Phosphoenolpyruvate (2)
2ADP → **2ATP**: second ATP-forming reaction
Pyruvate (2)
$2NAD^+$
**2 Lactate**

2

**“Principles of Biochemistry” Lehninger, Worth**

The end product is not ethanol

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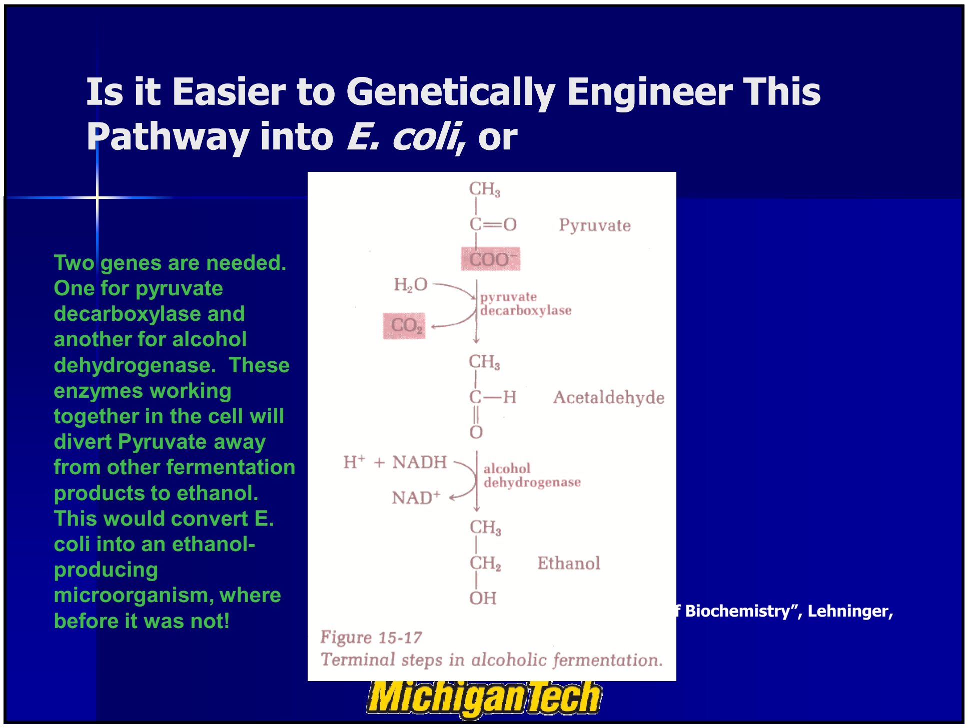

# Is it Easier to Genetically Engineer This Pathway into *E. coli*, or

Two genes are needed. One for pyruvate decarboxylase and another for alcohol dehydrogenase. These enzymes working together in the cell will divert Pyruvate away from other fermentation products to ethanol. This would convert E. coli into an ethanol-producing microorganism, where before it was not!

$CH_3-C(=O)-COO^-$ Pyruvate

$H_2O$ → $CO_2$: pyruvate decarboxylase

$CH_3-C(=O)-H$ Acetaldehyde

$H^+ + NADH$ → $NAD^+$: alcohol dehydrogenase

$CH_3-CH_2-OH$ Ethanol

Figure 15-17
*Terminal steps in alcoholic fermentation.*

**f Biochemistry”, Lehninger,**

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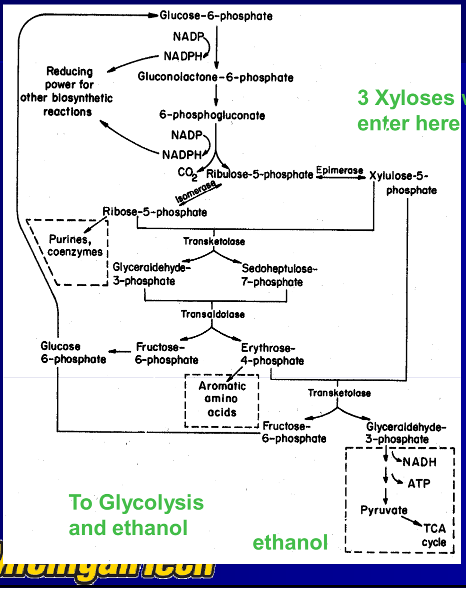
## Is it Easier to Genetically Engineer This Pathway into *S. cerviciae* or *Z. mobilis*?

This is the Pentose Phosphate pathway in E. coli. This pathway is obviously more complicated, containing many more enzyme-catalyzed reactions than the two-step pathway on the previous slide. The pathway for other 5C sugars (arabanose, mannose, galactose) would be similar.



3 Xyloses would enter here

To Glycolysis and ethanol

ethanol

"Bioprocess Engineering: Basic Concepts Shuler and Kargi, Prentice Hall, 2002

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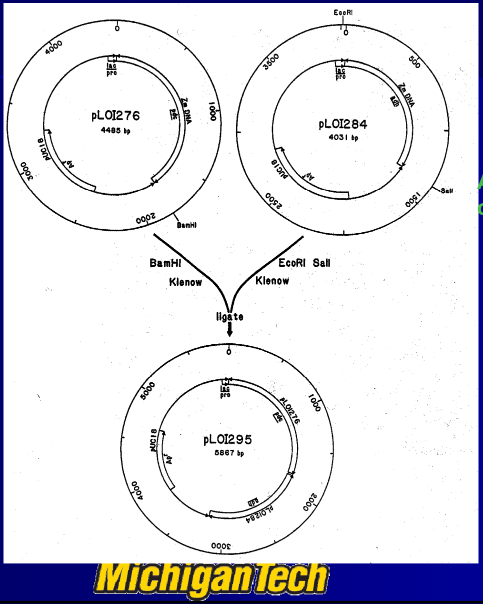
## Genetic Engineering of Ethanol Production in *E. coli*

A plasmid for Pyruvate decarboxylase (pdc)



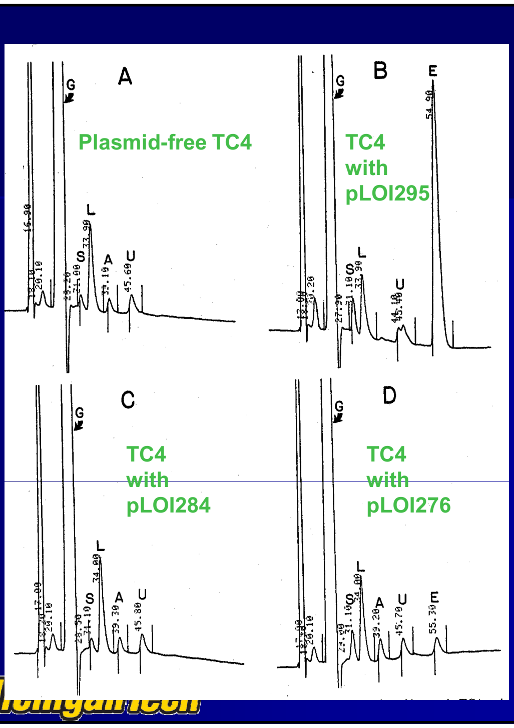
A plasmid for Alcoho dehydrogenase (adh)

Ingram, Conway, Clark, Sewell, and Preston, "Genetic engineering of ethanol production in E. coli", App. Environ. Microbio., 1987, 53(10), 2420-2425.

# Ethanol Production in Sealed Cultures of *E. coli* TC4

High Performance Liquid Chromatography Profiles

G = glucose
S = succinate
L = lactic acid
A = acetic acid
U = unknown
E = ethanol



Ingram, Conway, Clark, Sewell, and Preston, "Genetic engineering of ethanol production in E. coli", App. Environ. Microbio., 1987, 53(10), 2420-2425.

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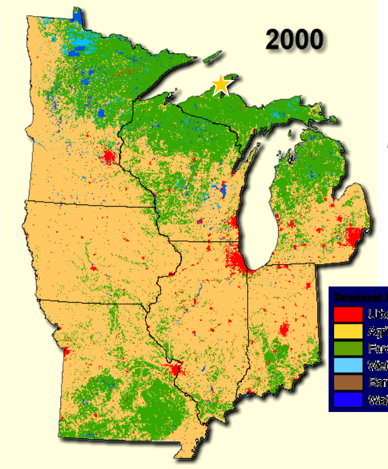
# Questions?



Midwestern land cover (USFS North Central Research Station image)