

# Biotechnological Routes to Biomass Conversion



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# The Unique Role of Biomass

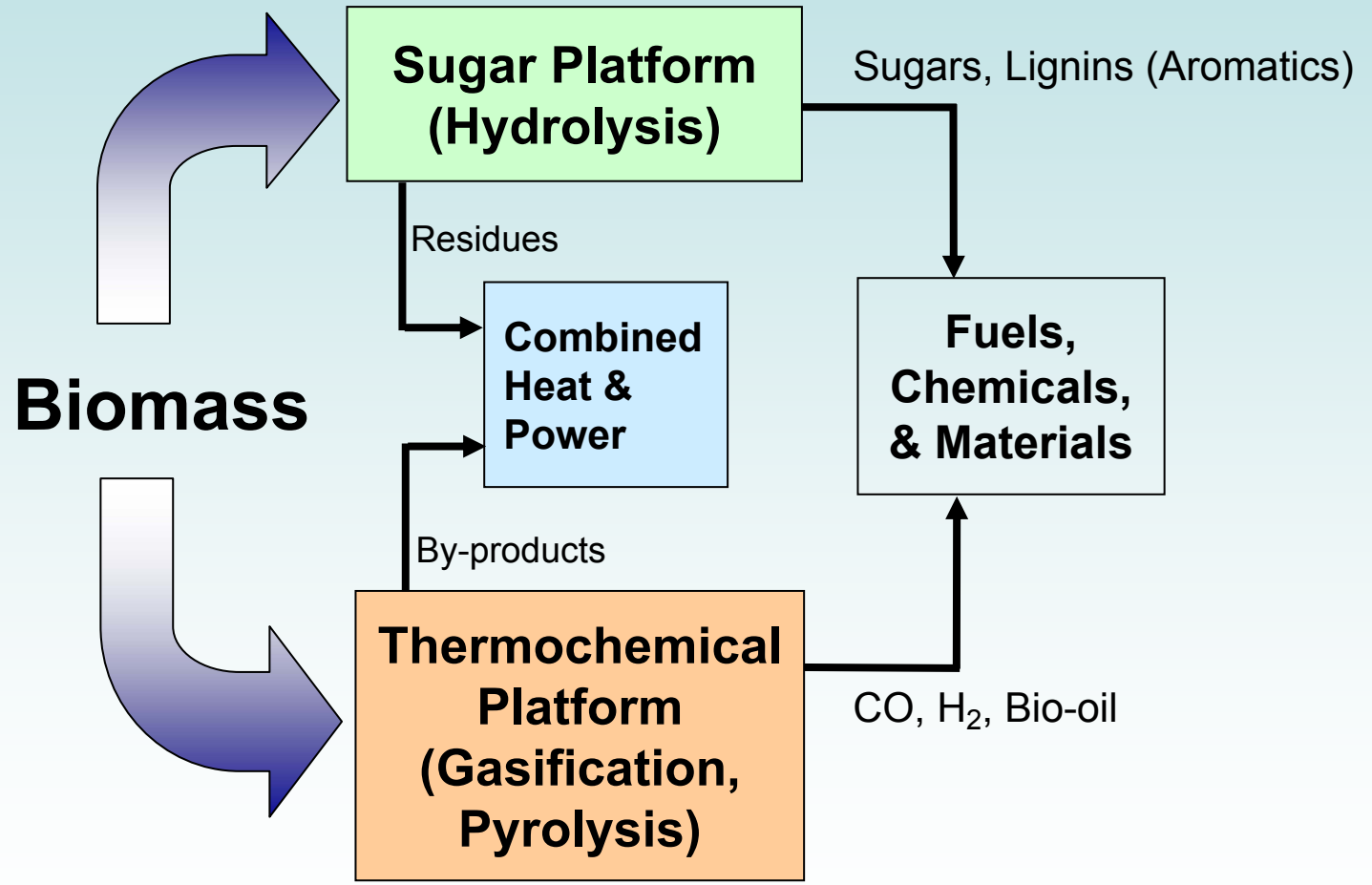
*While the growing need for sustainable electric power can be met by other renewables...*



*Biomass is our only renewable source of carbon-based fuels and chemicals*



# Biomass Conversion Technology “Platforms”



# Outline

- Biomass Basics
- Overview of Conversion Options
- Details of Enzyme-based Technology
- Biorefining Now and in the Future

# Biomass Feedstock Types

- “Starchy”: Grains (e.g., corn and wheat)
  - “Oily”: Seeds (e.g., soya and rape)
  - “Fibrous”: Lignocellulose (e.g., ag and forestry residues, grasses, trees, etc.)
- 
- *Emphasis of today’s presentation will be conversion of **lignocellulosic** biomass*
    - *Comparison to illustrate the differences between starchy and fibrous feedstocks: corn grain versus corn stover*

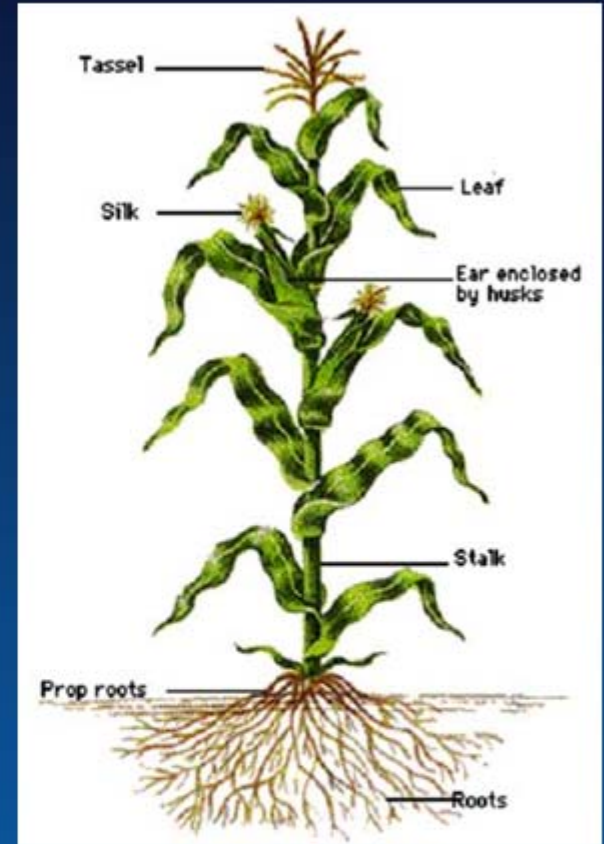
# Corn Grain vs. Corn Stover



<http://maize.agron.iastate.edu/corngrows.html>



<http://www.bisonfarm.com/images/fsp-corn.jpg>



## GRAIN



<http://amica.csustan.edu/key/corn.jpg>

## STOVER



# Biomass Basics

- Grain contains
  - $\geq 80\%$  carbohydrates, dry basis
  - Major component is starch
- Lignocellulosic biomass contains
  - 60-70% carbohydrates, dry basis
  - Major components are cellulose, hemicellulose, and lignin
- Biomass types exhibit differences in
  - Macro structure and cell wall architecture
  - Types and levels of lignins and hemicelluloses
  - Types and levels of minor constituents

# Composition: Grain vs. Stover

<b>Component</b>	<b>Corn Kernel (Grain)</b>	<b>Corn Stover (Lignocellulose)</b>
<b>Starch</b>	<b>72-73</b>	<b>Trace</b>
<b>Cellulose/Hemicellulose</b>	<b>10-12</b>	<b>63-77</b>
<b>Lignin</b>	<b>Trace</b>	<b>10-16</b>
<b>Other Sugars</b>	<b>1-2</b>	<b>3-6</b>
<b>Protein</b>	<b>8-10</b>	<b>1-3</b>
<b>Oil/Other Extractives</b>	<b>4-5</b>	<b>3-6</b>
<b>Ash</b>	<b>1-2</b>	<b>5-7</b>
<b>Cellulose</b>		<b>34-39</b>
<b>Xylan/Arabinan</b>		<b>22-26</b>
<b>Galactan/Mannan</b>		<b>1-2</b>
<b>Acetate &amp; Uronics</b>		<b>6-10</b>
<b>Total</b>	<b>96-104</b>	<b>85-115</b>



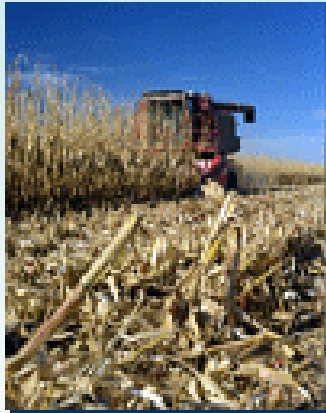
# Biomass Resources and Key Issues



## Wood Residues

Sawdust  
Wood waste  
Pulp mill wastes

- **Quality**
  - Composition
  - Ease of Conversion



## Agricultural Residues

Corn stover  
Rice hulls  
Sugarcane bagasse  
Animal waste

- **Cost**
  - Production
  - Collection and Transportation
  - Quantity Available



## Energy Crops

Switchgrass  
Hybrid poplar  
Willow

- **Sustainability**
  - Land, Air and Water Resources

# Biomass Composition



Hardwoods



Grasses



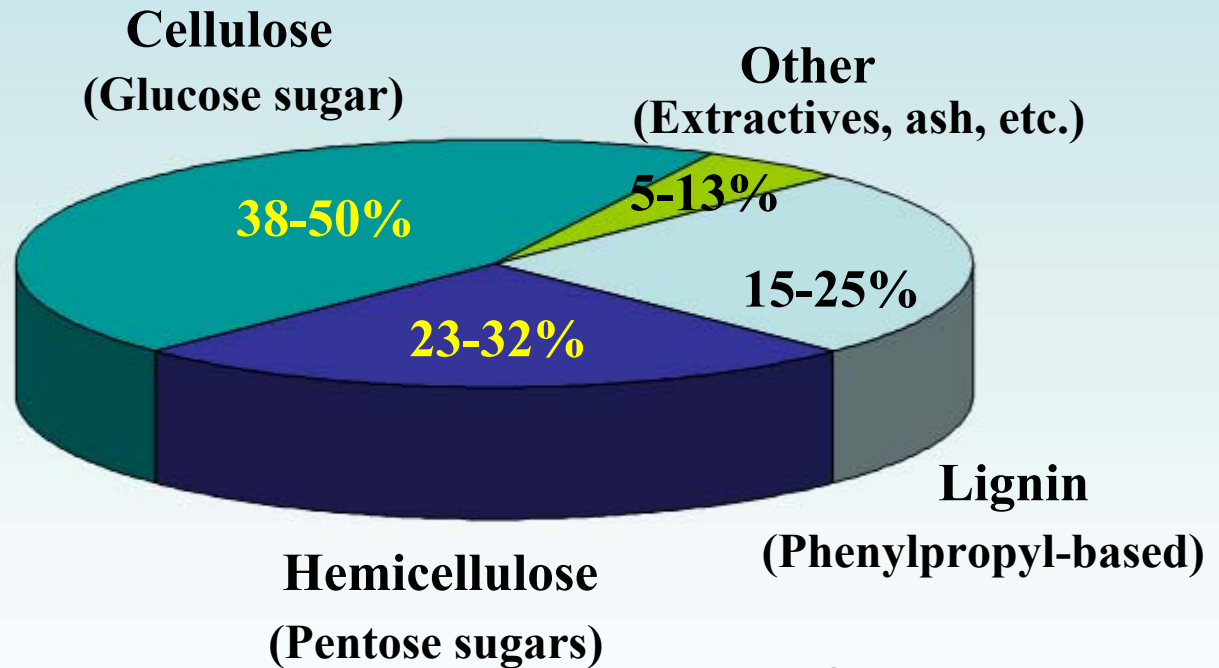
Crop residues



MSW



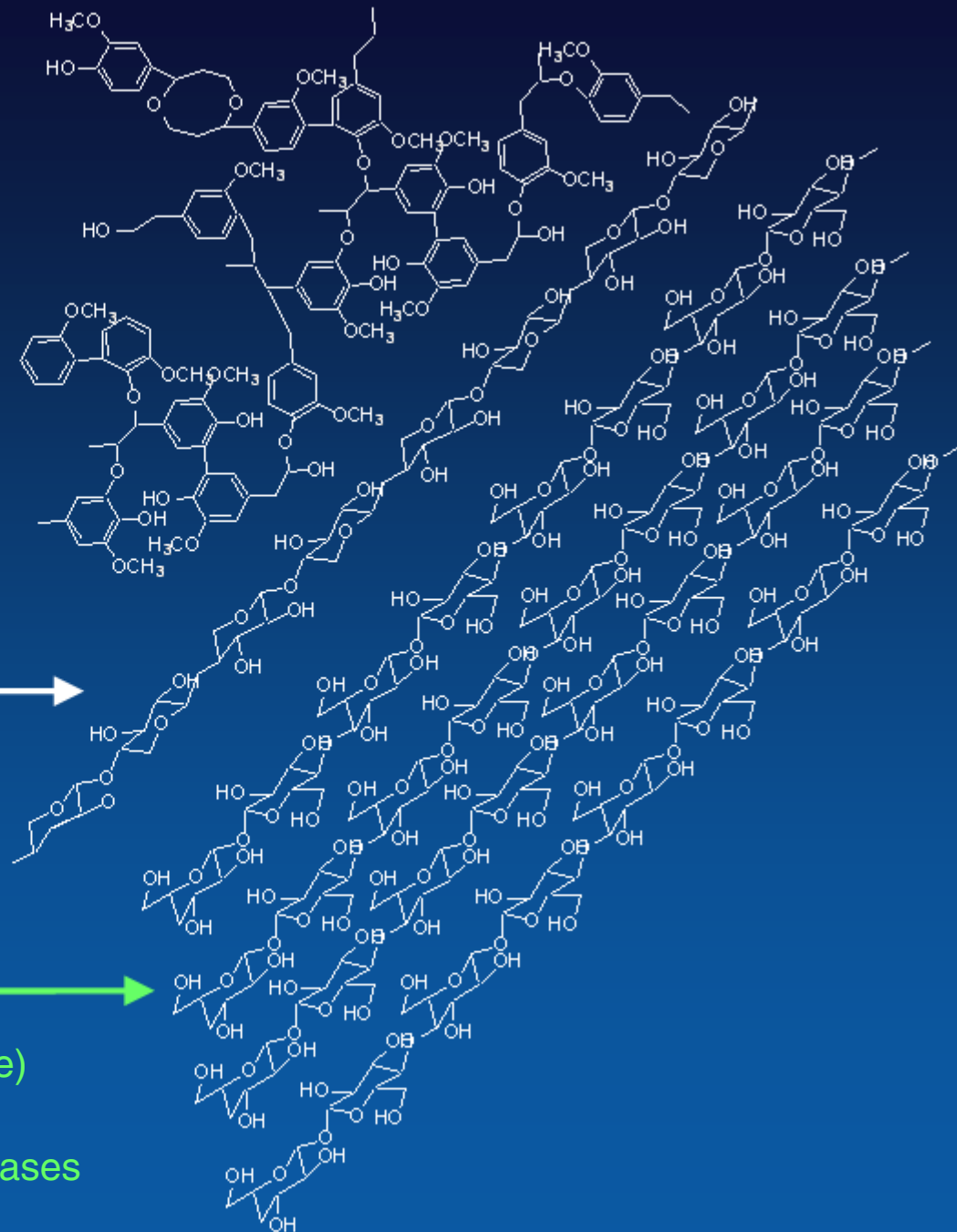
Softwoods



# Major Plant Cell Wall Components

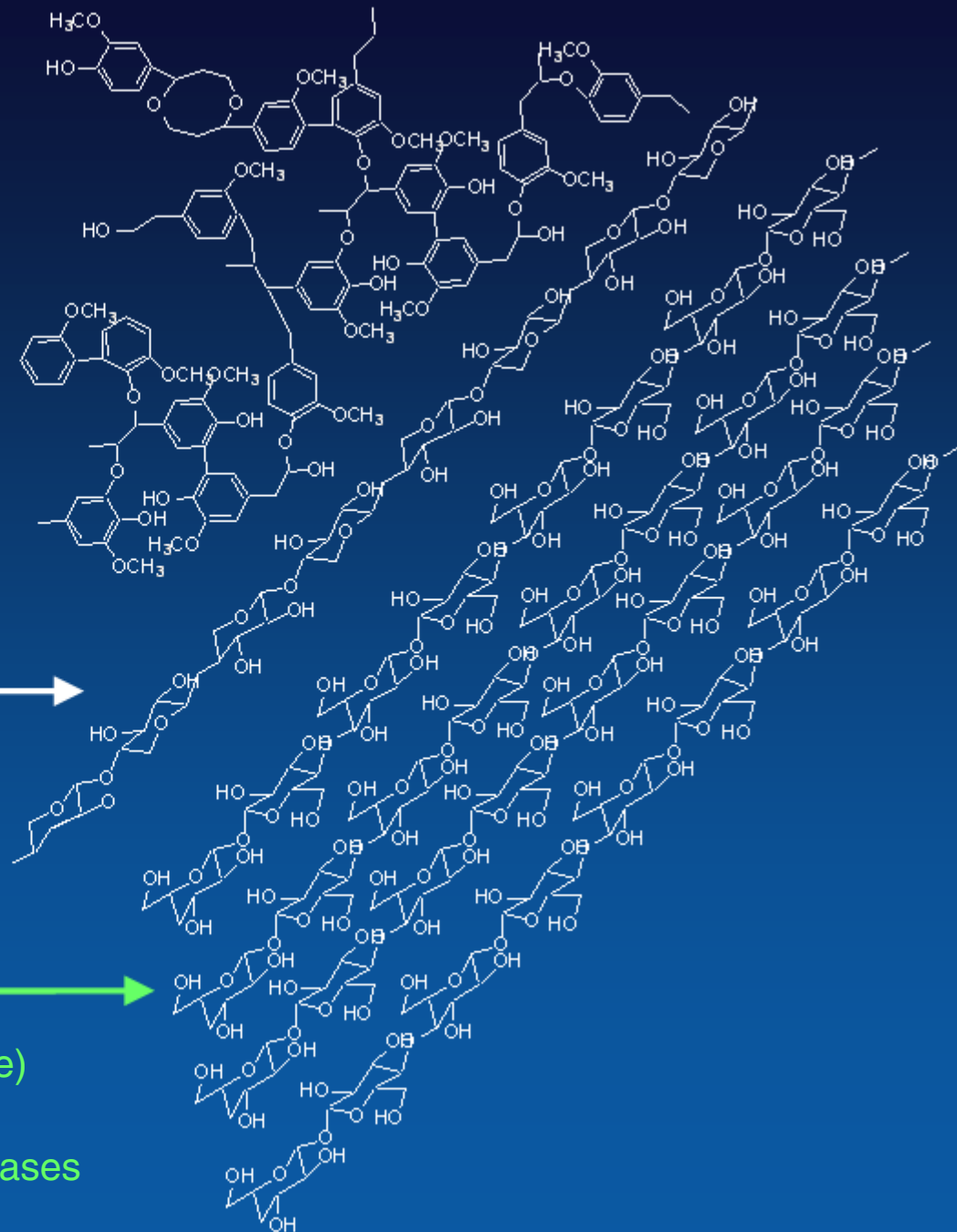
Lignin: 10-25%

- Complex aromatic structure
- Resistant to biochemical conversion
- Different depolymerization chemistry



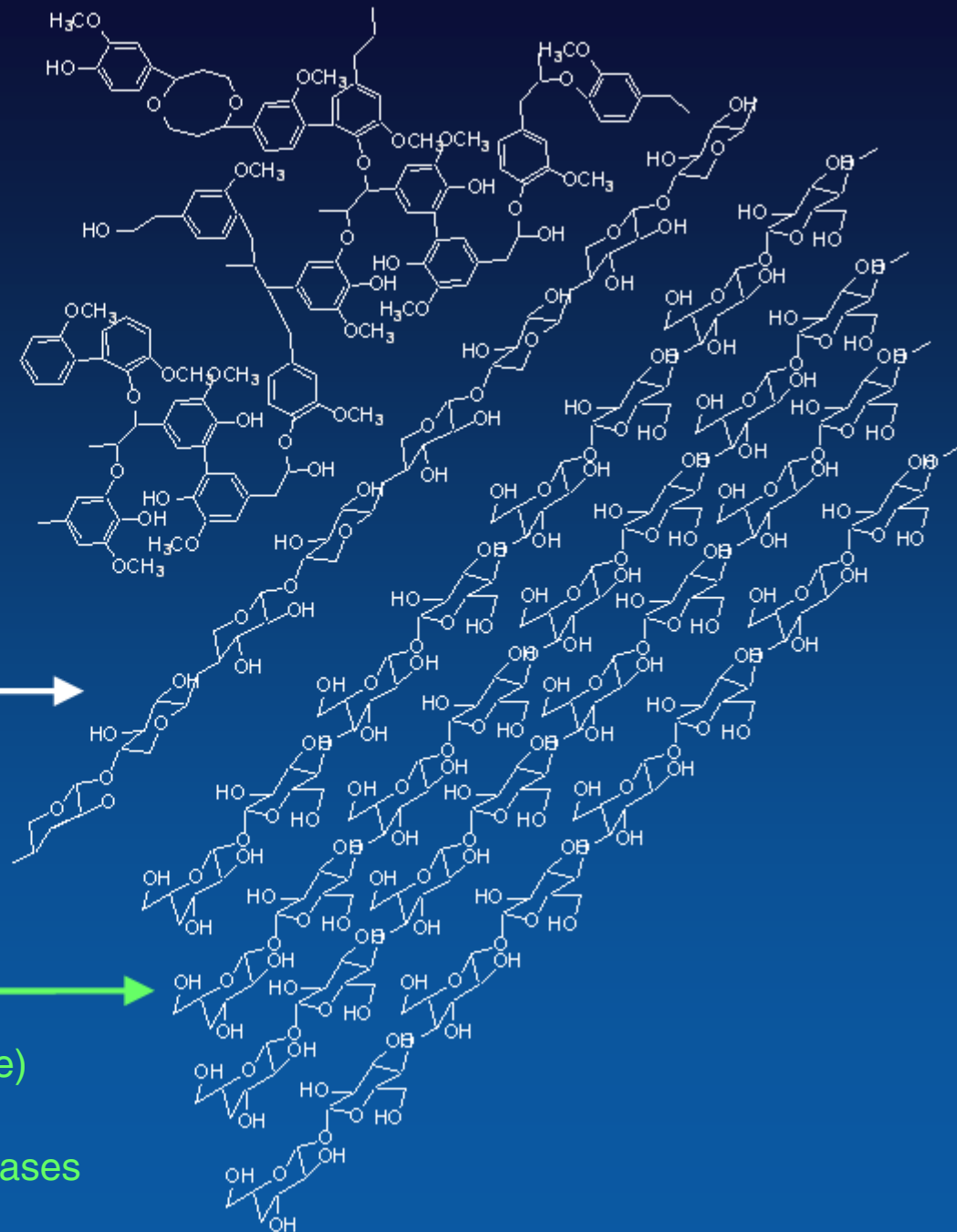
Hemicellulose: 15-30%

- Heteropolymer of pentoses and hexoses
- Variably substituted (acetyl, uronics)
- More easily depolymerized



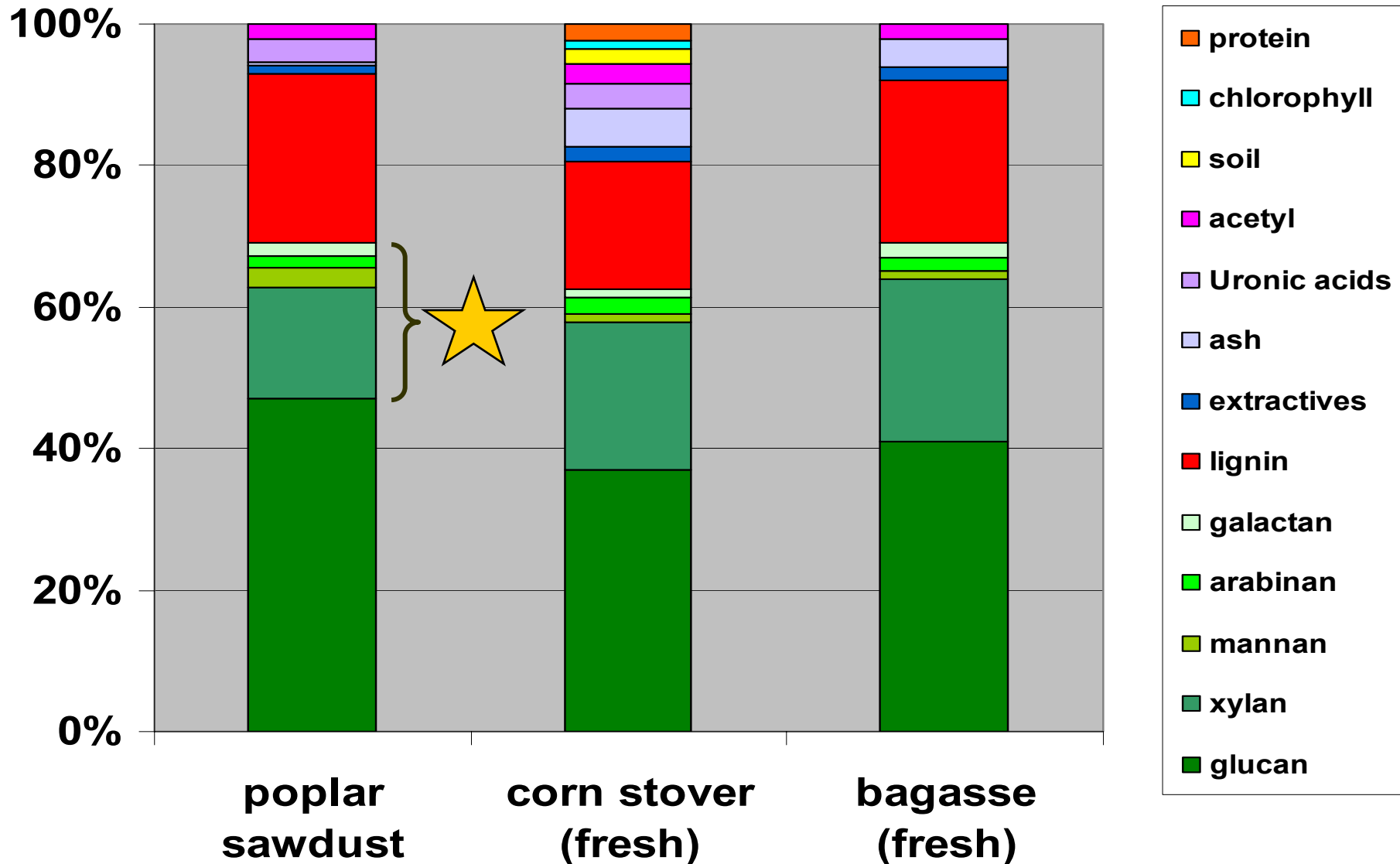
Cellulose: 30-50%

- Crystalline polymer of glucose (cellobiose)
- Difficult to chemically hydrolyze
- Susceptible to enzymatic attack by cellulases



# Not All Biomass is Created Equal!

Important Compositional and Structural Differences Exist





# Biomass Structure

- Surface and structural property measurement are key to developing a sound understanding of recalcitrance and conversion mechanisms
  - Very difficult system to study
    - Extremely heterogeneous at both macro- and micro-scales (ultrastructure complexity)
  - Tools and techniques emerging
    - E.g., NREL's Biomass Surface Characterization Laboratory, NMR Laboratory, etc.

# Biomass Surface Characterization Laboratory



TEM  
Tecnai G<sup>2</sup> Quanta 400 FEG



SEM  
Quanta 400 FEG

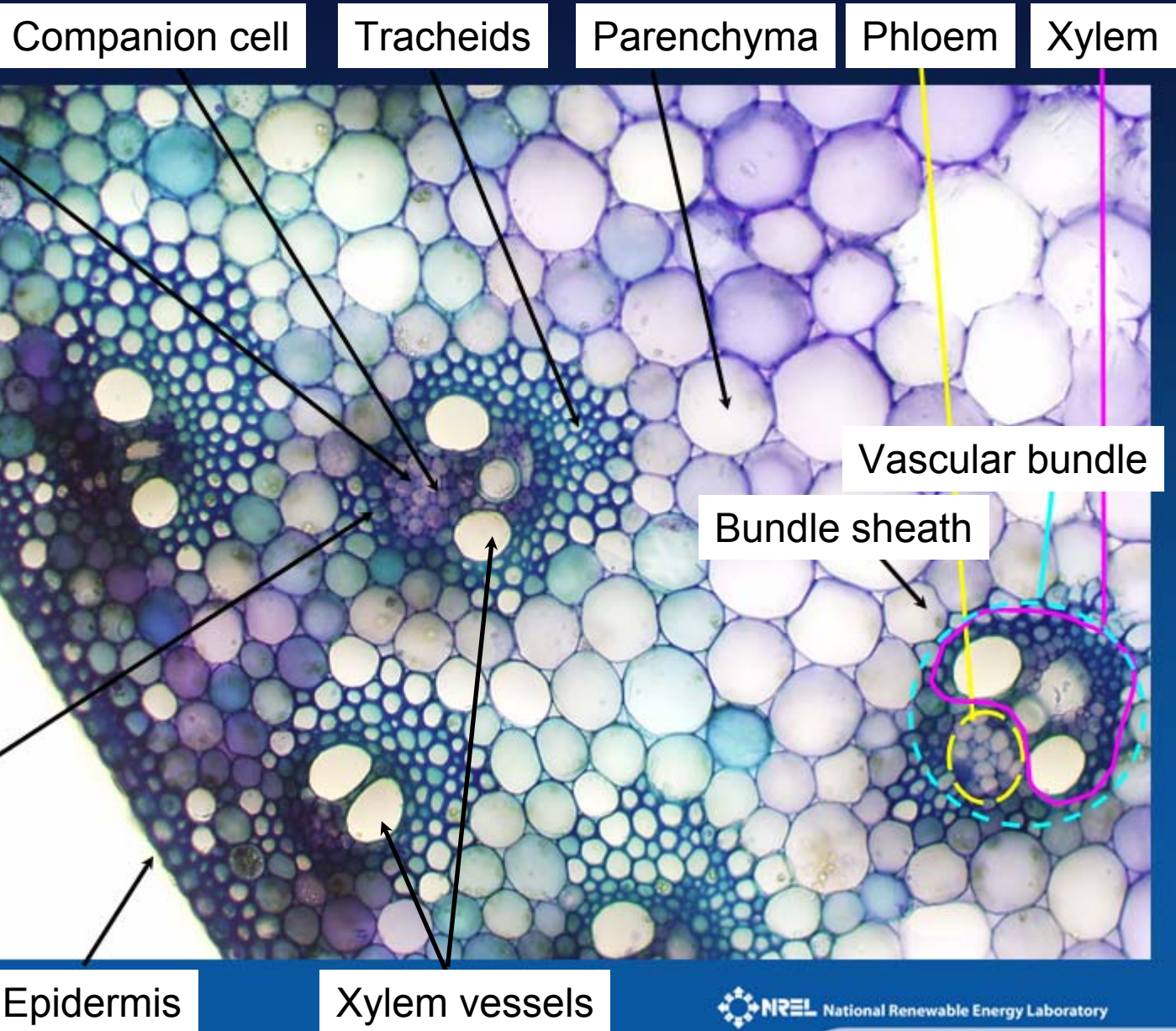


AFM  
MultiMode PicoForce



NSOM  
AURORA-3

# Heterogeneity Across a Single Corn Stem\*



Light microscopy  
Toluidine Blue O  
200x

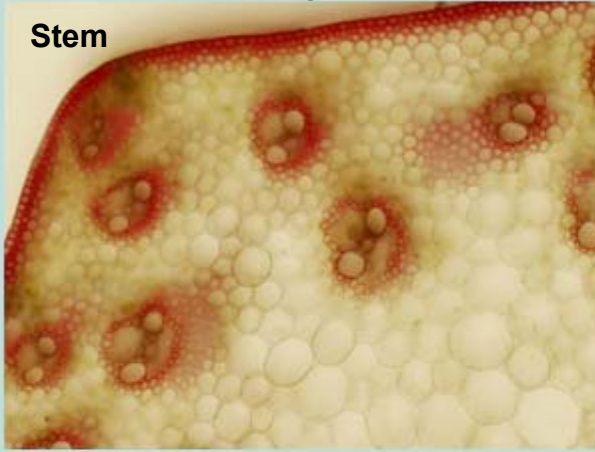
Schlerenchyma

\*Photomicrograph courtesy of Stephanie Porter (NREL)

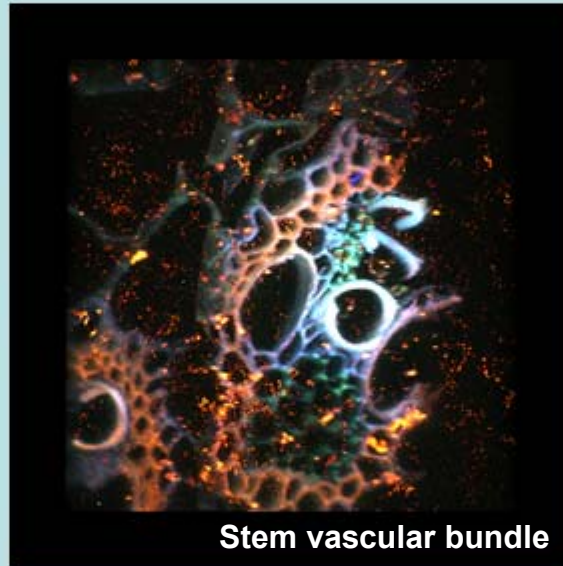
# Structural Complexity at Many Scales\*

White light, 100x

Stem

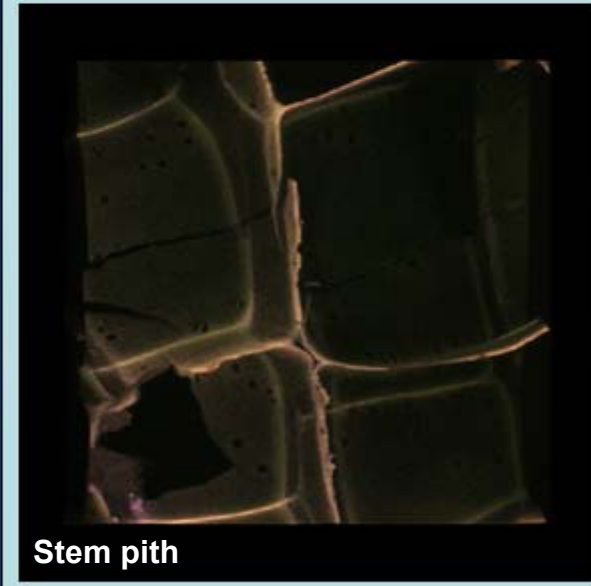


UV Fluorescence, 600x



Stem vascular bundle

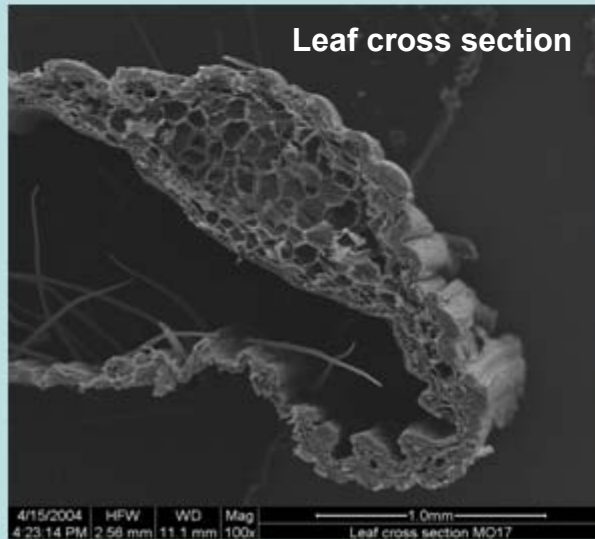
Confocal, 1000x



Stem pith

SEM, 100x

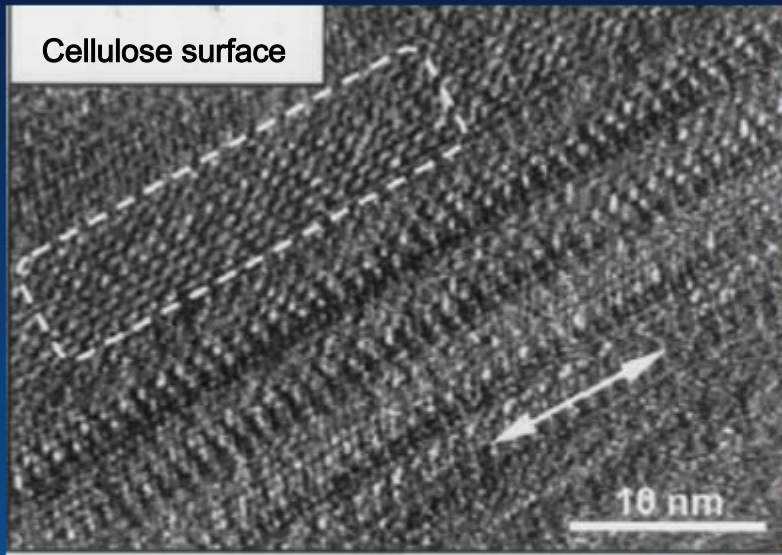
Leaf cross section



\*Images courtesy of S. Porter (NREL)



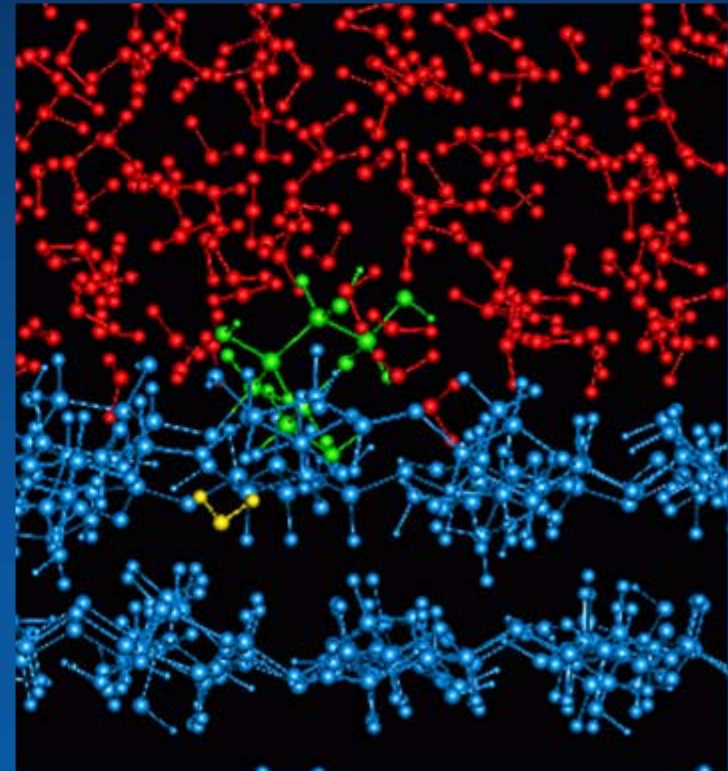
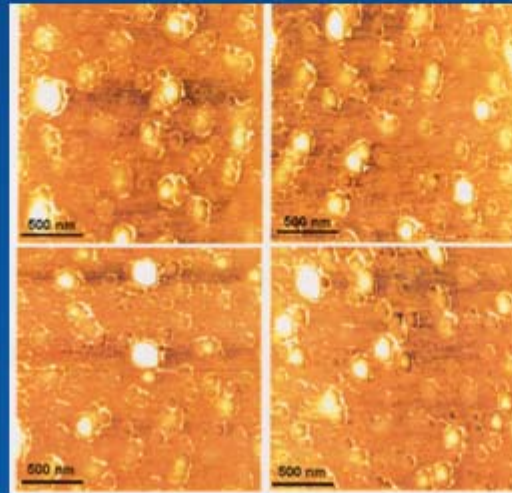
# Advanced imaging facilities (such as NREL's BSCL) provide new tools to study the fundamentals of biomass conversion processes



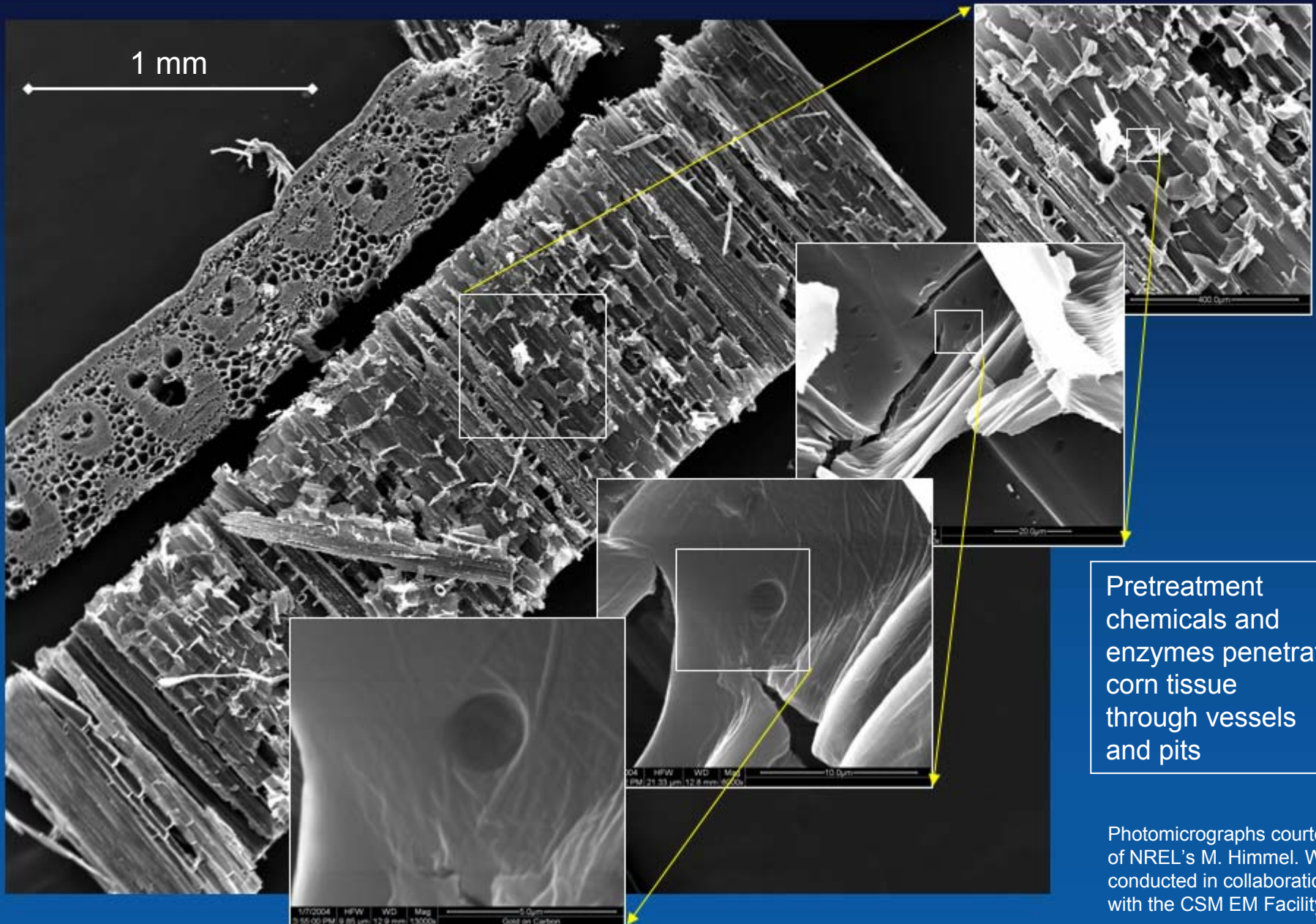
Monitor cellulose surfaces during pretreatment and enzymatic hydrolysis

Test molecular models

Visualize changes to biomass surfaces caused by various pretreatment processes



# SEM of Corn Stems – How small are pits?



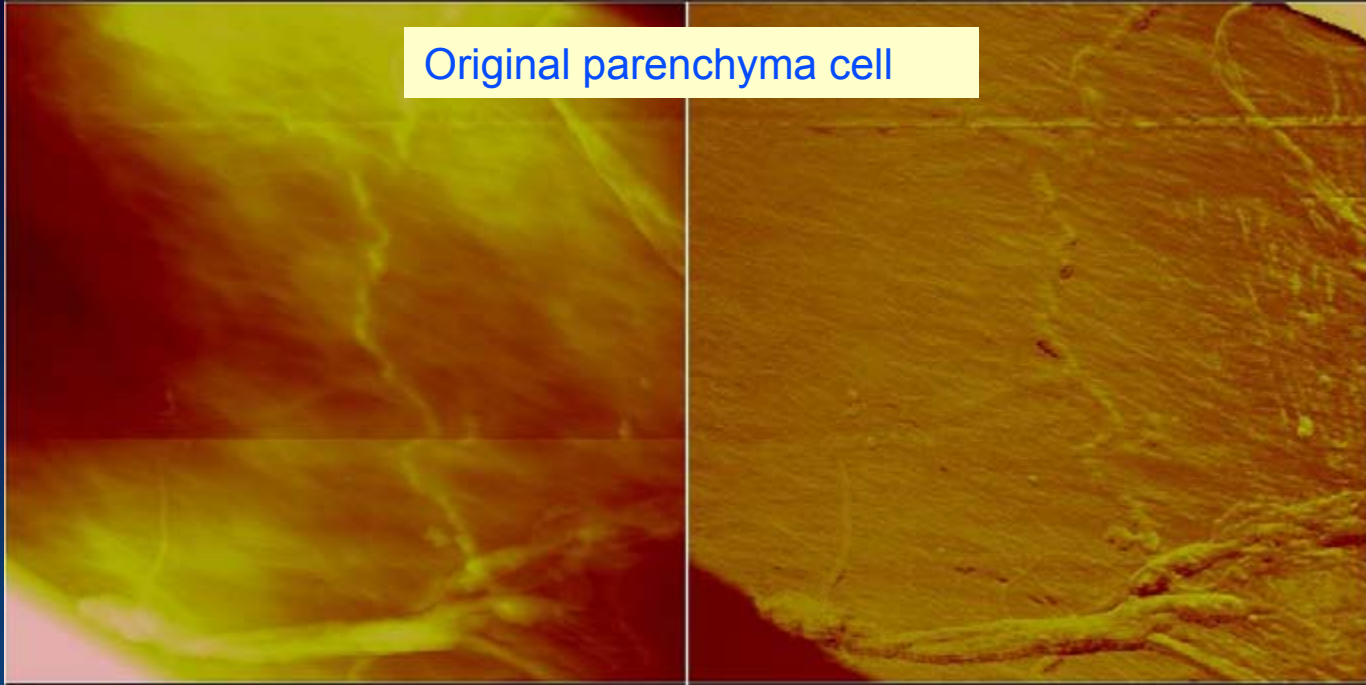


# AFM

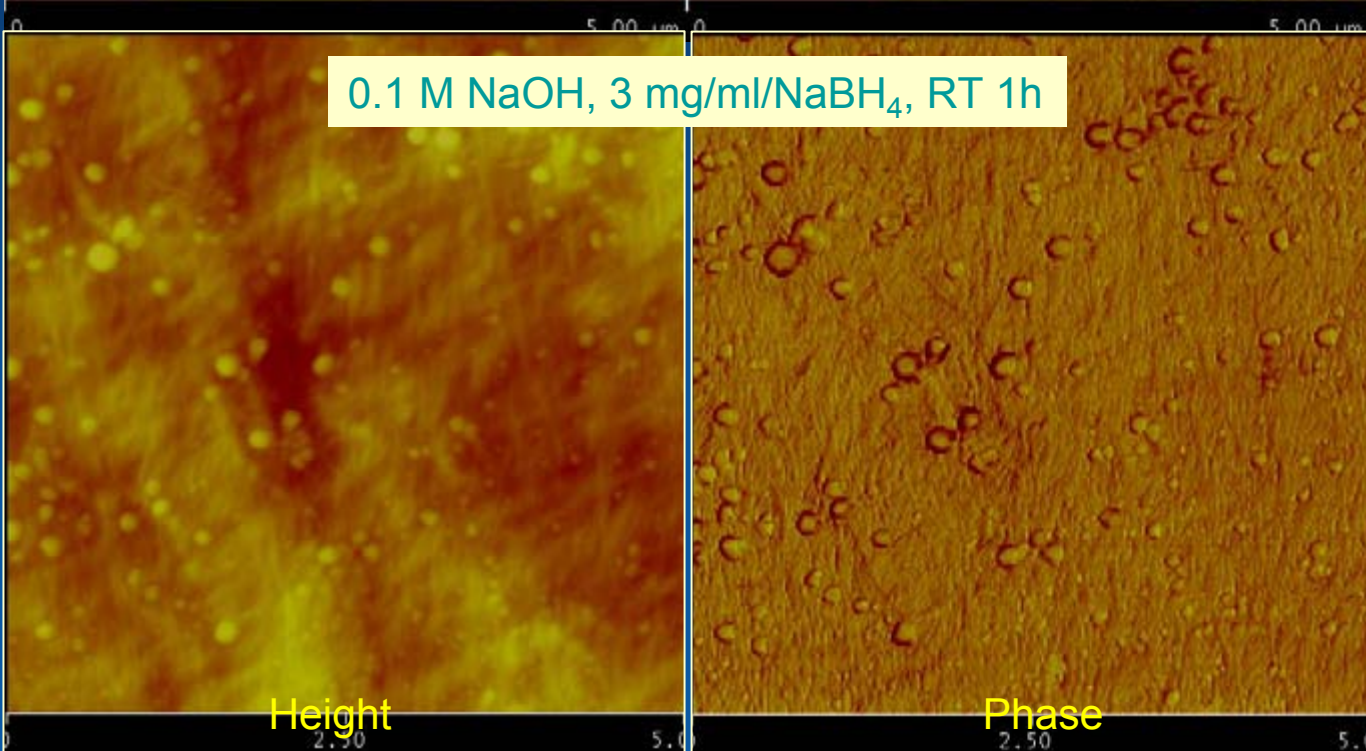
pith parenchyma  
cell cell-wall  
structure

Tapping mode  
Scan size: 5x5 $\mu$ m

Original parenchyma cell



0.1 M NaOH, 3 mg/ml/NaBH<sub>4</sub>, RT 1h



# Outline

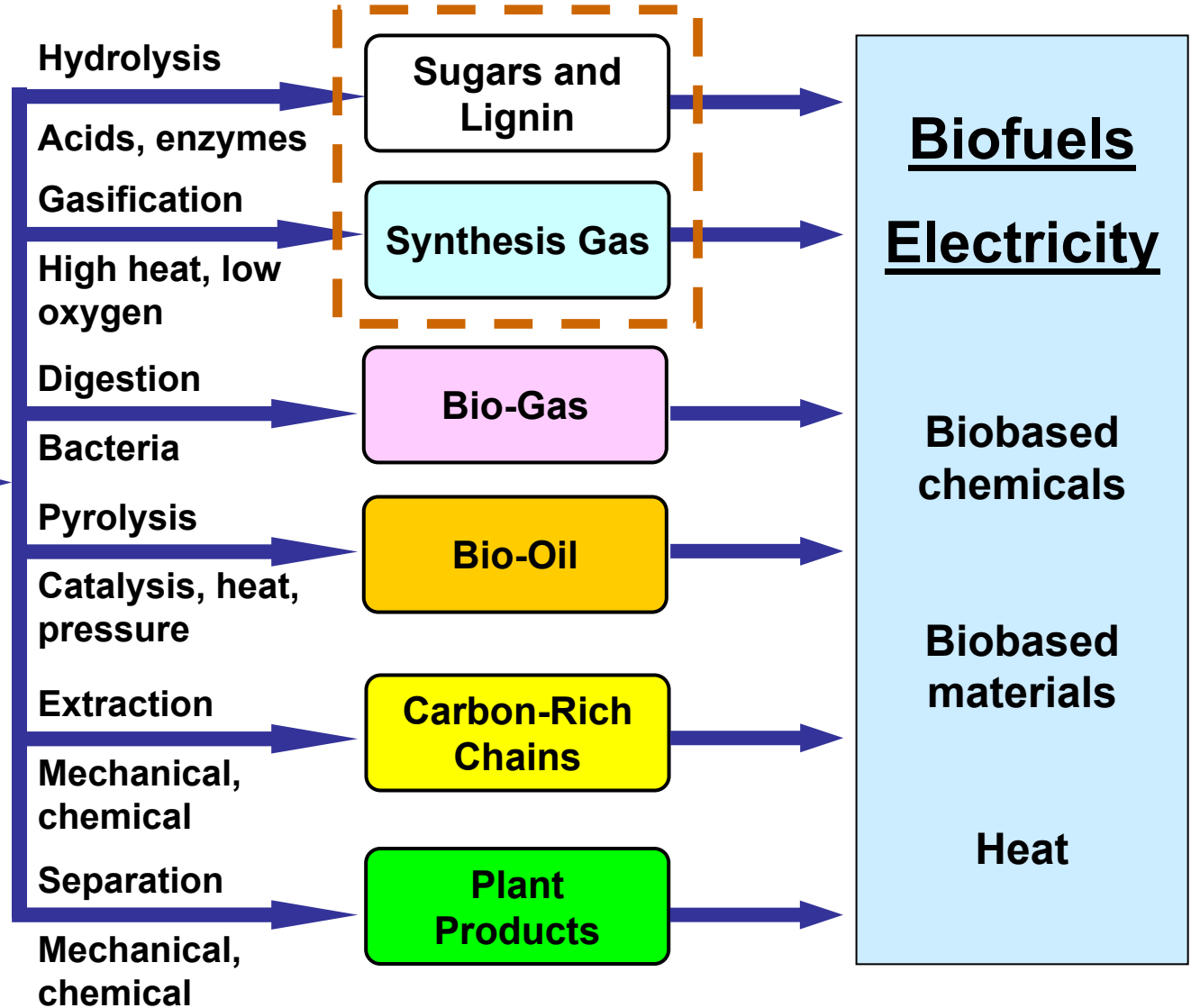
- Biomass Basics
- Overview of Conversion Options
- Details of Enzyme-based Technology
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# Biomass Energy Options




Feedstock production, collection, handling & preparation



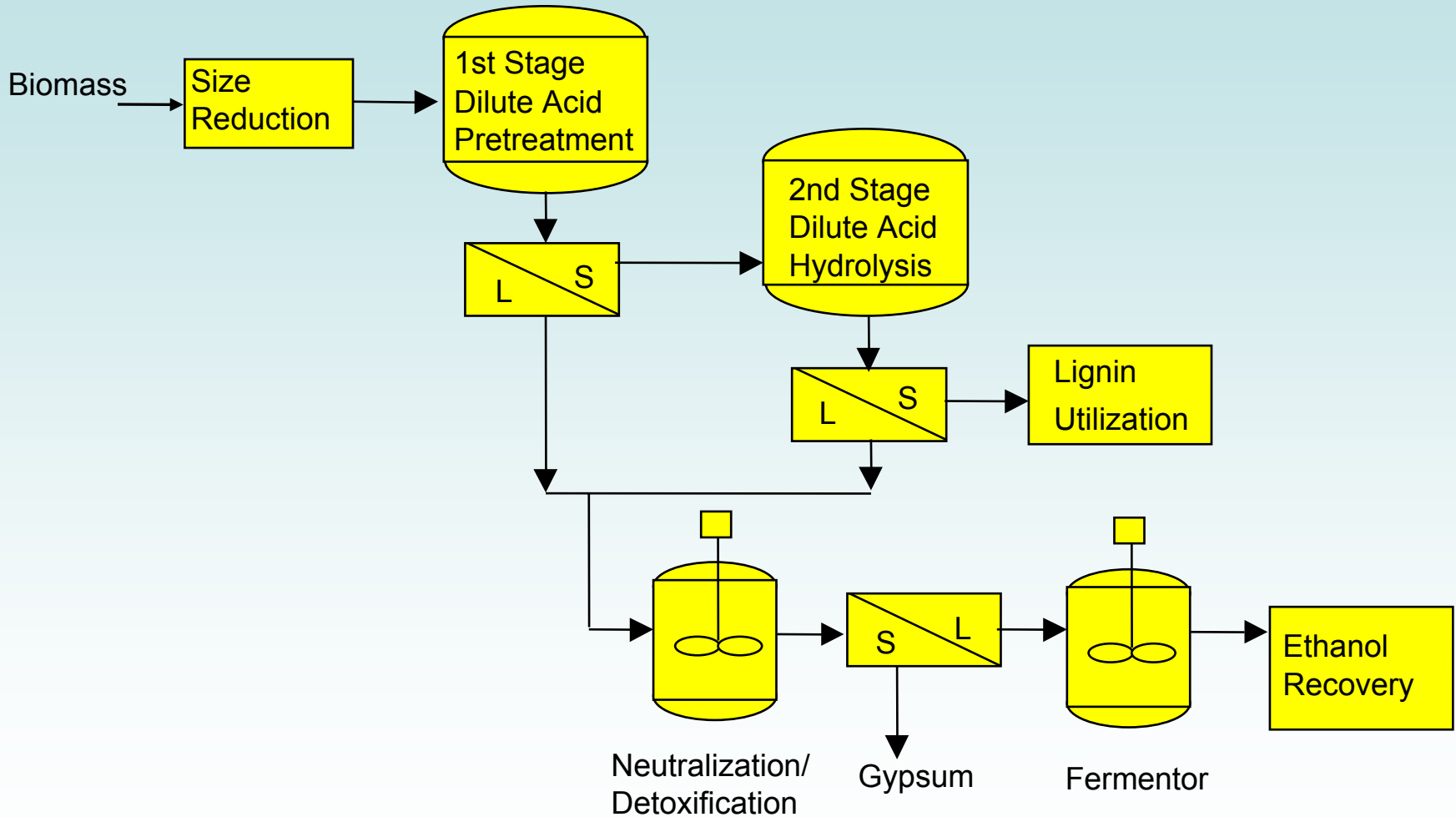
# Biomass Conversion (or Fractionation)

- Approaches
  - Mechanical
    - e.g., milling, comminution, decompression
  - Thermal
    - e.g., hot water, steam, heat
  - Chemical
    - e.g., acids, alkalis, solvents
  - Biological
    - e.g., cellulases, hemicellulases, ligninases
- *Most processing schemes employ a combination of methods*

# Process Technology Options

- Major categories of biomass conversion process technology
  - Sugar Platform
    - Dilute acid cellulose conversion
    - Concentrated acid cellulose conversion
    - Enzymatic cellulose conversion (*jump directly to this*  ?)
      - Using any of a variety of different primary fractionation or “pretreatment” methods
  - Syngas Platform
    - Gasification followed by synthesis gas fermentation

# Two-Stage Dilute Acid Process

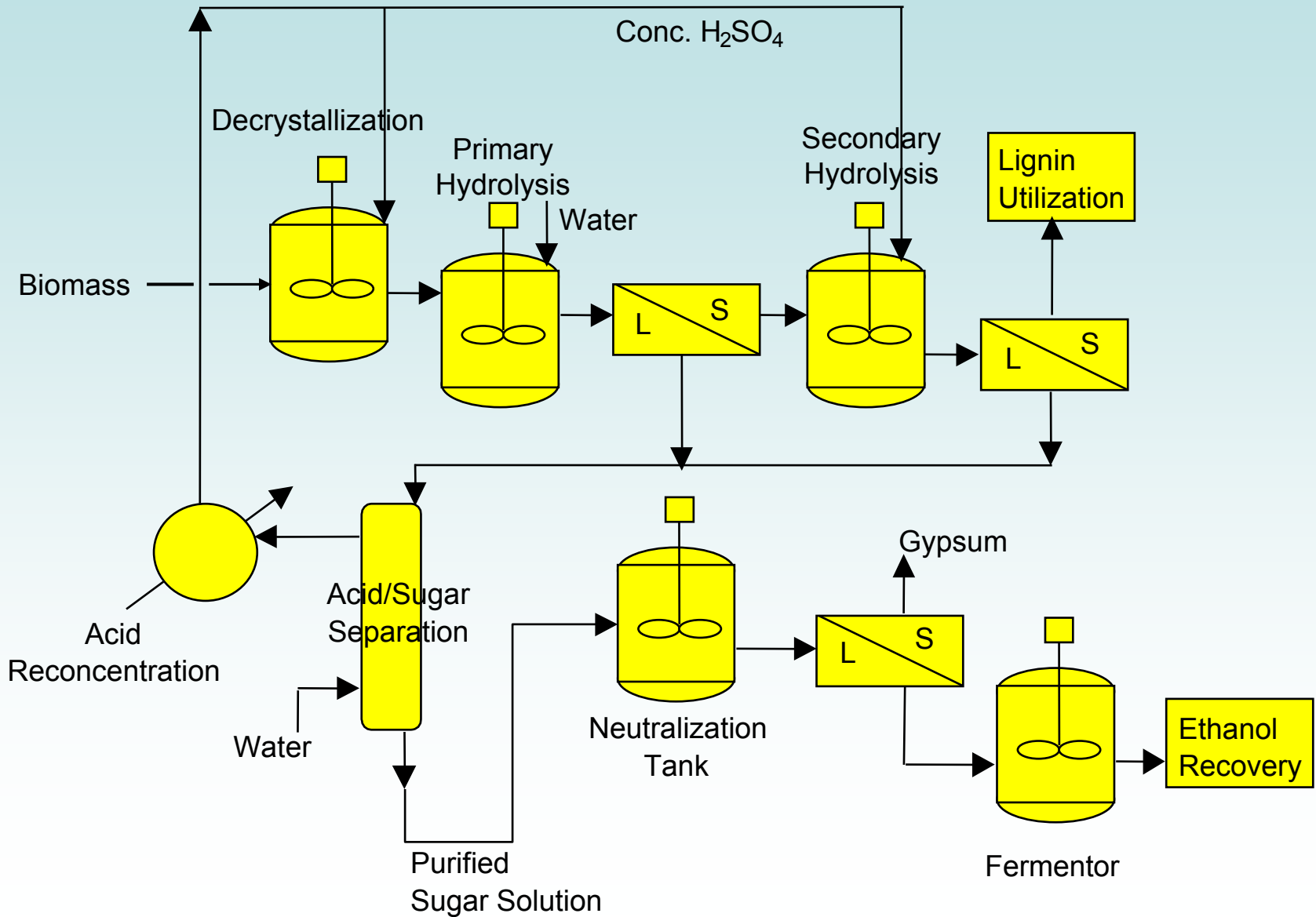




# Dilute Acid Hydrolysis

- Driving Forces
  - Adapt existing infrastructure, use recycled equip.
  - Exploit recombinant fermentation technology for hexose *and* *pentose* sugar conversion
- Strengths
  - Proven: oldest, most extensive history of all wood sugar processes, with the first commercial process dating back to 1898.
- Active Companies/Institutions include
  - BC International
  - Swedish government

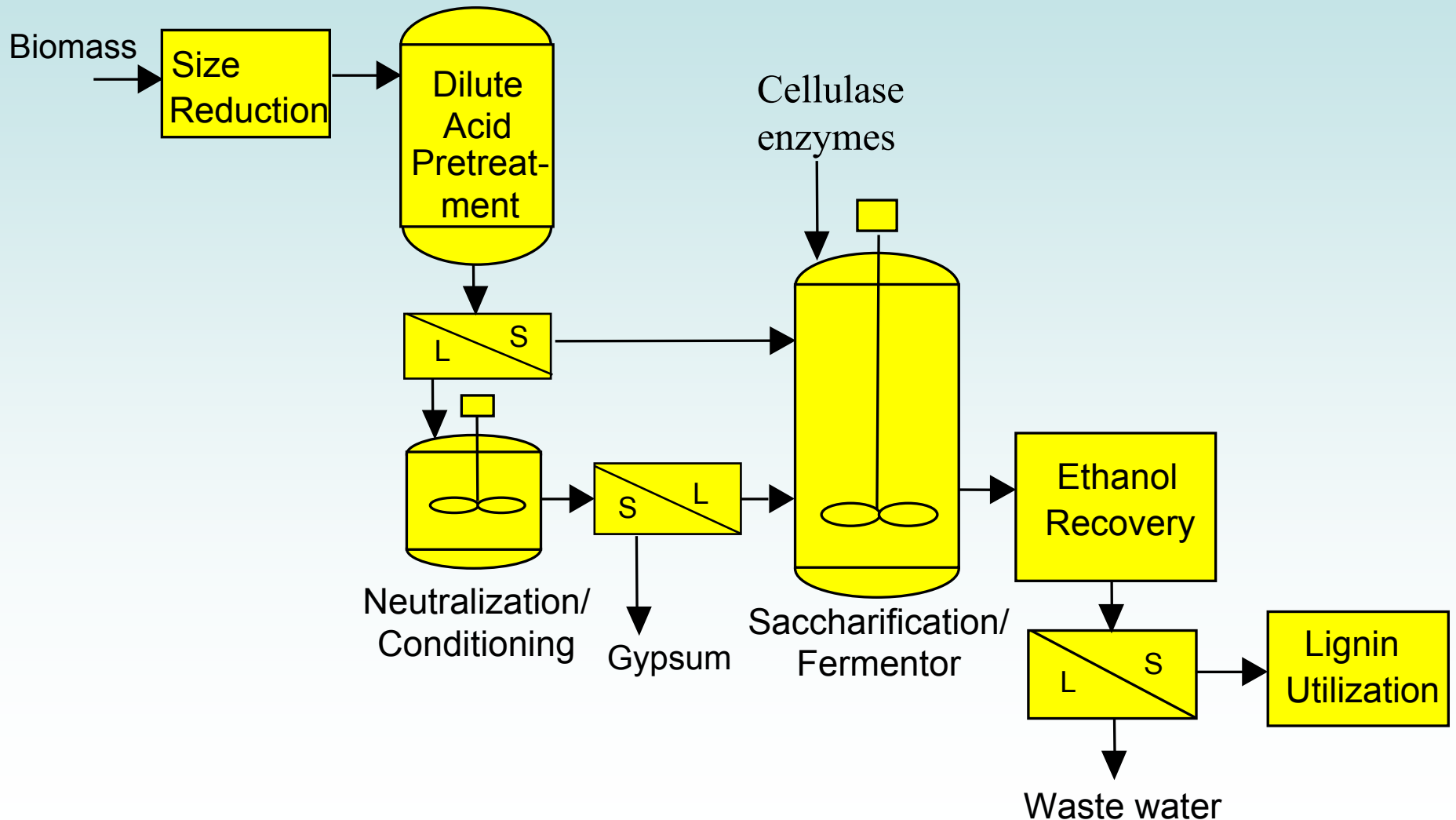
# Concentrated Acid Process



# Concentrated Acid Process

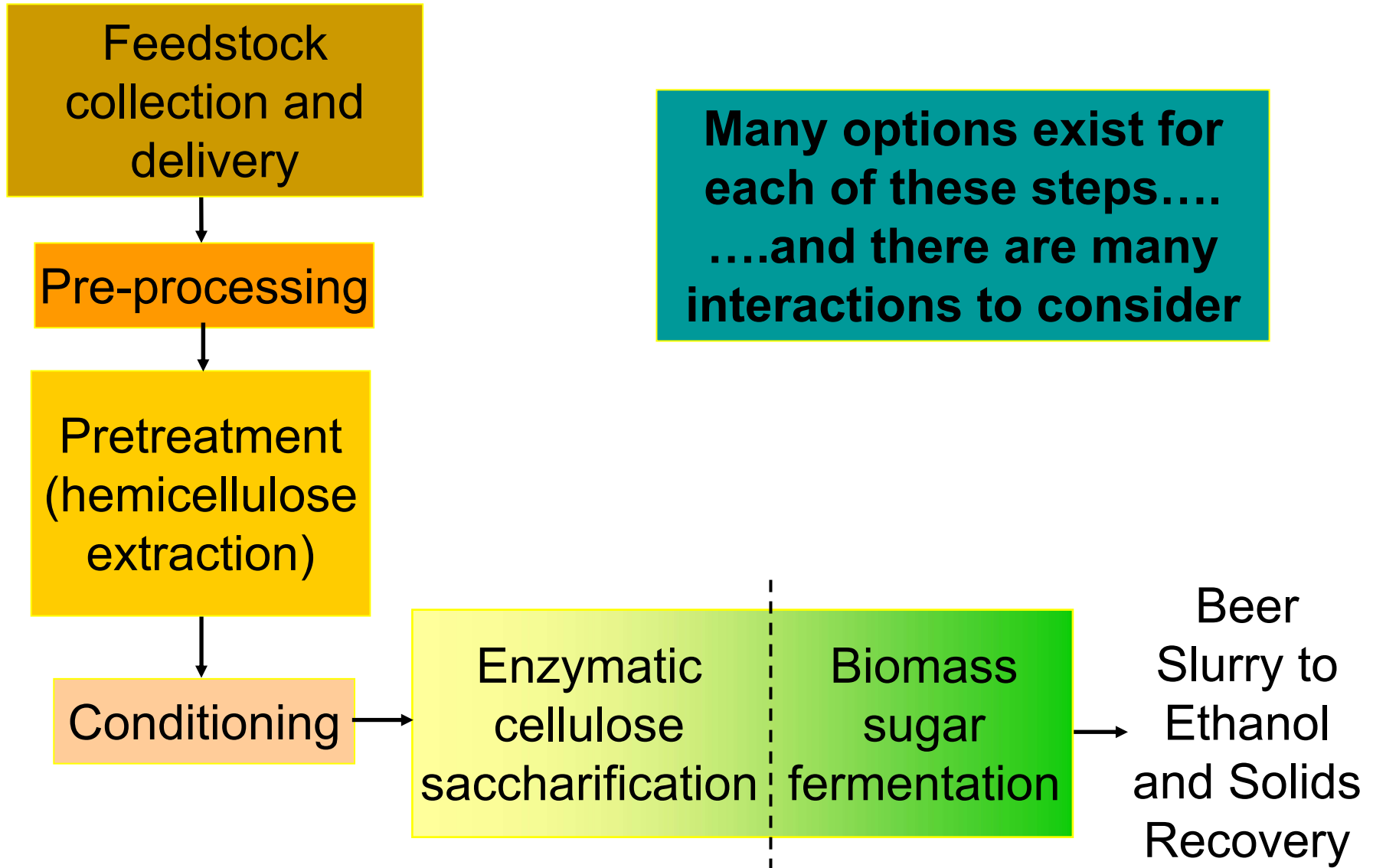
- Driving Forces
  - Cost effective acid/sugar separation and recovery technologies
  - Tipping fees for biomass
- Strengths
  - Proven: large scale experience dates back to Germany in the 1930s; plants still may be operating in Russia today.
  - Robust: able to handle diverse feedstocks
- Active Companies include
  - Arkenol
  - Masada Resources Group

# Historical Enzymatic Process





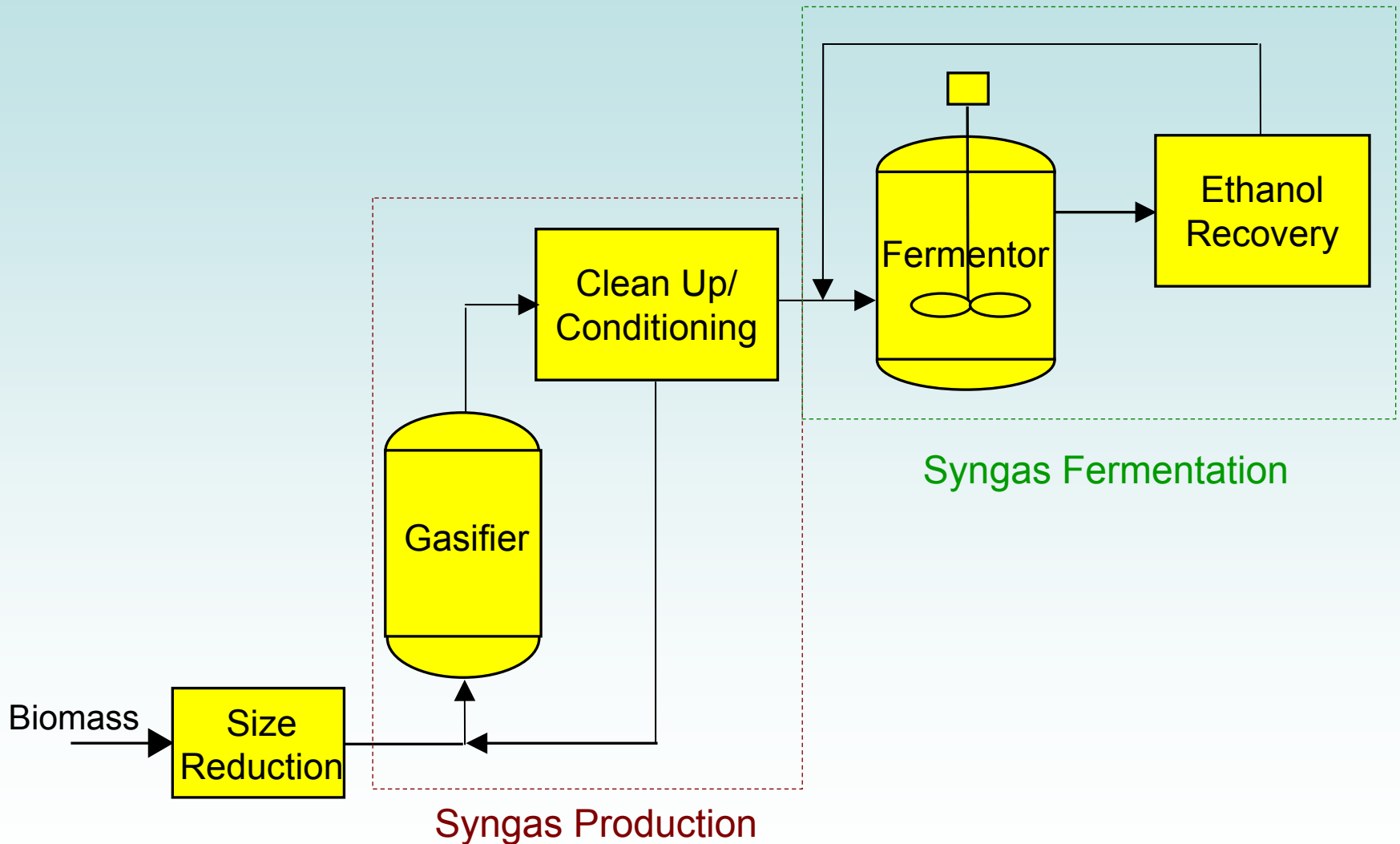
# Evolving Enzymatic Process



# Enzymatic Process

- Driving Forces
  - Exploit lower cost cellulases under development
  - Conceptually compatible with many different fractionation/pretreatment approaches
- Strengths
  - Potential for higher yields due to less severe processing conditions
  - Focus of USDOE's core R&D
- Active companies include
  - Iogen/PetroCanada, BC International, SWAN Biomass, and many others, including some of the recent Bioenergy Initiative solicitation awardees

# Syngas Fermentation Process



# Syngas Fermentation

- Bacterial fermentation of CO, CO<sub>2</sub> and H<sub>2</sub> to ethanol



- Syngas fermentation strains and processes remain relatively poorly characterized compared to other routes; many issues need to be resolved
  - Overall process economics
  - Required performance targets for
    - Gasification, e.g., yield = f(gas mixture)
    - Syngas fermentation, e.g., ethanol prod. yield, titer, and rate



# Syngas Fermentation Process

- Driving Forces
  - While unproven, may enable higher yields through conversion of non-carbohydrate fractions (e.g., lignin) to syngas components
- Strengths
  - Build off previous gasification/clean up knowledge
  - Ability to process a diverse range of feedstocks to a common syngas intermediate
- Active groups include
  - Bioresource Engineering Inc.
  - Oklahoma State
  - Mississippi State

# Status of Conversion Options

- Many options based on Sugar and Syngas Platform technology routes exist and are being pursued
- Sugar Platform technologies are at a more advanced development stage because of their longer history
- Recent programmatic emphasis has been on Enzymatic Hydrolysis route
  
- Further information on process options is available at:
  - [http://www.eere.energy.gov/biomass/sugar\\_platform.html](http://www.eere.energy.gov/biomass/sugar_platform.html)
    - USDOE EERE Biomass Program web site
  
- Also see:
  - <http://www.nrel.gov/biomass/publications.html>
    - Biomass research publications (several searchable databases)
  - <http://www.bioproducts-bioenergy.gov/>
    - Joint USDOE-USDA Biomass R&D Initiative

# Process Development Challenges

- Processing at high solids levels
- Understanding process chemistries
- Closing carbon, mass & energy balances
  - Requires accurate measurement/analysis methods
- Identifying critical process interactions
  - Integration efforts must focus on key issues
- Producing realistic intermediates and residues
  - Essential to evaluate potential coproduct values

# Commercialization Challenges

- Demonstrated market competitiveness
  - Compelling economics with acceptable risk
- Established feedstock infrastructure
  - Collection, storage, delivery & valuation methods
- Proven societal & environmental benefits
  - Sustainable
  - Supportive policies



# Lessons Learned from Past Pioneer Processing Plant Efforts

⇒ ***Accurately estimating cost & performance is the key to success!\****

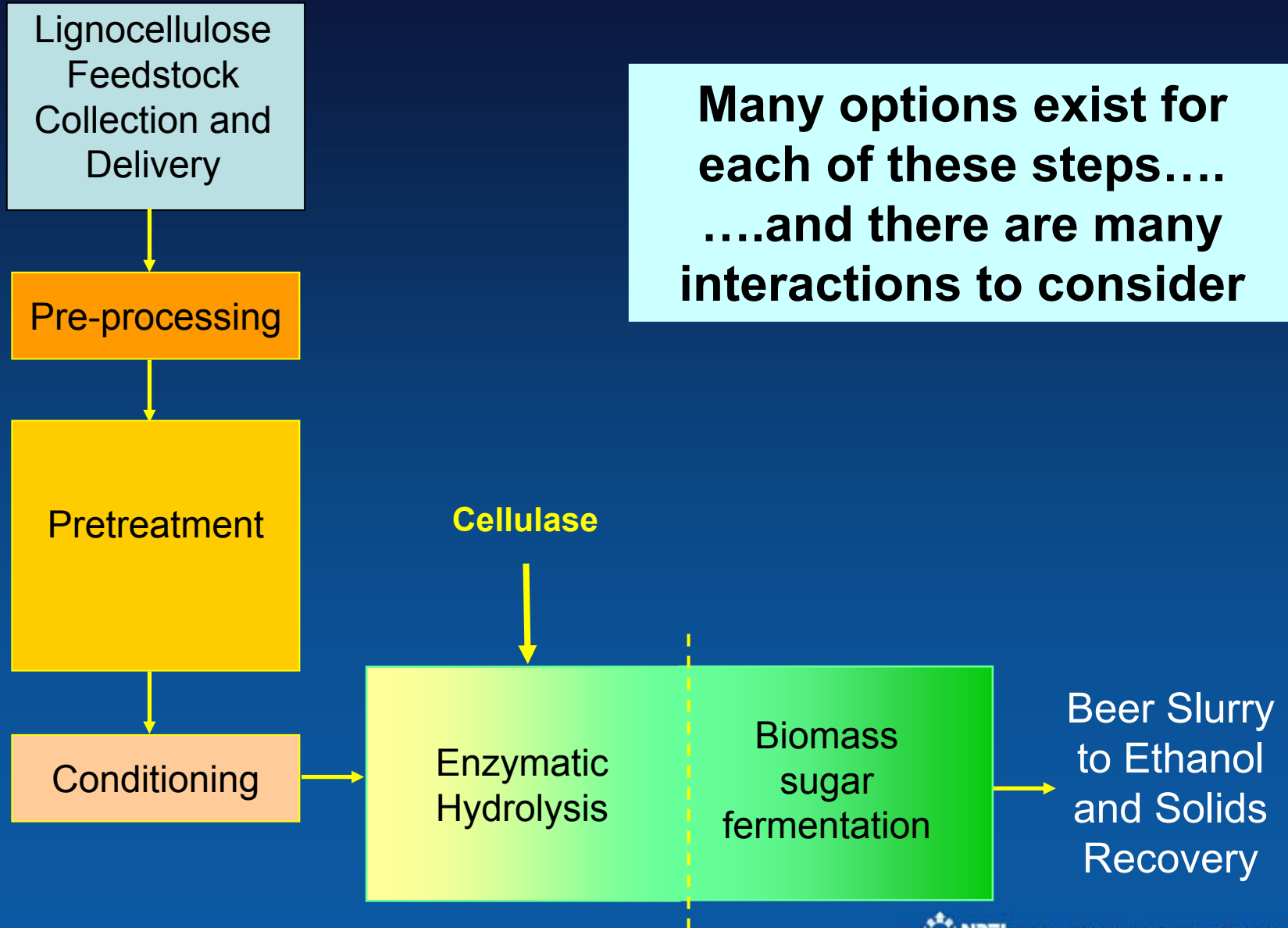
- Plant cost growth strongly correlated with:
  - Process understanding (*integration issues*)
  - Project definition (*estimate inclusiveness*)
- Plant performance strongly correlated with:
  - Number of new steps
  - % of heat and mass balance equations based on data
  - Waste handling difficulties
  - Plant processes primarily solid feedstock

\* *“Understanding Cost Growth and Performance Shortfalls in Pioneer Process Plants”, a 1981 Rand Corp. study for the USDOE*

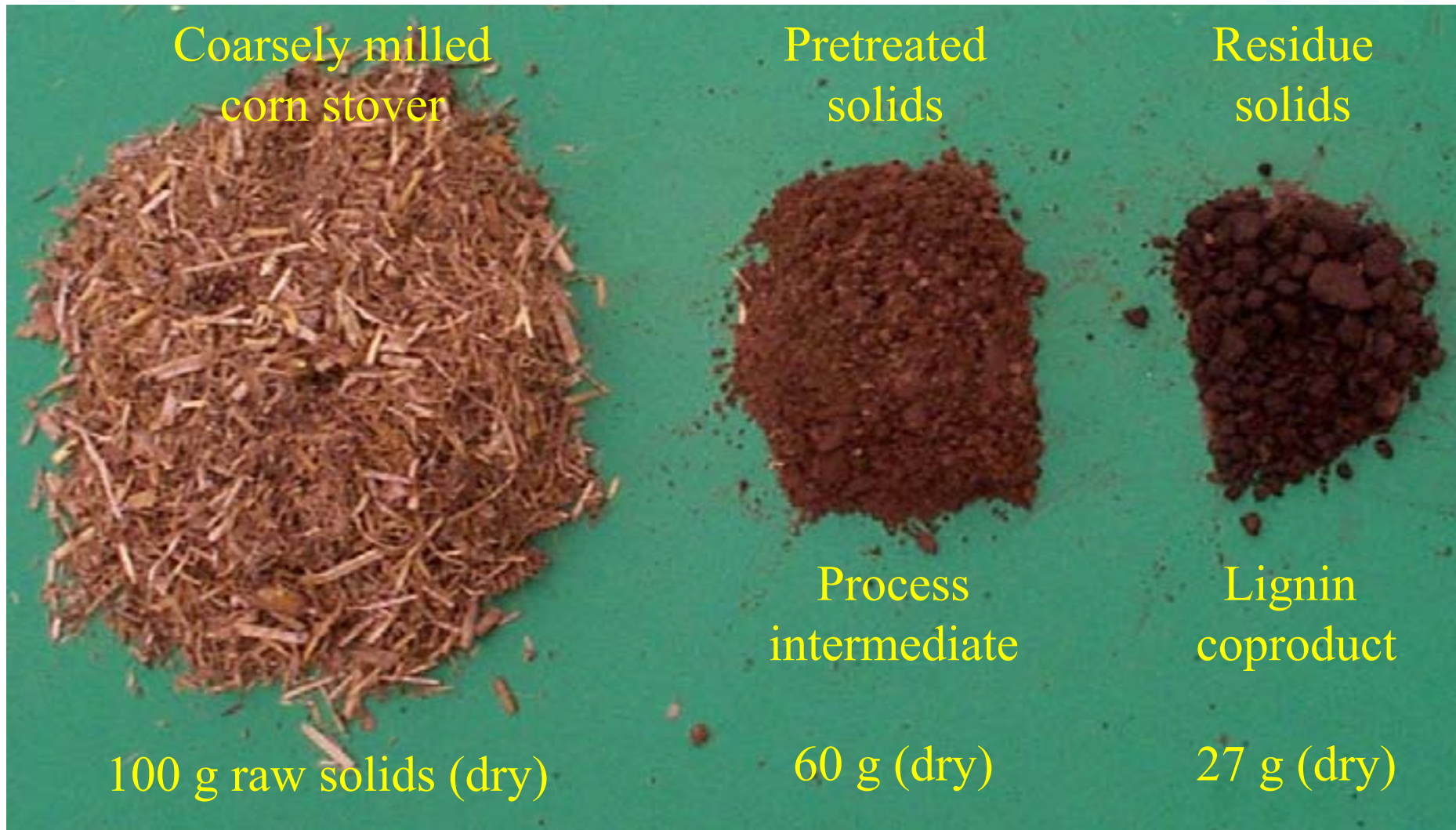
# Outline

- Biomass Basics
- Overview of Conversion Options
- Details of Enzyme-based Technology
- Biorefining Now and in the Future

# Enzymatic Process for Producing Ethanol



# Conversion is Technically Feasible...



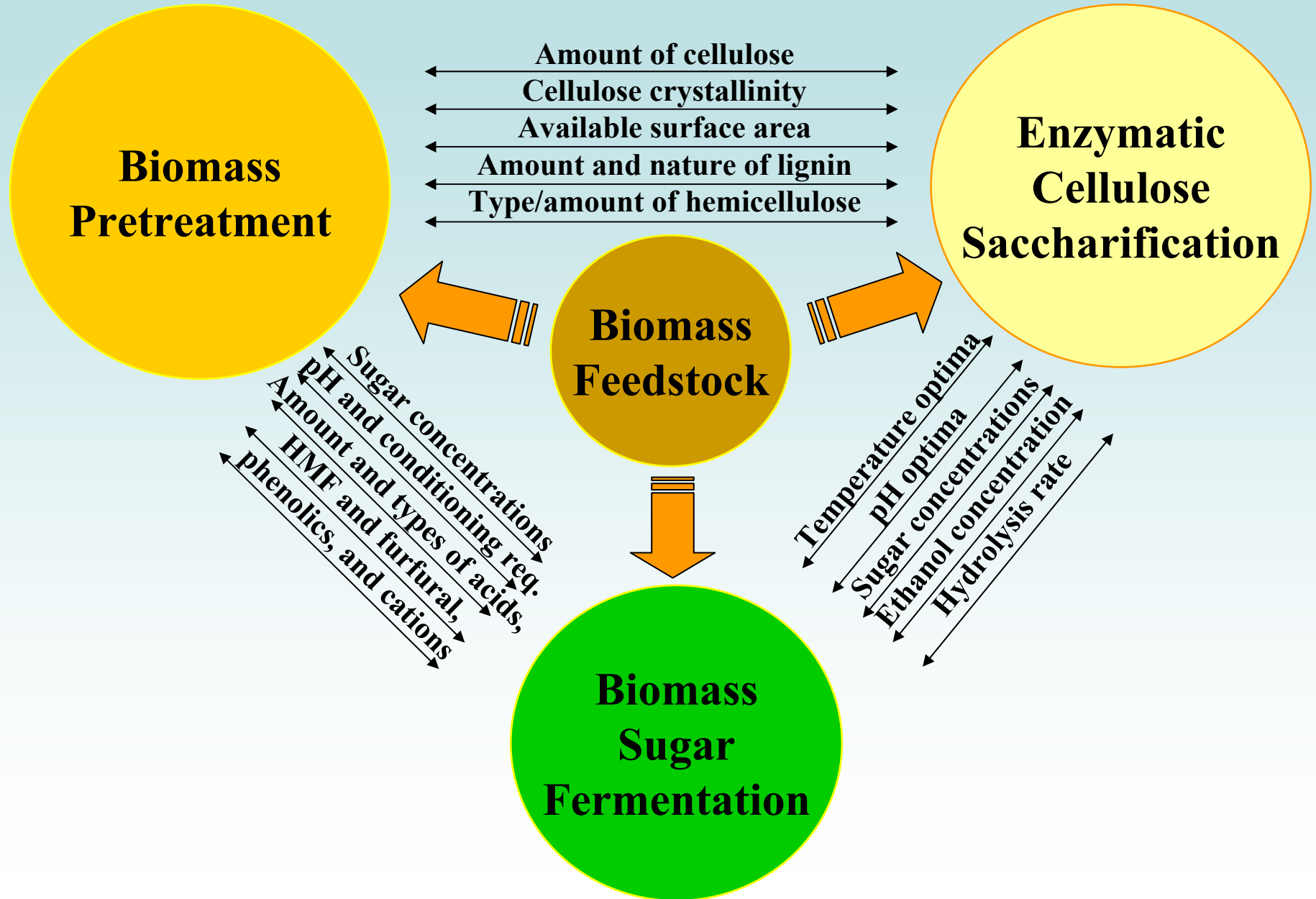
***...the Challenge is Making it Economical!***

# Technical Barriers

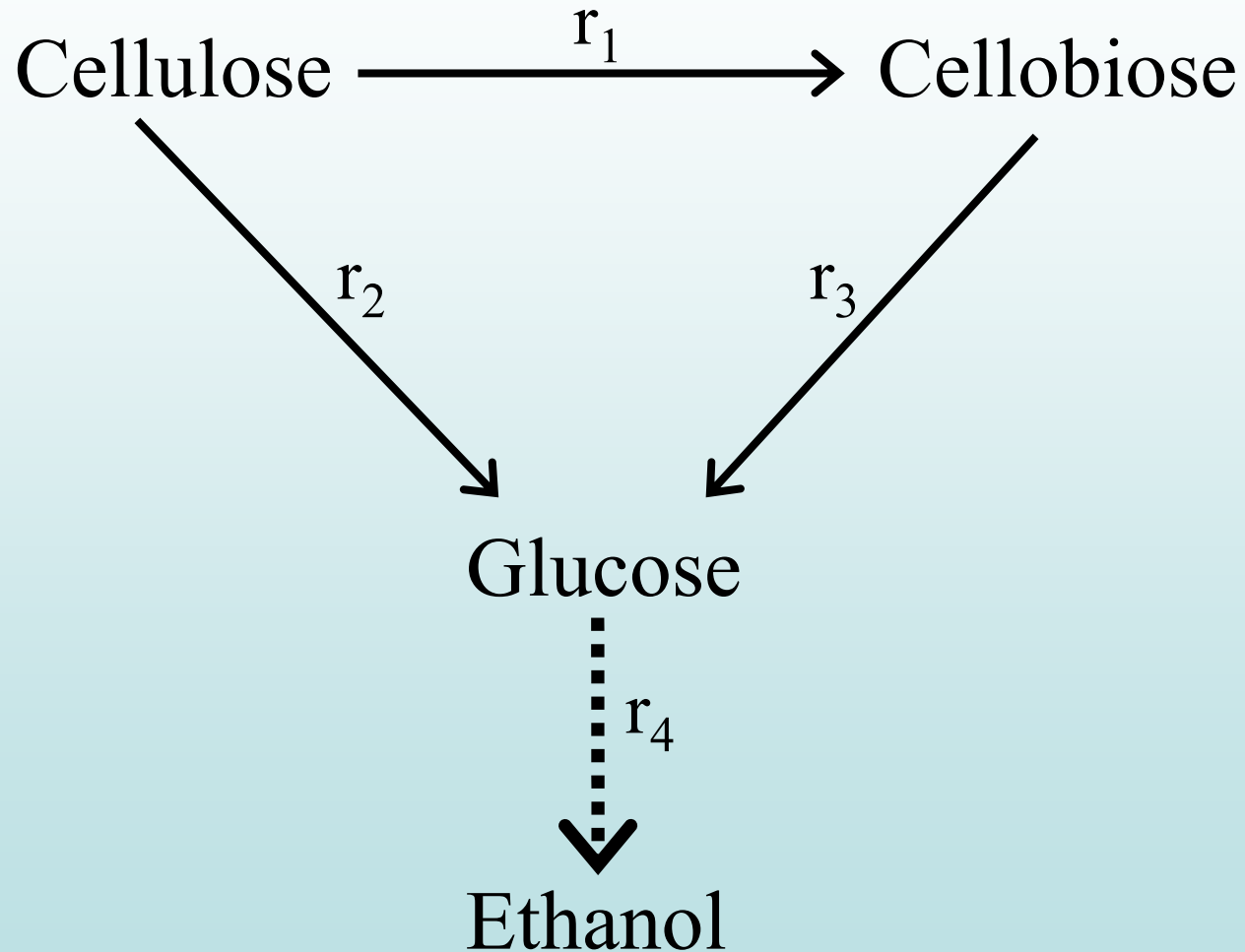
- Feedstock Valuation and Delivery
  - Analytical methods/sensors
  - Supply systems
  - Soil sustainability
- Biomass Recalcitrance to Conversion
  - Pretreatment
  - Enzymatic hydrolysis
  - Pentose fermentation
- Process Integration
  - Solids handling
  - ***Interactions***
  - Process chemistry



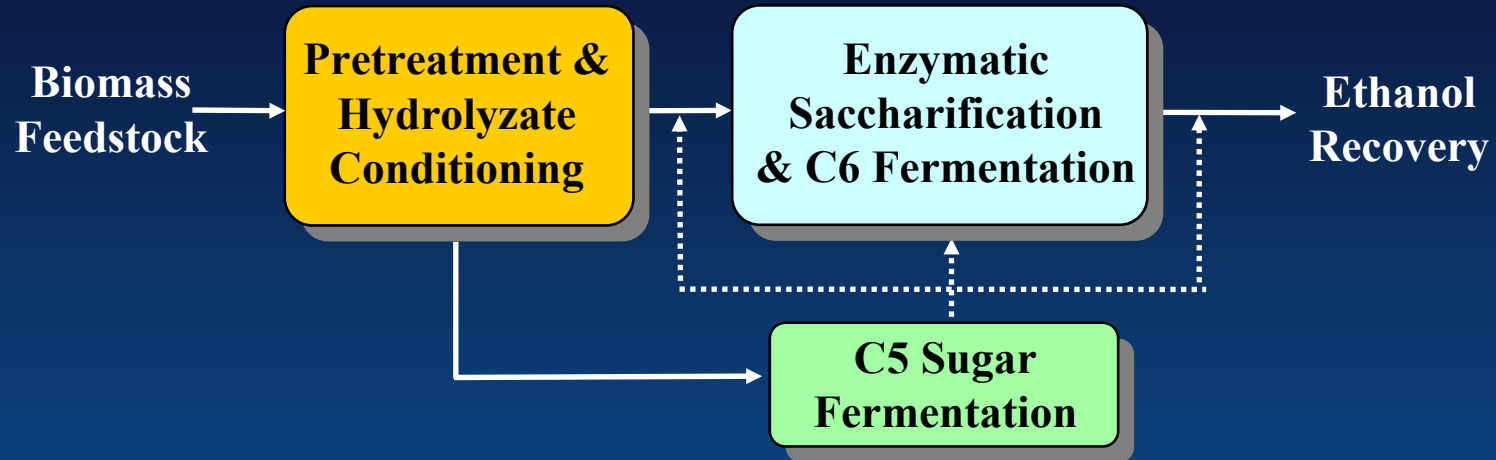
# Understanding Integration Issues



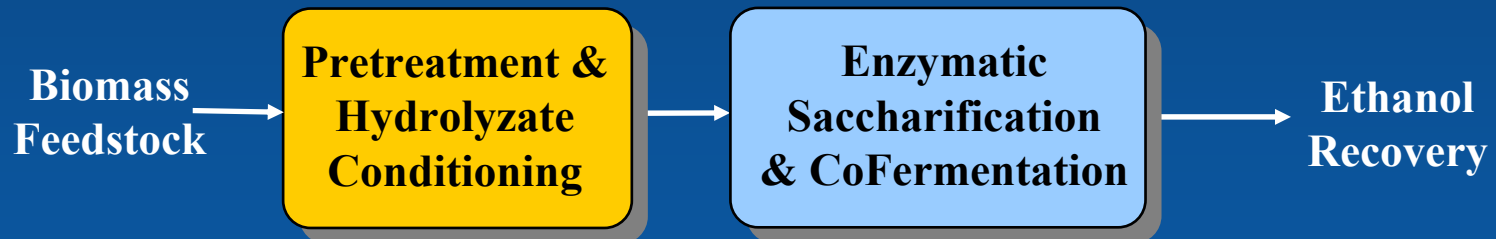
# Cellulose Conversion in SSF



# Enzymatic Hydrolysis Configurations Using Simultaneous Saccharification & Fermentation

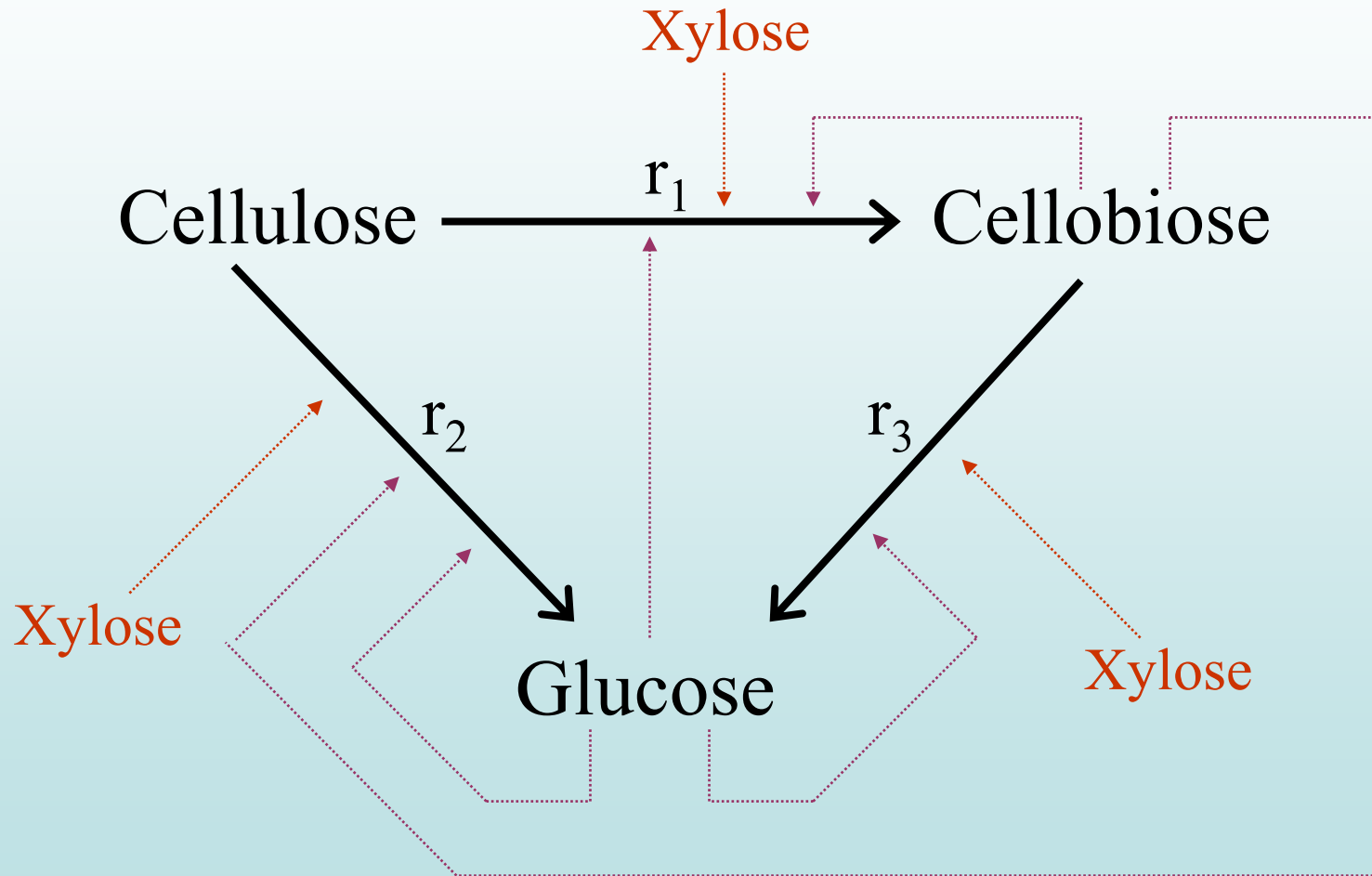


Separate C5 and C6 Sugar Fermentation (SSF or SSCF)

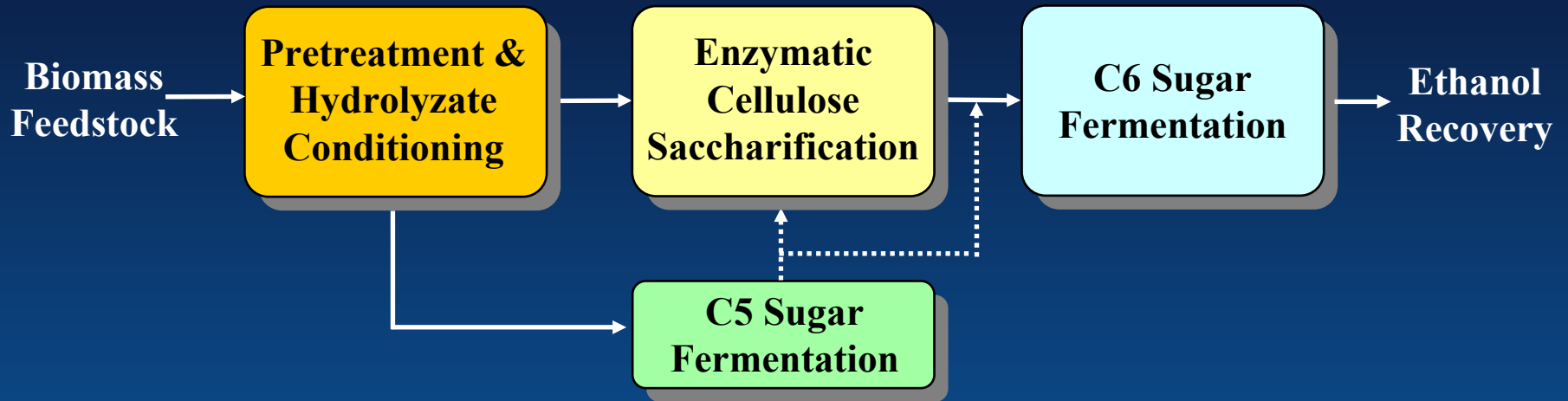


SSF with Combined C5 and C6 Sugar CoFermentation (SSCF)

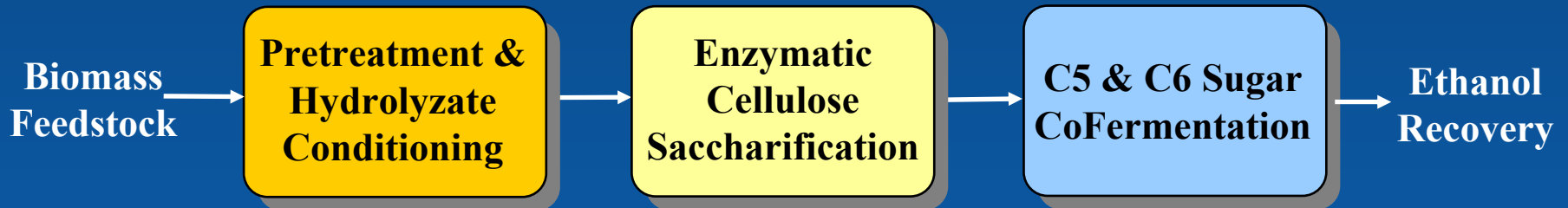
# Cellulose Conversion in SHF



# Process Configurations Based on Sequential Hydrolysis and Fermentation



SHF with Separate C5 and C6 Sugar Fermentation



SHF with Combined C5 and C6 Sugar Fermentation

# Comparing the Attributes of SSF and SHF Process Configurations

## Simultaneous (SSF/SSCF)

- Minimize enzyme inhibition by accumulating sugars
- Achieve high cellulose conversion yields
- Reduce process complexity via “one step” approach
- Increase pentose utilization and fermentative strain robustness through sustained production and co-utilization of glucose
- Minimize the potential for contaminant outgrowth by maintaining a low free sugar concentration

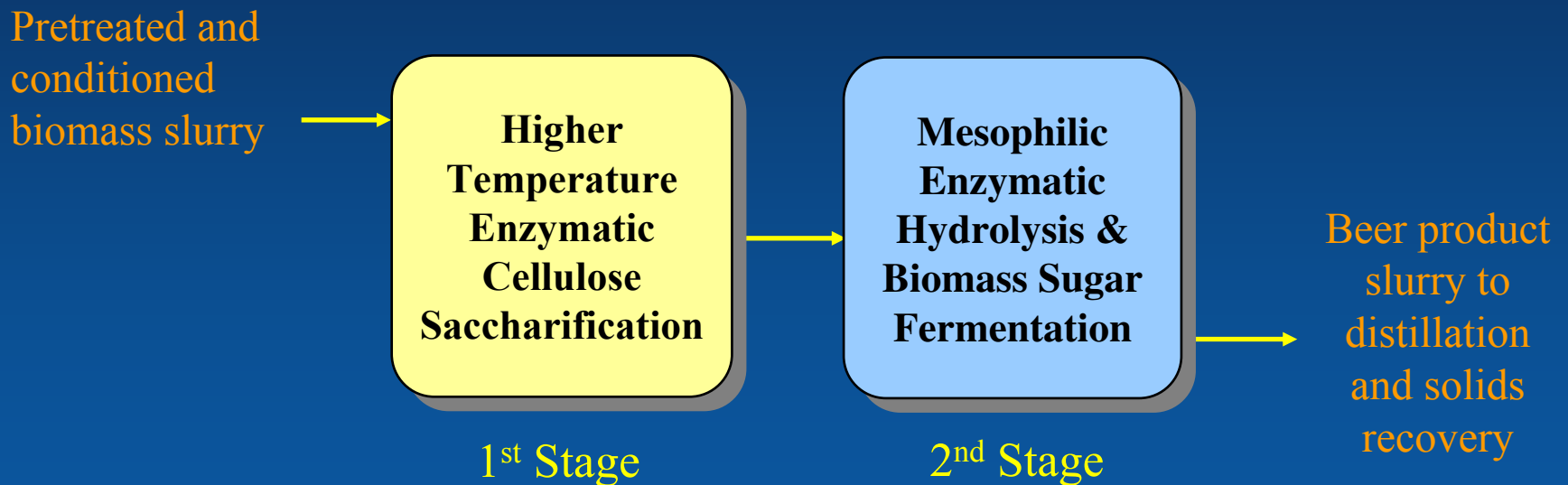
## Sequential (SHF)

- Run enzymatic hydrolysis and fermentation at their respective temperature and pH optima
  - *large benefits possible when optima are significantly different*
- Generate intermediate sugar product(s)
  - *Upgrade for sale or use as substrates to manufacture other value-added products...enable multi-product biorefineries*
- Easier mixing in fermentation
  - *Lower levels of solids in fermentation (or absence of solids if S/L separation used prior to fermentation)*



# Probable Commercial Configuration

- Anticipate exploiting next generation thermostable cellulases using a two stage hybrid hydrolysis and fermentation process that leverages the strengths of both SSF and SHF
  - Stage 1: Operate at high temperature to exploit enzymes' thermostability
  - Stage 2: Operate as SSF/SSCF to achieve high cellulose conversion yield



Hybrid Hydrolysis and Fermentation (HHF)

# Technical Barriers

- Feedstock Valuation and Delivery
  - Analytical methods/sensors
  - Supply systems
  - Soil sustainability
- Biomass Recalcitrance to Conversion
  - Pretreatment
  - Enzymatic hydrolysis
  - Pentose fermentation
- Process Integration
  - Solids handling
  - Interactions
  - ***Process chemistry***

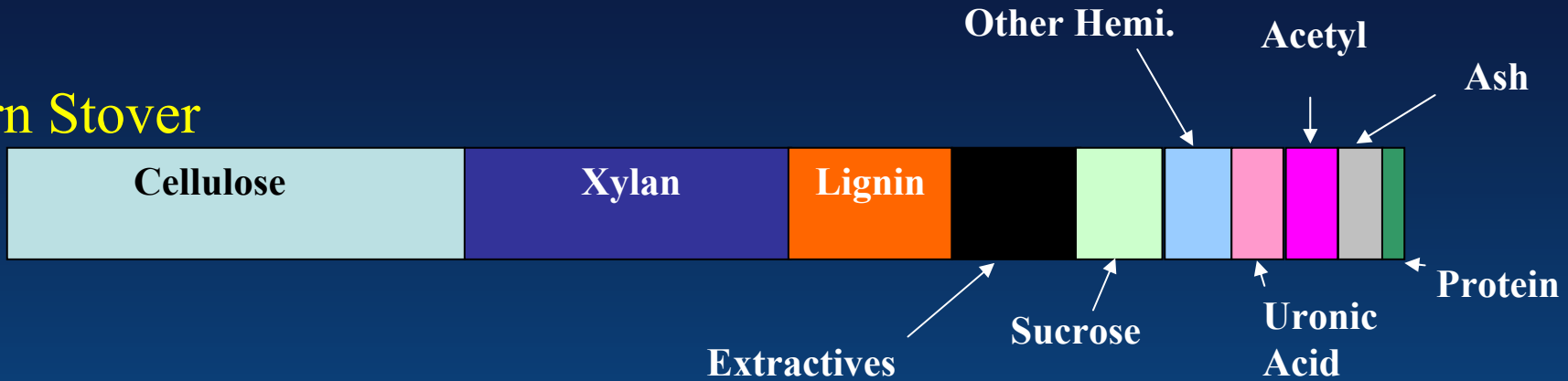
# Biomass Chemistry and Ultrastructure

- Our understanding of biomass chemistry and structure and of conversion mechanisms continues to grow, but many issues remain unknown
  - Further work needed to advance analysis tools and fundamental understanding of biomass ultrastructure and process chemistry during conversion processes

# Tracking Composition and Mass

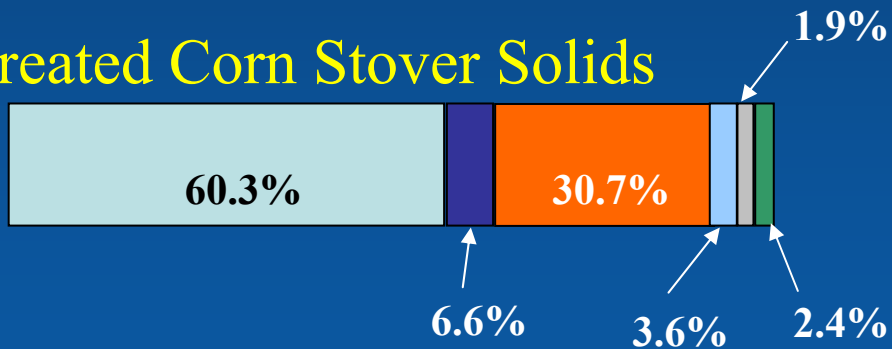
## Pretreatment Example

### Corn Stover

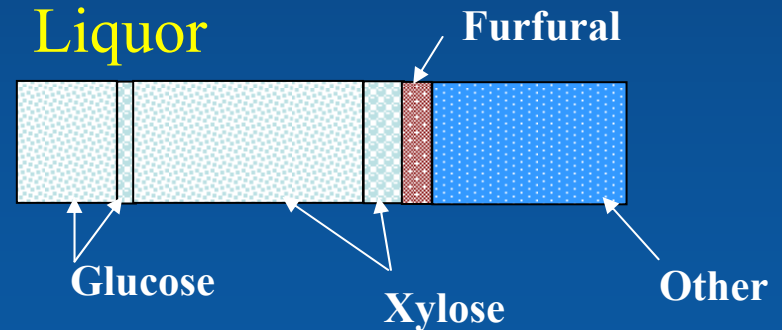


### Pretreatment

### Pretreated Corn Stover Solids



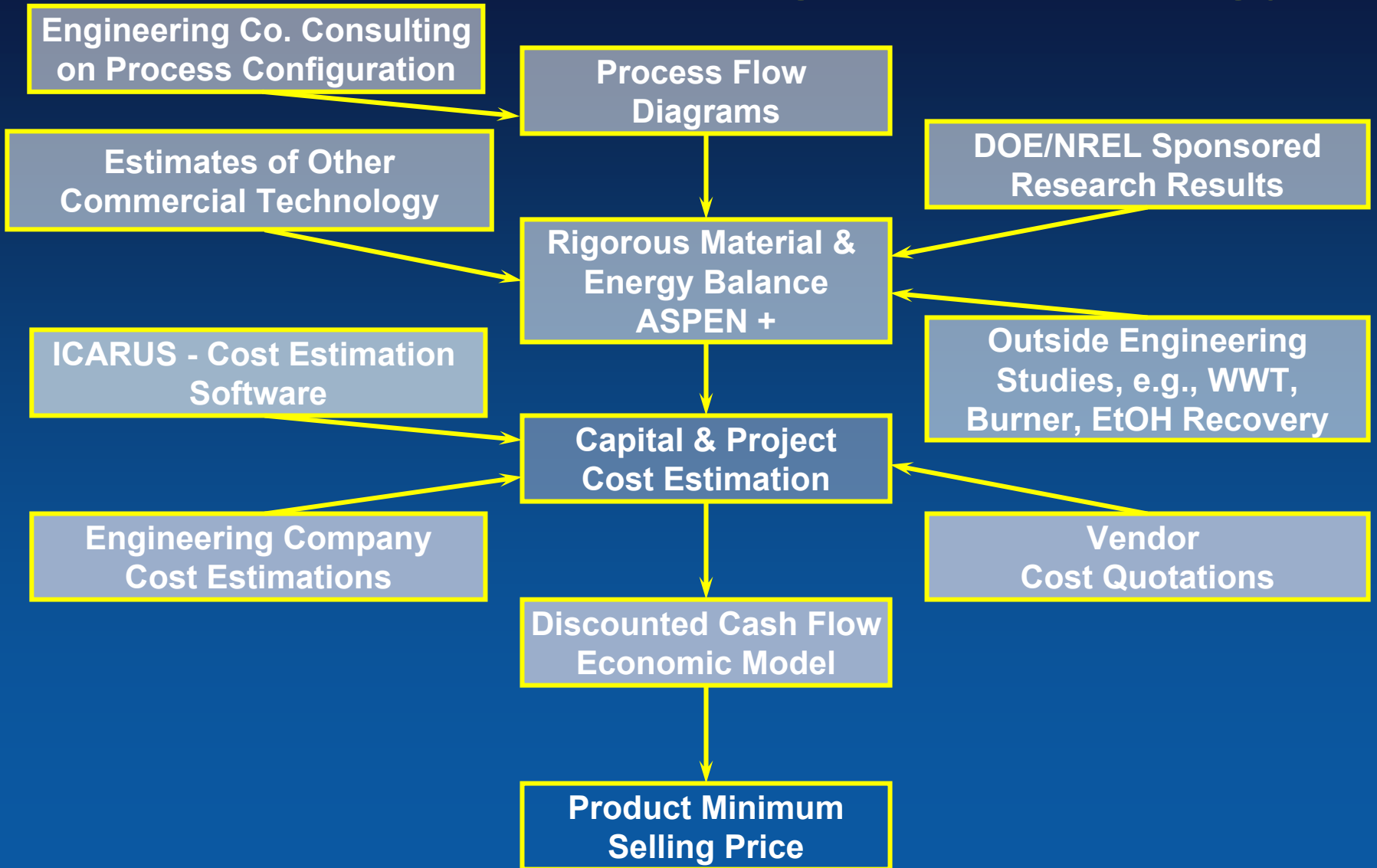
### Liquor



# The Role of Technoeconomic Analysis

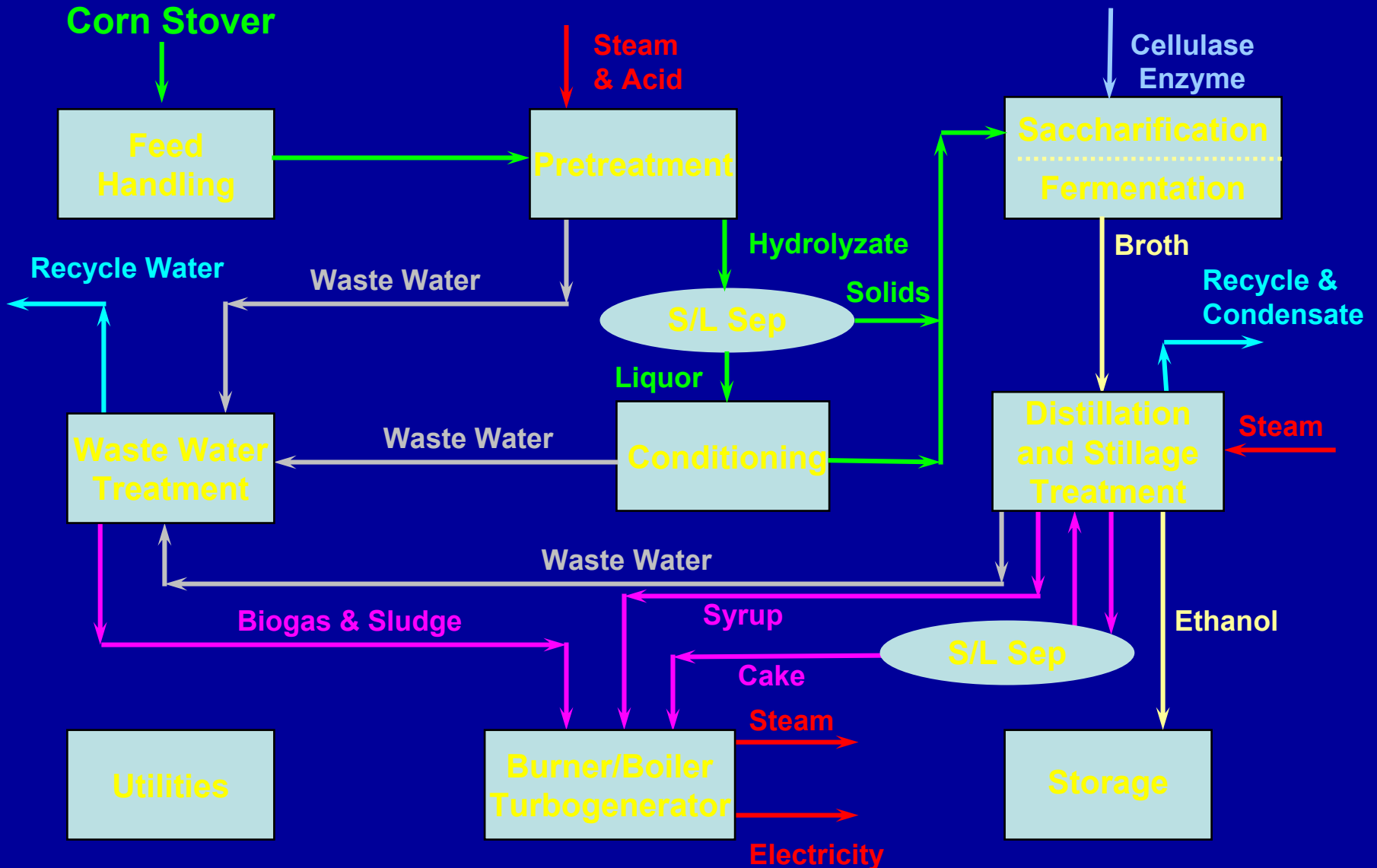
- Quantify relative impacts of process improvements
- Identify research directions with largest cost reduction potential, or highest perceived benefit/investment ratio

# Process Design and Economic Modeling Methodology





# Developing Inclusive Cost Estimates



# Projected Economics – Example

Plant Size Basis: 2000 MT Dry Corn Stover/Day

Assumed Corn Stover Cost: \$35/dry ton

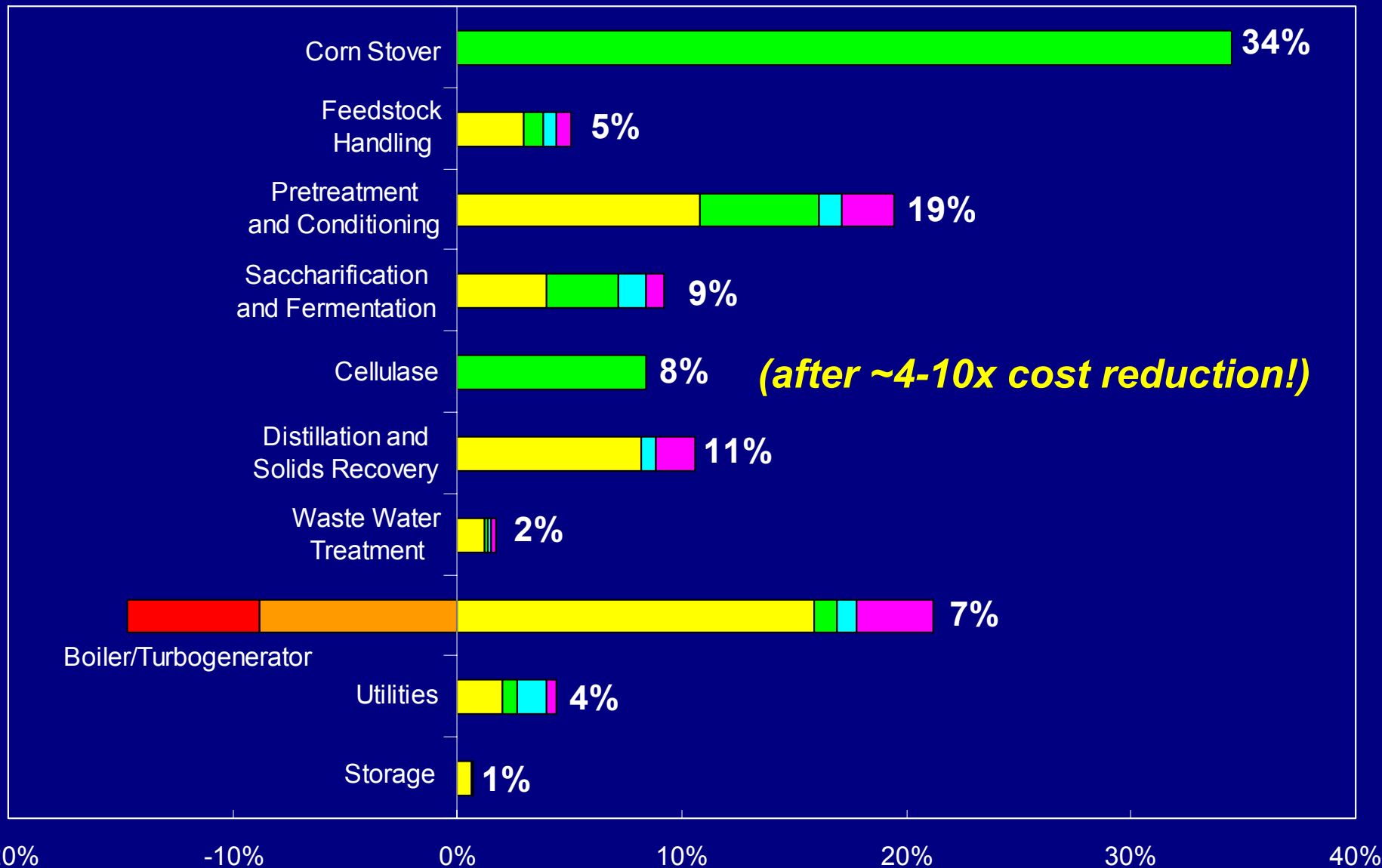
Assumed Enzyme Cost: \$0.11/gallon of produced ethanol

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<b>Economic Parameter (Units, \$1999)</b>	<b>Value</b>
Min. Ethanol Selling Price (\$/gal)	\$1.28
Ethanol Production (MM gal/yr)	59.9
Ethanol Yield (gal/dry ton)	77.5
Total Project Investment (\$ MM)	\$198
TPI per annual gallon (\$/gal)	\$3.31

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# Corn Stover Case - % Costs by Area



# Highlight Economic Findings

- Enzymatic ethanol production costs dominated by
    - Feedstock
    - Enzymes - cellulases
    - Capital equipment throughout the plant
  - Syngas production costs dominated by
    - Feedstock
    - Capital equipment
- ⇒ ***Current USDOE and NBC (ANL, INEEL, NREL, ORNL, and PNNL) Biomass Program efforts focused on decreasing these key cost centers***

# Economic Modeling Highlights, cont'd

- Estimated operating costs are becoming competitive, although capital costs remain high
  - Process intensification and the ability to produce additional value-added coproducts are both approaches being pursued to reduce the capitalization/financing burden

⇒ *There has been significant progress in reducing projected sugar platform costs through a variety of approaches, including co-location, feedstock valuation, enzyme cost reduction, high solids processing, etc.*

- Selected highlights follow....

# Potential to Reduce Capital Costs through Co-location – An Example

Economic Parameter (Units, \$1999)	Process Case	Dry-mill Co-location	Coal-fired Power Plant Co-location
MESP (\$/gal)	<b>\$1.30</b>	<b>\$1.23</b>	<b>\$1.18</b>
EtOH Production (MM gal/yr)	60	30 / 30	60
EtOH Yield (gal/dry ton stover) (gal/bushel corn)	77.5	77.5 2.85	77.5
TPI (\$ MM)	<b>\$200</b>	<b>\$109 / \$70</b>	<b>\$130</b>
TPI per Annual Gallon (\$/gal)	\$3.34	\$1.83 / \$1.16	\$2.17
Net Operating Costs (\$/gal)	\$0.73	\$0.72	\$0.82



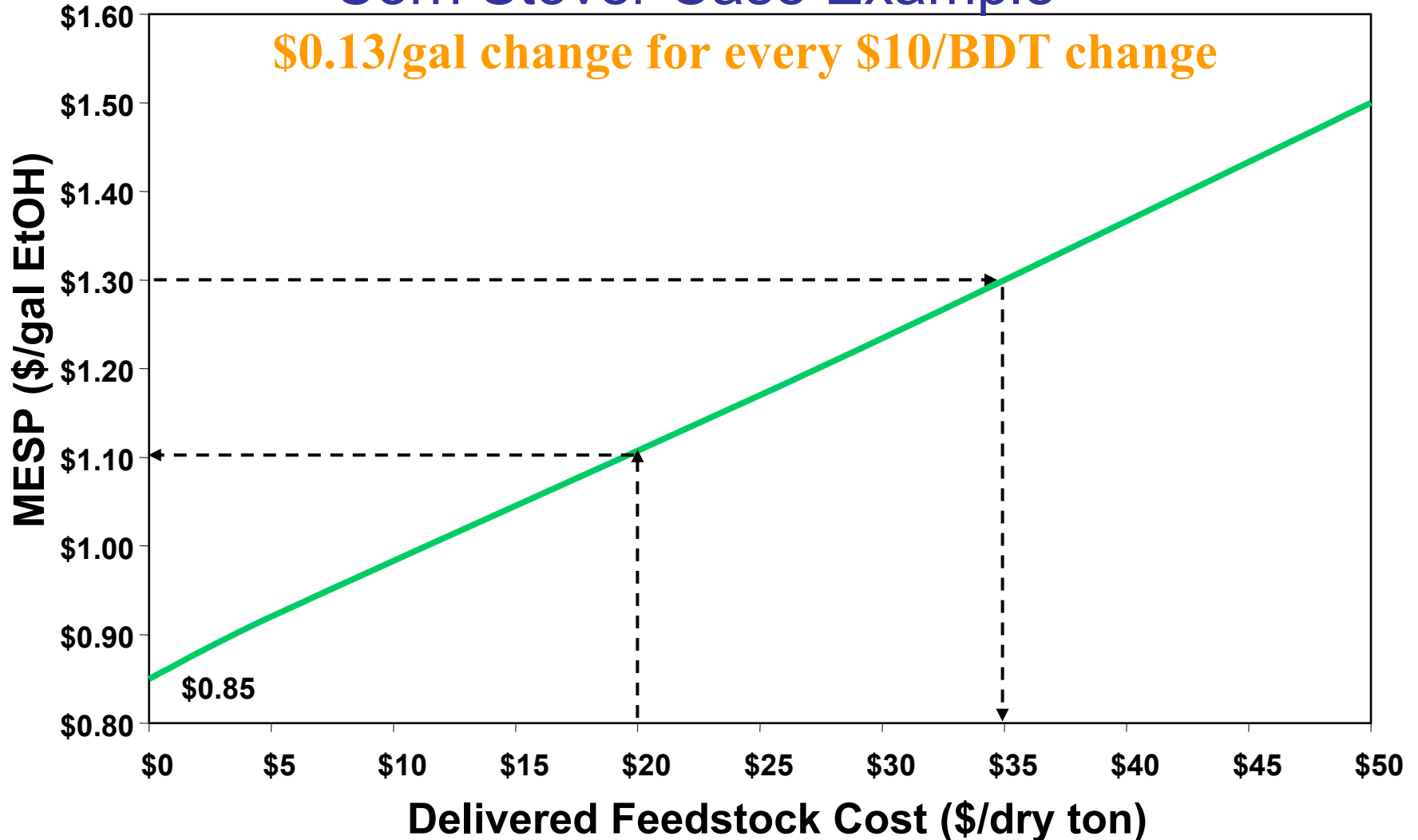
# Towards a Low Cost Feedstock Infrastructure

- Reducing feedstock cost is a significant opportunity
  - Apply innovative harvesting & storage methods
    - Whole stalk harvest?
    - Dry or wet densification?
  - Value the feedstock based on its composition
    - In-field or point-of-delivery rapid compositional analysis, e.g., using calibrated Near InfraRed Spectroscopy (NIRS)

⇒ *Application of NIRS shows that significant knowledge gaps remain about the magnitude and sources of feedstock compositional variability*

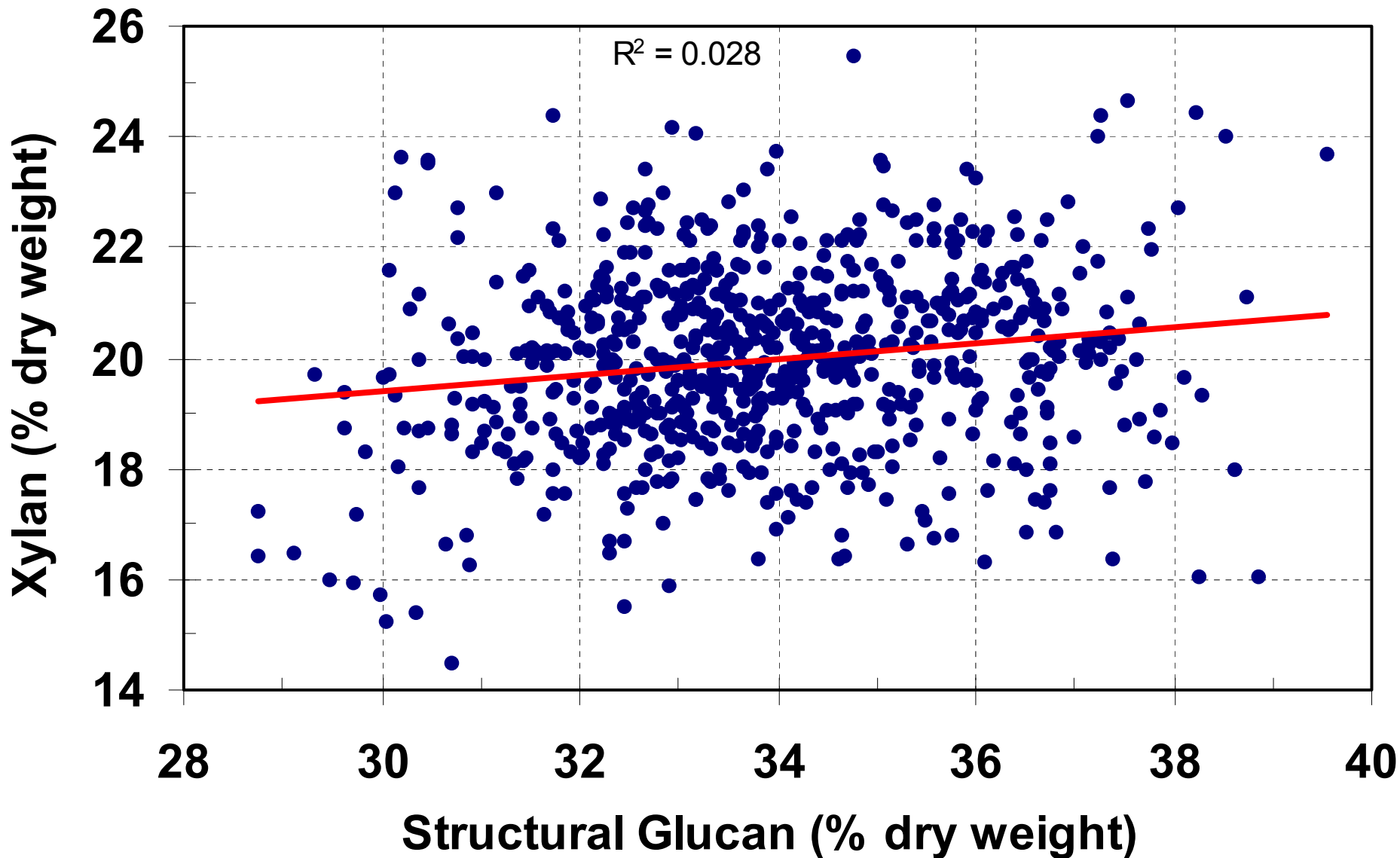
# Impact of Reducing Feedstock Cost

## Corn Stover Case Example

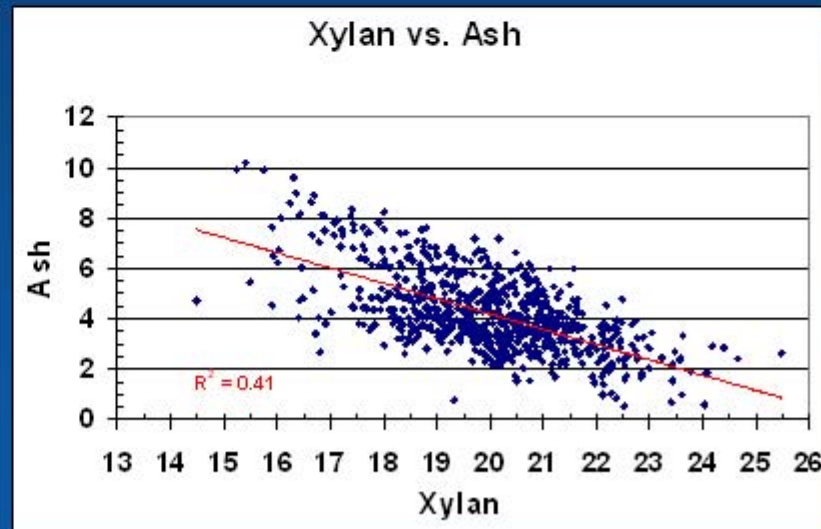
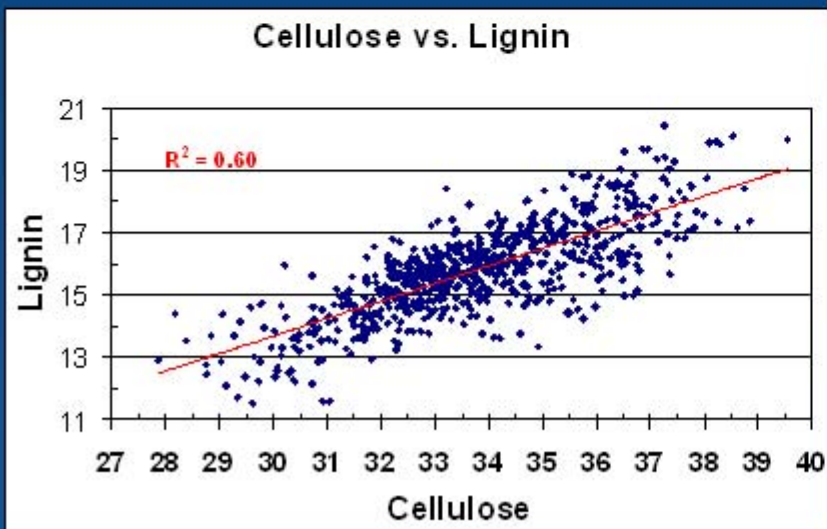
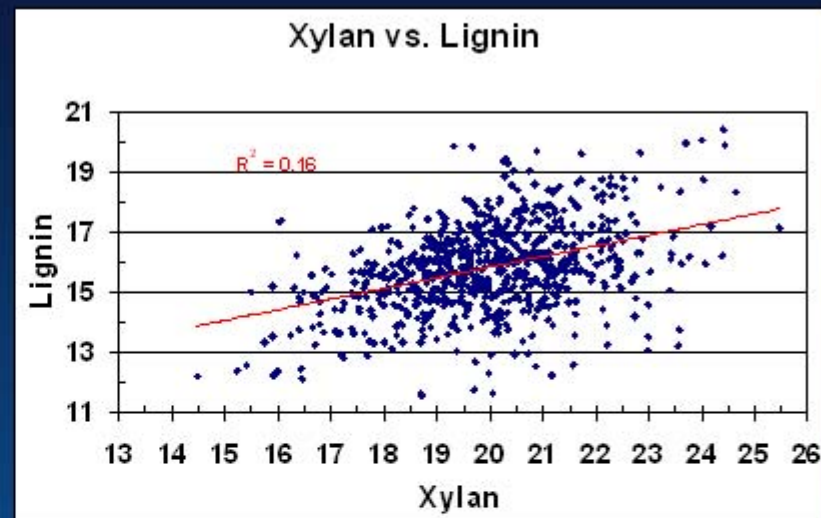
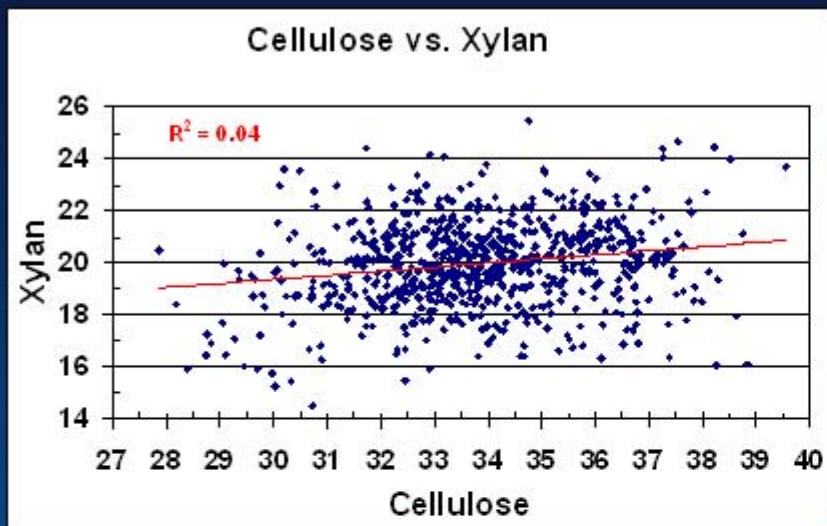


# Substantial Feedstock Variability

NIR Composition of 731 corn stover samples from the 2001 harvest



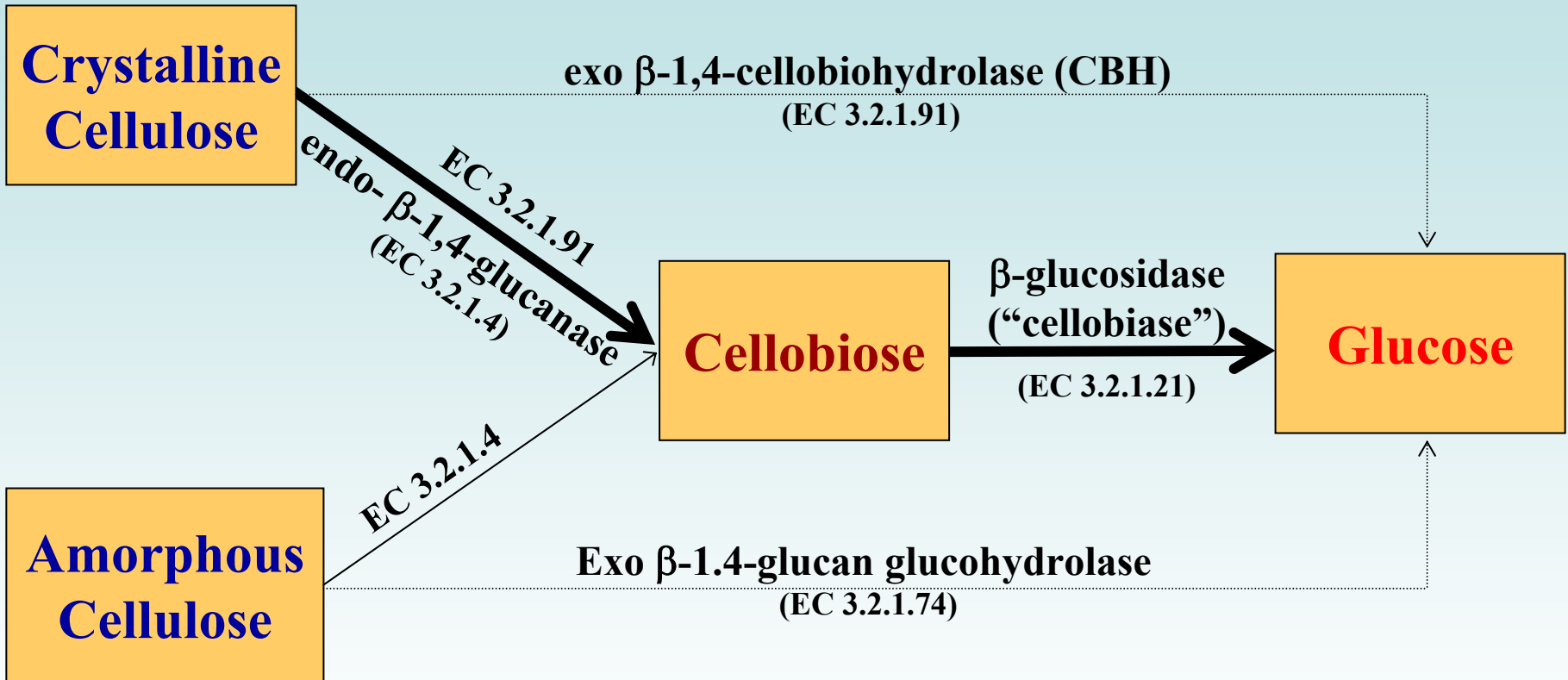
# Corn Stover Variability



# Reducing Cellulase Cost

- Objective: Reduce cost of cellulases for biomass conversion applications to enable large volume sugar platform technology
  - The program's enzyme cost target is \$0.10/gallon ethanol or less
- NREL's role:
  - Issue subcontracts to industry and facilitate their success
  - Supply "standard" pretreated feedstock
  - Develop cost metric to translate enzyme performance into economic terms, i.e., enzyme cost (\$/gallon EtOH)
  - Experimentally validate key results
  - Review/Audit key results that can't be independently validated
  - Provide supporting information, consultation, and guidance as requested or needed to facilitate subcontractor success

# Multi-enzyme Cellulase System

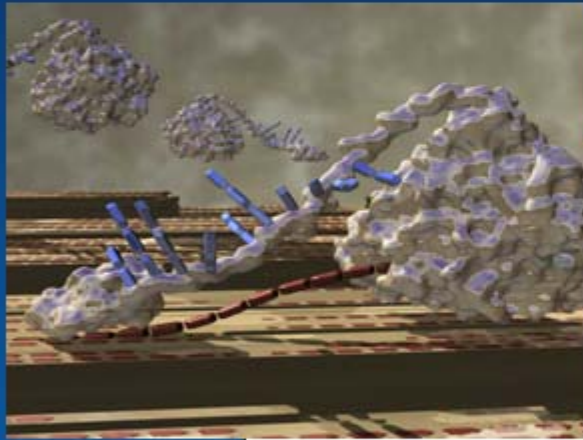


**Bold Main Hydrolysis Reactions Proceed via**

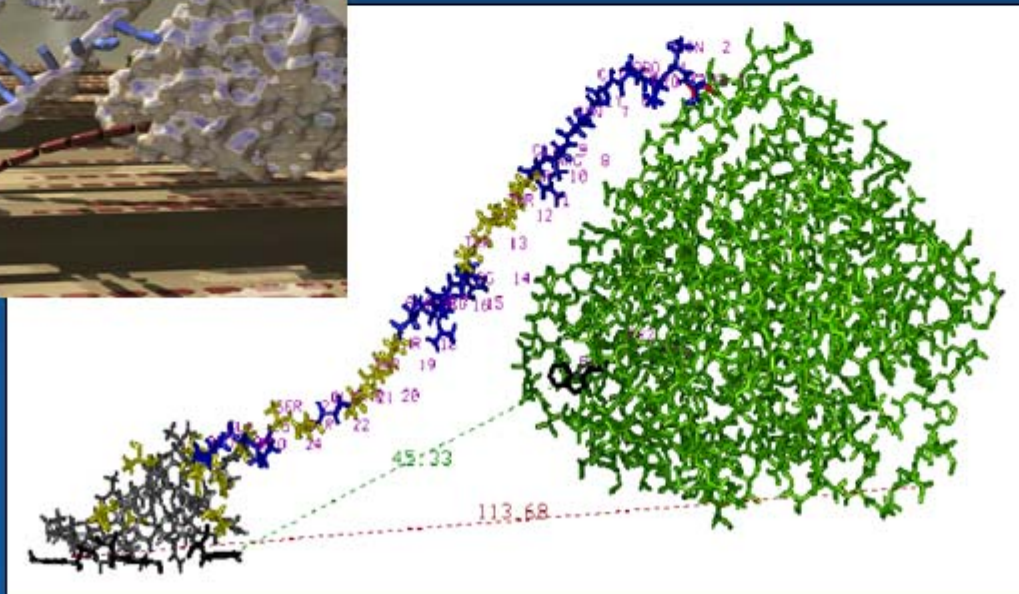
**“Endo” → “Exo” → “ $\beta$ -G”**

# NREL's Enzymatic Hydrolysis Partnerships

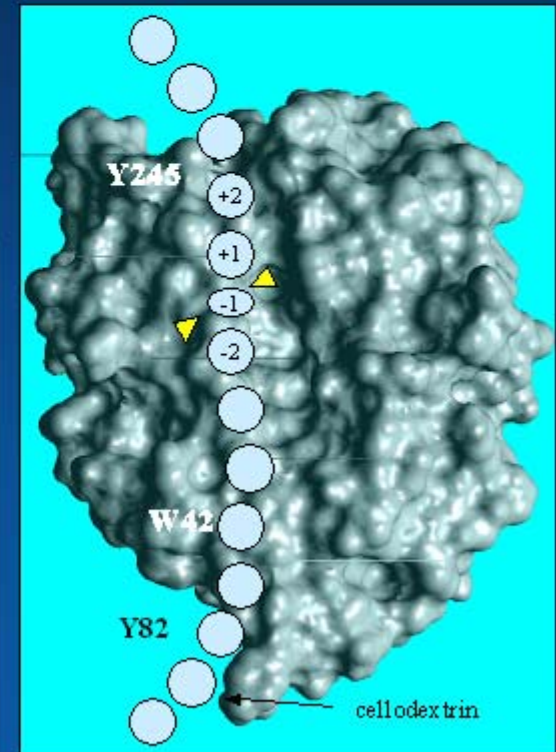
- 4-year Partnerships with Genencor & Novozymes
  - Enzyme biochemistry and specific activity
  - Cellulase - cellulose surface interaction
  - Lower the cost of enzyme



CBH1 from *T. reesei*



E1 from *A. cellulotiticus*





# Metrifying Enzyme Cost Reduction

$$C_E = \frac{E_P E_L}{B_N Y}$$

Where:

- $C_E$  = Enzyme cost (\$/gal ethanol)
- $E_P$  = Enzyme price (\$/L product) (subcontractor supplied)
- $E_L$  = Enzyme loading (g protein/g cellulose entering hydrolysis) (measured)
- $B_N$  = Enzyme concentration in product (g protein/L product) (measured)
- $Y$  = Ethanol Process Yield (gal EtOH/g cellulose entering hydrolysis)  
(calculated from process model; a constant)

➤ see Andy Aden and Mark Ruth's tech memo #4988 for further details

# Approach

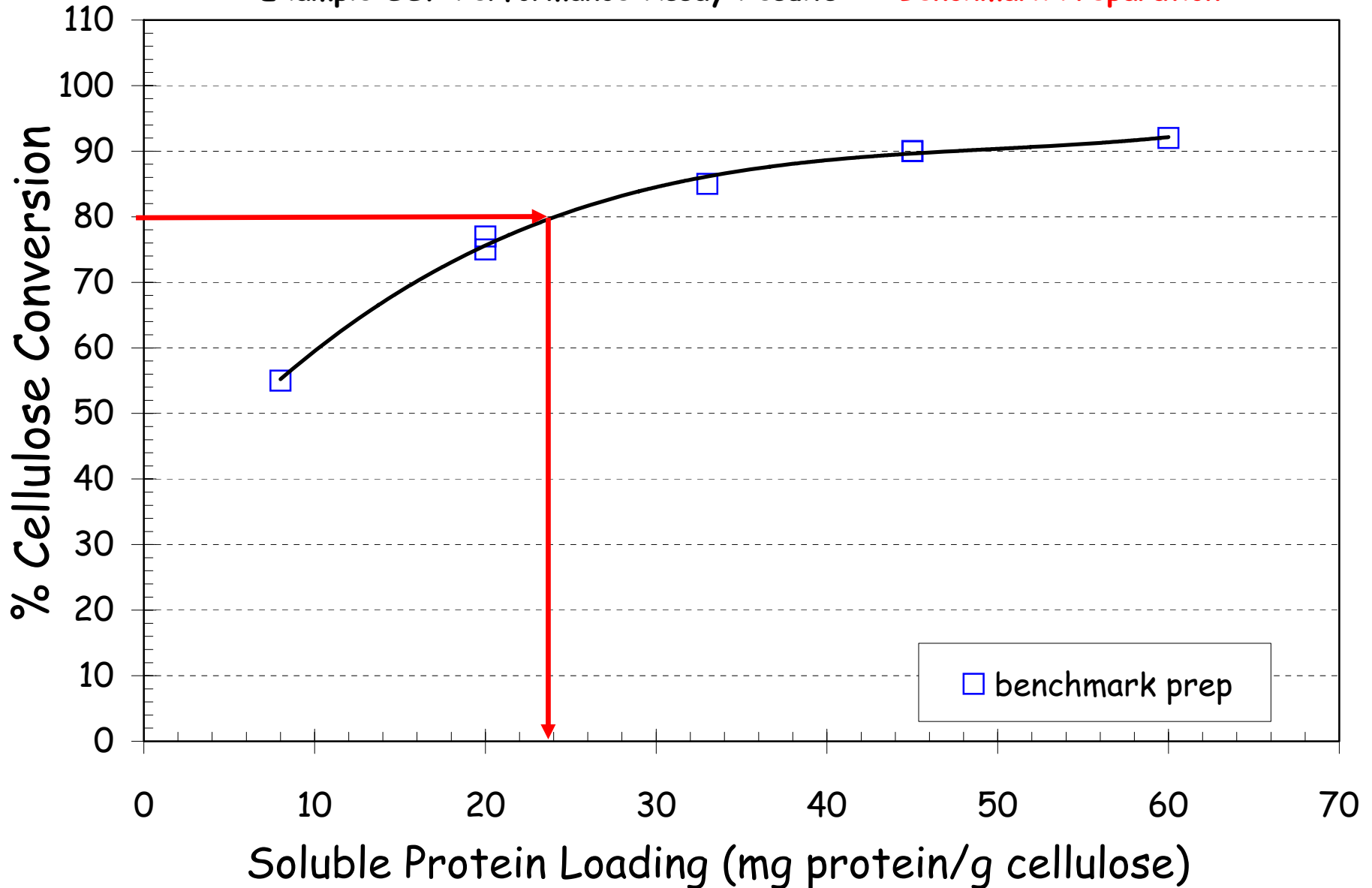
1. Measure enzyme concentration,  $B_N$ 
  - Use accepted protein measurement method (Pierce BCA)
2. Measure required enzyme loading on “standard” pretreated corn stover (PCS) substrate,  $E_L$ 
  - Use variation of traditional shakeflask SSF digestibility test
3. Calculate  $C_E$  using subcontractor supplied  $E_P$  and metric  $Y$

$$C_E = \frac{E_P E_L}{B_N Y}$$

4. Compare  $C_E$  of improved preparations against subcontract benchmark
5. Repeat

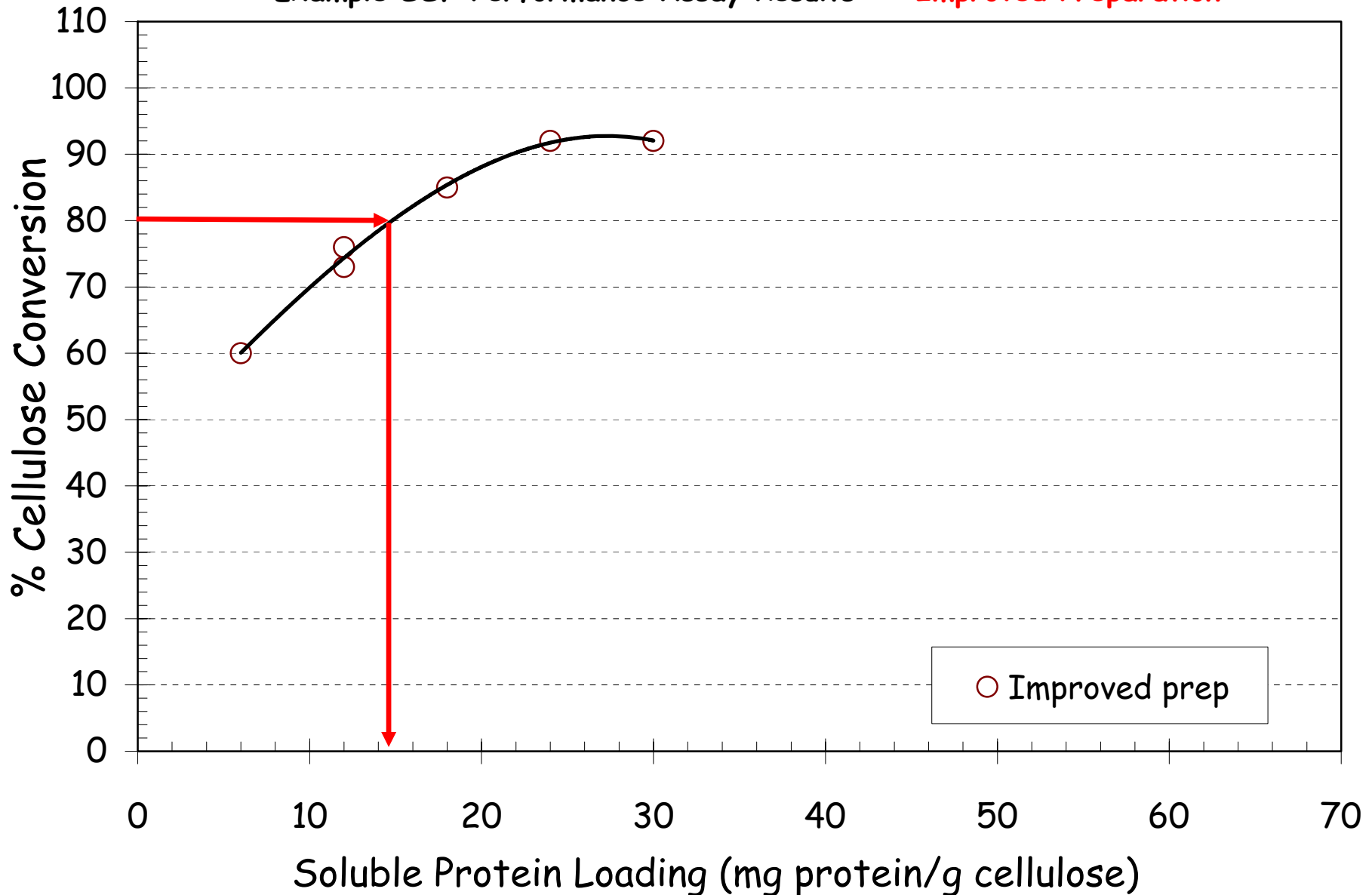
# Benchmarking Performance

Example SSF Performance Assay Results -- **Benchmark Preparation**



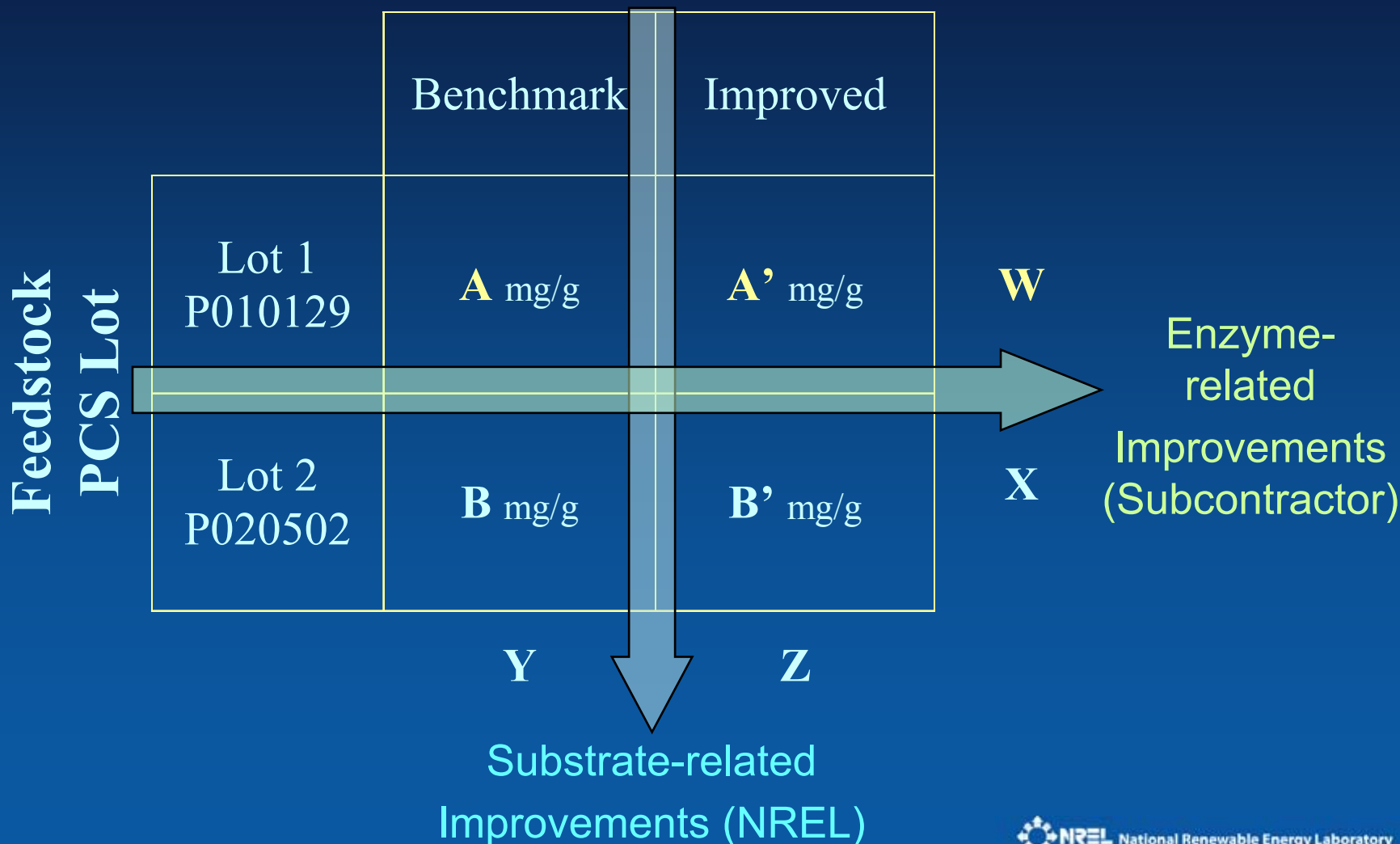
# Measuring Improvement

Example SSF Performance Assay Results -- Improved Preparation



# Overall Improvement Matrix

## Enzyme Preparation

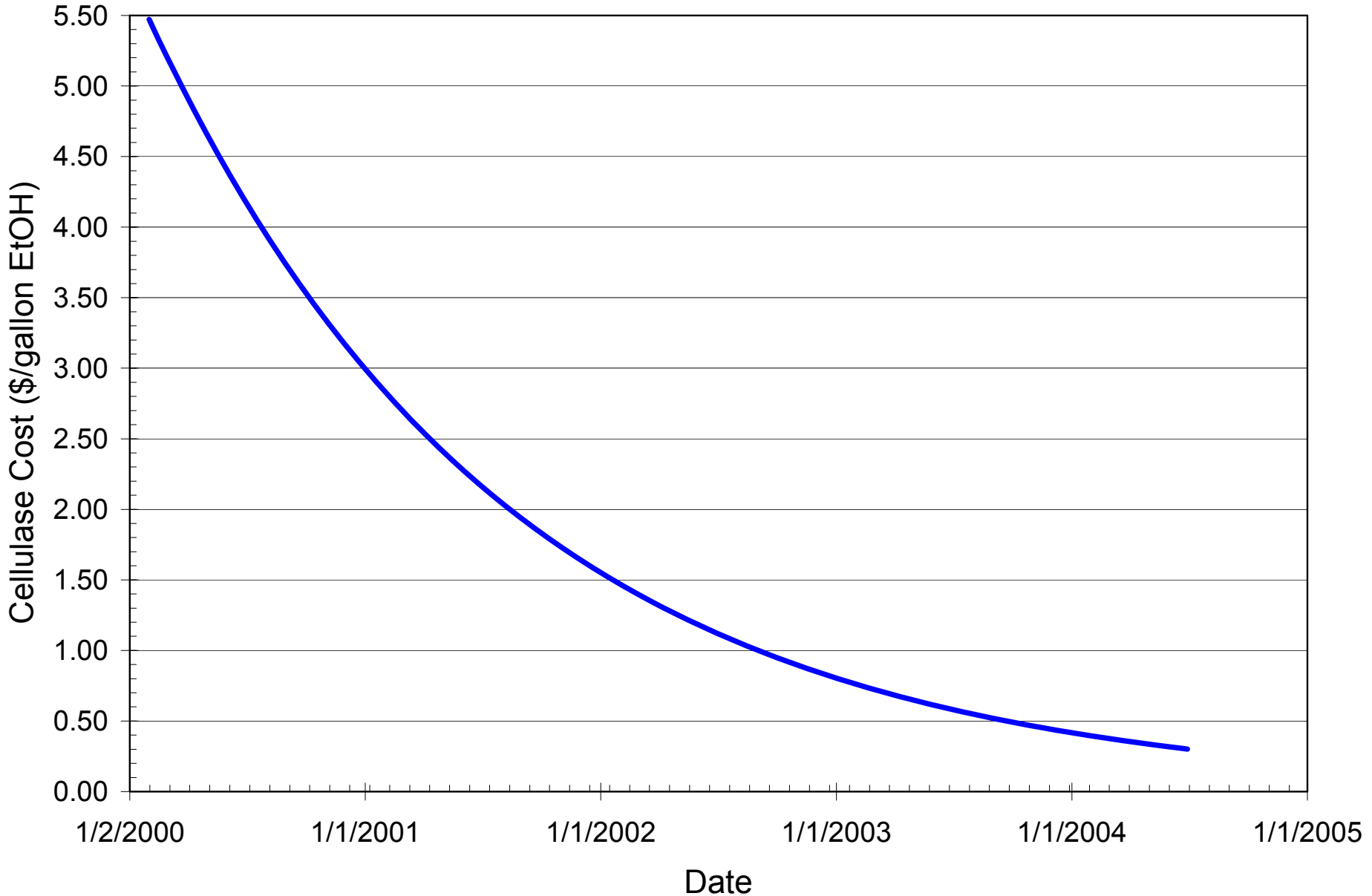


# Industry-led Cellulase Cost Reduction

- Similar Subcontracts set up with Genencor and Novozymes to reduce cost of commodity cellulases by tenfold or greater
  - 3 year periods of performance + 1 year extensions
  - 20% cost share by industry
  - Annual performance milestones with ultimate 3 yr 10X goal relative to benchmark established at start of subcontracts; in extensions, goal adjusted to reaching an enzyme cost of \$0.10/gallon of ethanol or less
- Status
  - Details proprietary. Both companies presented updates at a May '03 project review and have since issued press releases. See internet.
    - [http://www.ott.doe.gov/biofuels/enzyme\\_sugar\\_platform.html](http://www.ott.doe.gov/biofuels/enzyme_sugar_platform.html)
    - <http://www.genencor.com>
    - <http://www.novozymes.com>
      - Go to the companies press web site archives and search on “biomass”
- Highlights/Summary of Reported Accomplishments
  - Both companies exceeded 3 yr 10X cost reduction goal, decreasing estimated enzyme costs from ~\$5.00 to \$0.30-0.40 per gal EtOH
  - Cost reduction efforts continuing
    - One year extensions finished in 11/04 (Genencor) or 1/05 (Novozymes)

# Cellulase Costs Falling Rapidly

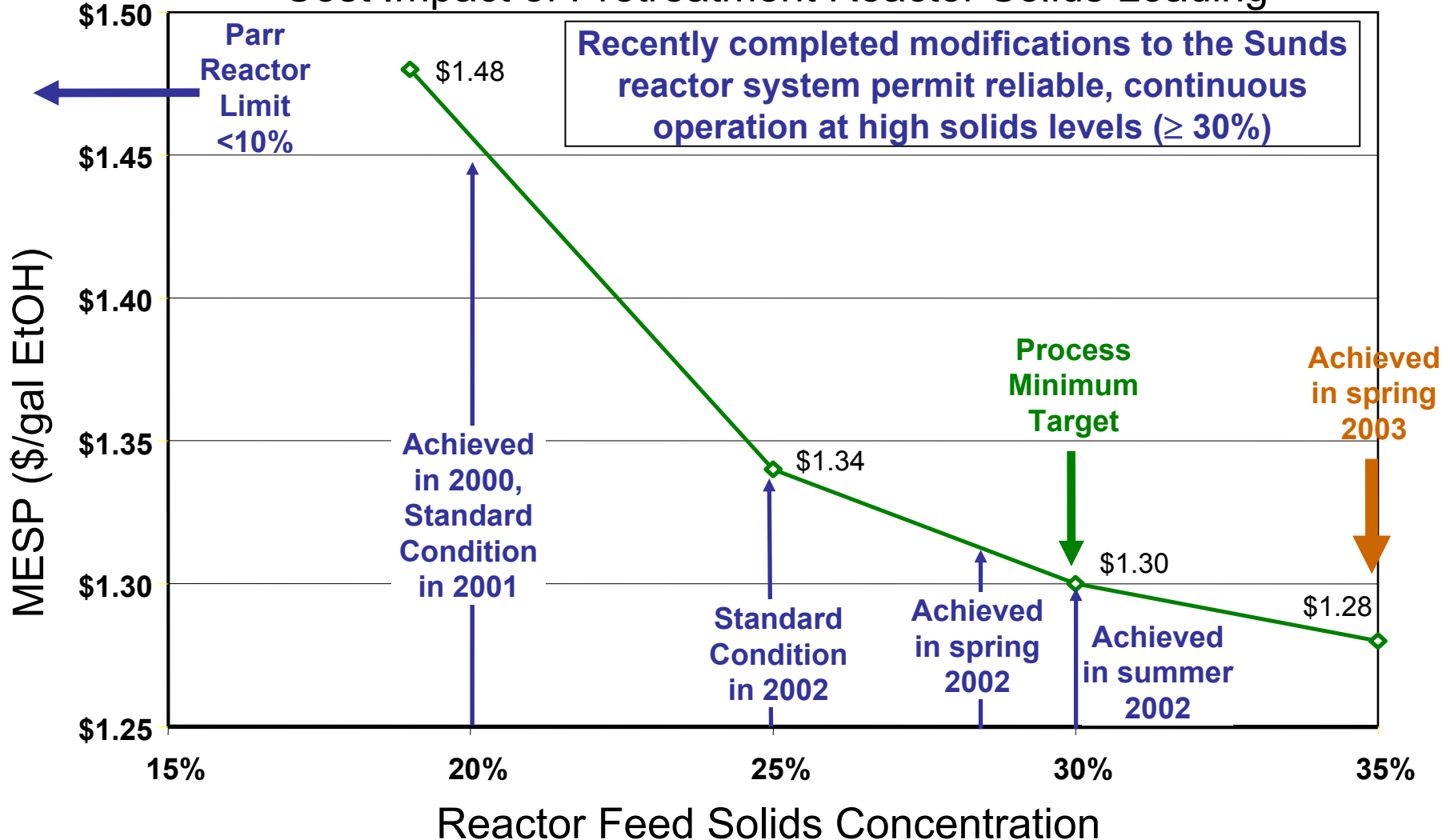
Excellent progress being made by industry through DOE subcontracts





# Reducing Performance Risk: Demonstrating High-solids Processing

## Cost Impact of Pretreatment Reactor Solids Loading



# Reducing Deployment Risk: Showing Base-line Engineering Feasibility

- Dilute-acid pretreatment showstoppers overcome
  - Some performance levels remain below targets

## Minimum Pretreatment Performance Targets

<i>Parameter</i>	<i>Achieved</i>	<i>Target</i>
<b>Catalyst Type</b>	<b>Dilute Acid</b>	<b>Dilute Acid</b>
<b>Reactor Solids Conc.</b>	<b>30-35 %</b>	<b>30 %</b>
<b>Residence Time</b>	<b>0.75-1.25 min</b>	<b>2 min</b>
<b>Acid Concentration</b>	<b>1.5 %</b>	<b>1.1 %</b>
<b>Temperature</b>	<b>190 °C</b>	<b>190 °C</b>
<b>Xylose Yield</b>	<b>80%</b>	<b>85%</b>
<b>Reactor Metallurgy</b>	<b>-----</b>	<b>Incoloy 825-clad</b>

- Process samples produced for evaluation
  - Pretreated solids and hemicellulose hydrolyzate liquors
  - Lignin-rich process residues

# Dilute Sulfuric Acid Pretreatment of Corn Stover



Stover  
harvested from  
northeastern  
Colorado in  
the fall of 2002

# Dilute Sulfuric Acid Pretreatment of Corn Stover



Pretreatment  
at solids  
loadings from  
25% to 35%

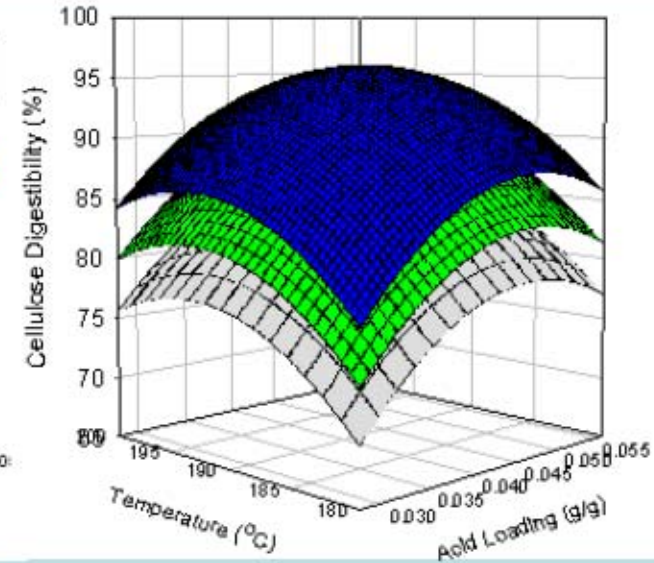
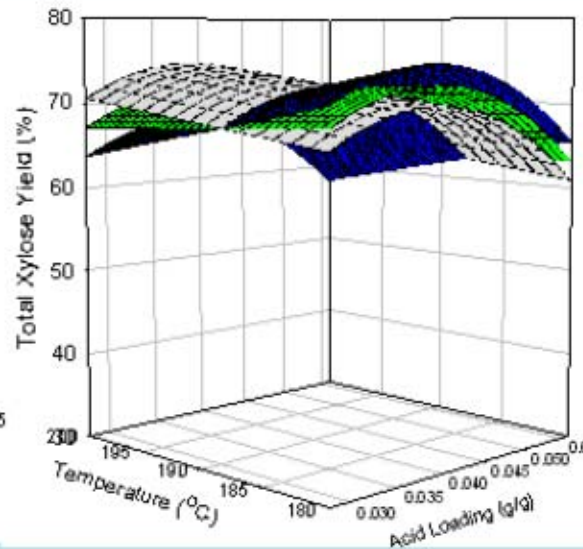
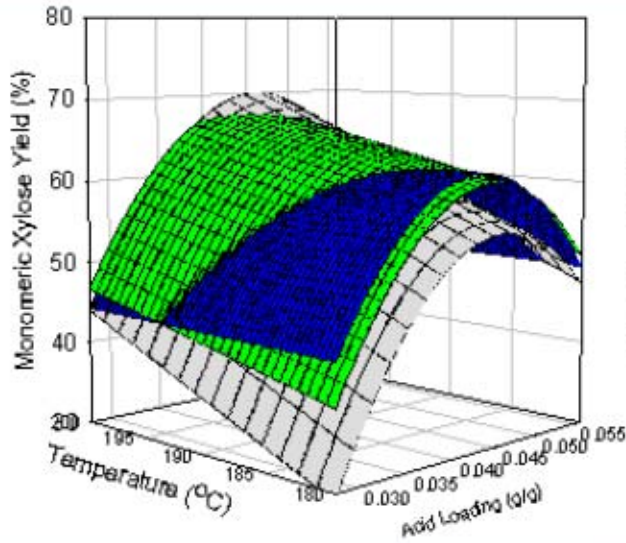


# High Solids Pretreatment Performance

Pilot-scale dilute acid pretreatment of corn stover at 25%-35% w/w solids

Xylan Solubilization as a Measure of Hemicellulose Extraction/Hydrolysis Efficiency

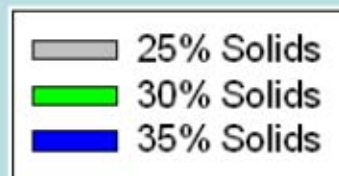
Enzymatic Digestibility of Pretreated Solids



Monomeric Xylose Yield

Total Xylose Yield

Cellulose Digestibility



# Examples of Corn Stover Dilute-acid Hemicellulose Hydrolyzate Liquors

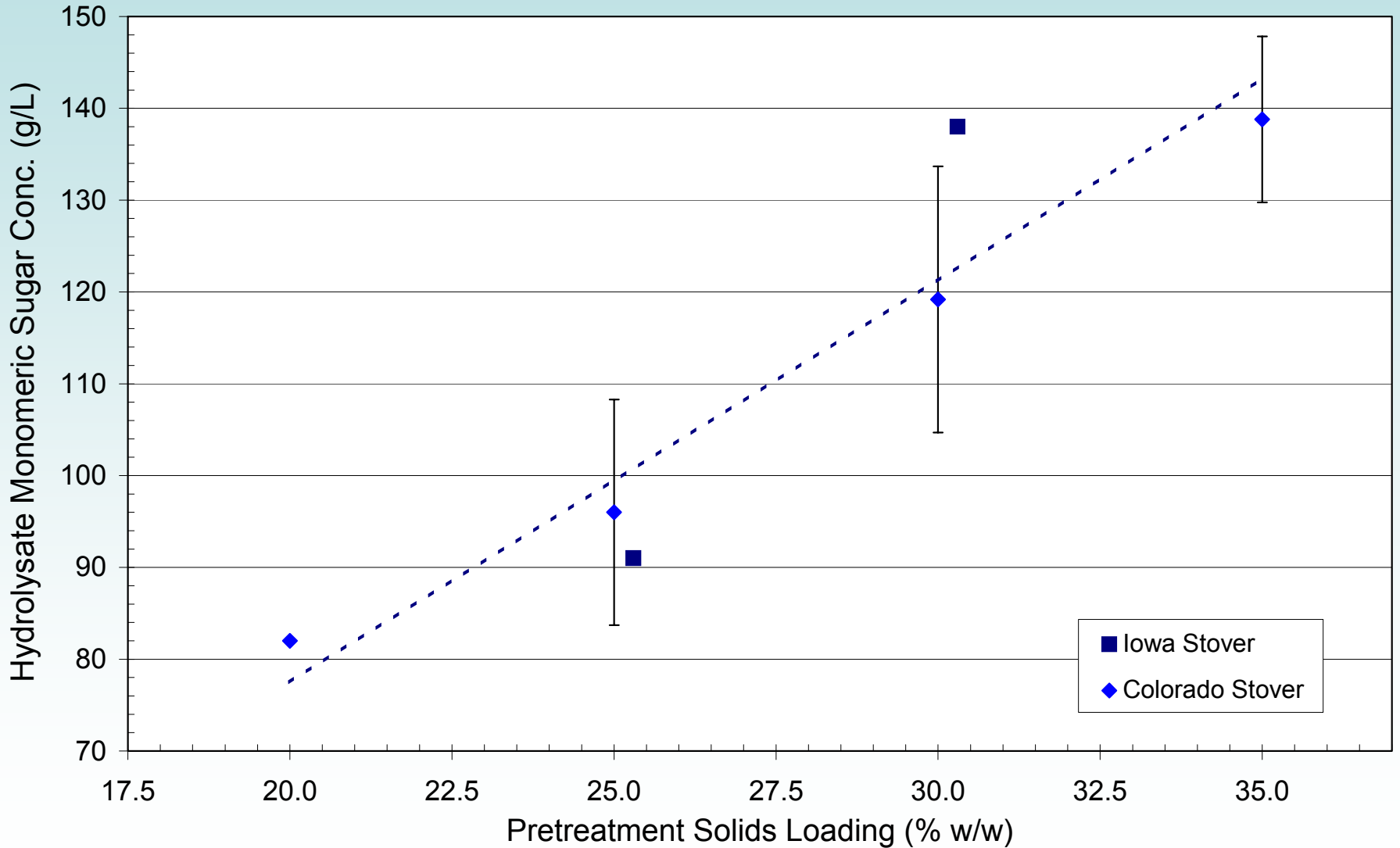
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Component	Concentration (g/L) (20% solids)	Concentration (g/L) (30% solids)
Glucose	9.24	17.7
<b>Xylose</b>	<b>59.7</b>	<b>93.6</b>
Arabinose	8.8	13.5
Galactose	4.6	7.1
Mannose	2.7	4.1
Oligomers	10.9	9.4
Furfural	1.5	2.4
Hydroxymethyl Furfural	0.3	0.5
Acetic Acid	7.1	11.5

---

# Sugar Concentration = f(Solids Loading)

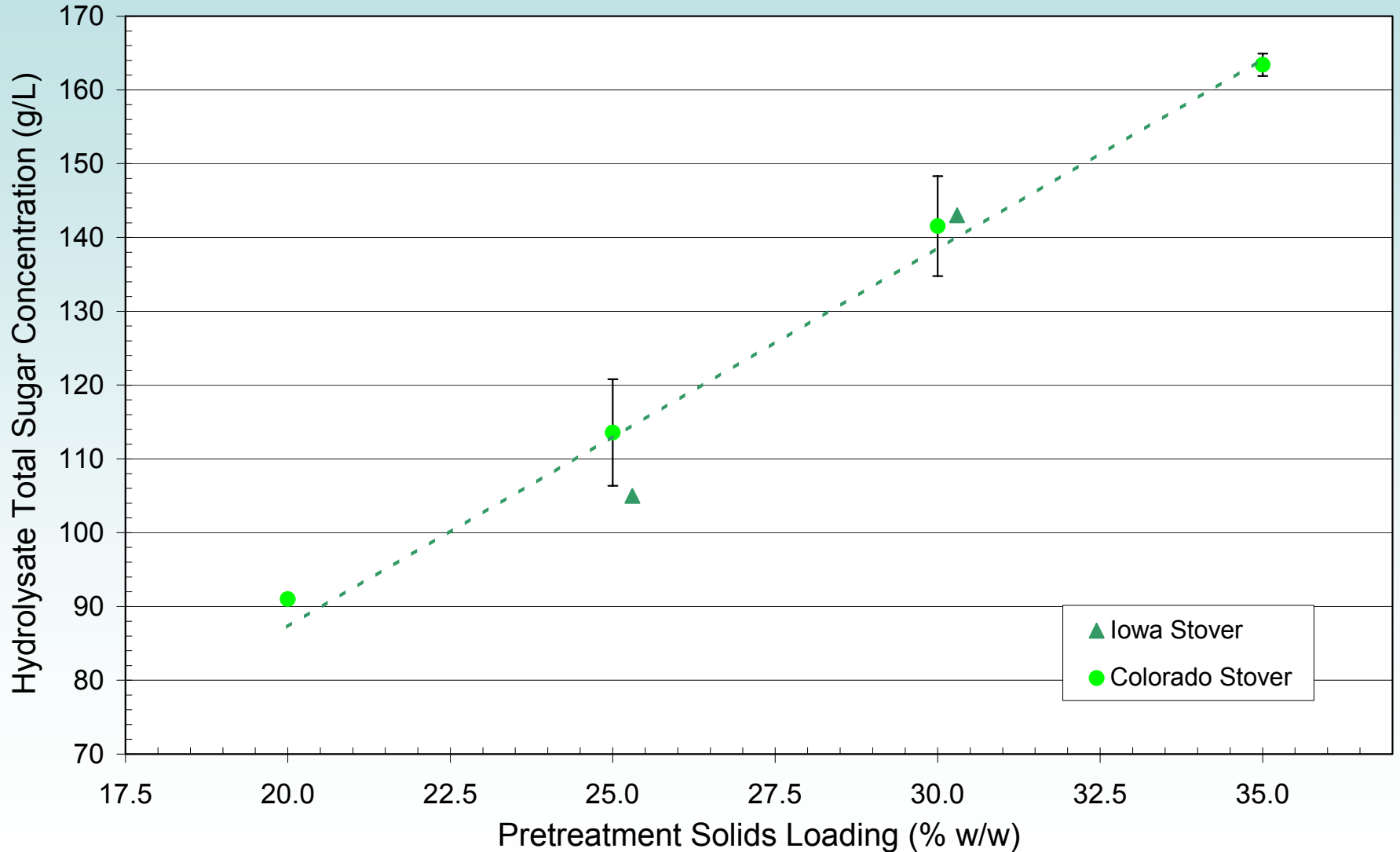
Ranges in Monomeric Sugar Concentrations





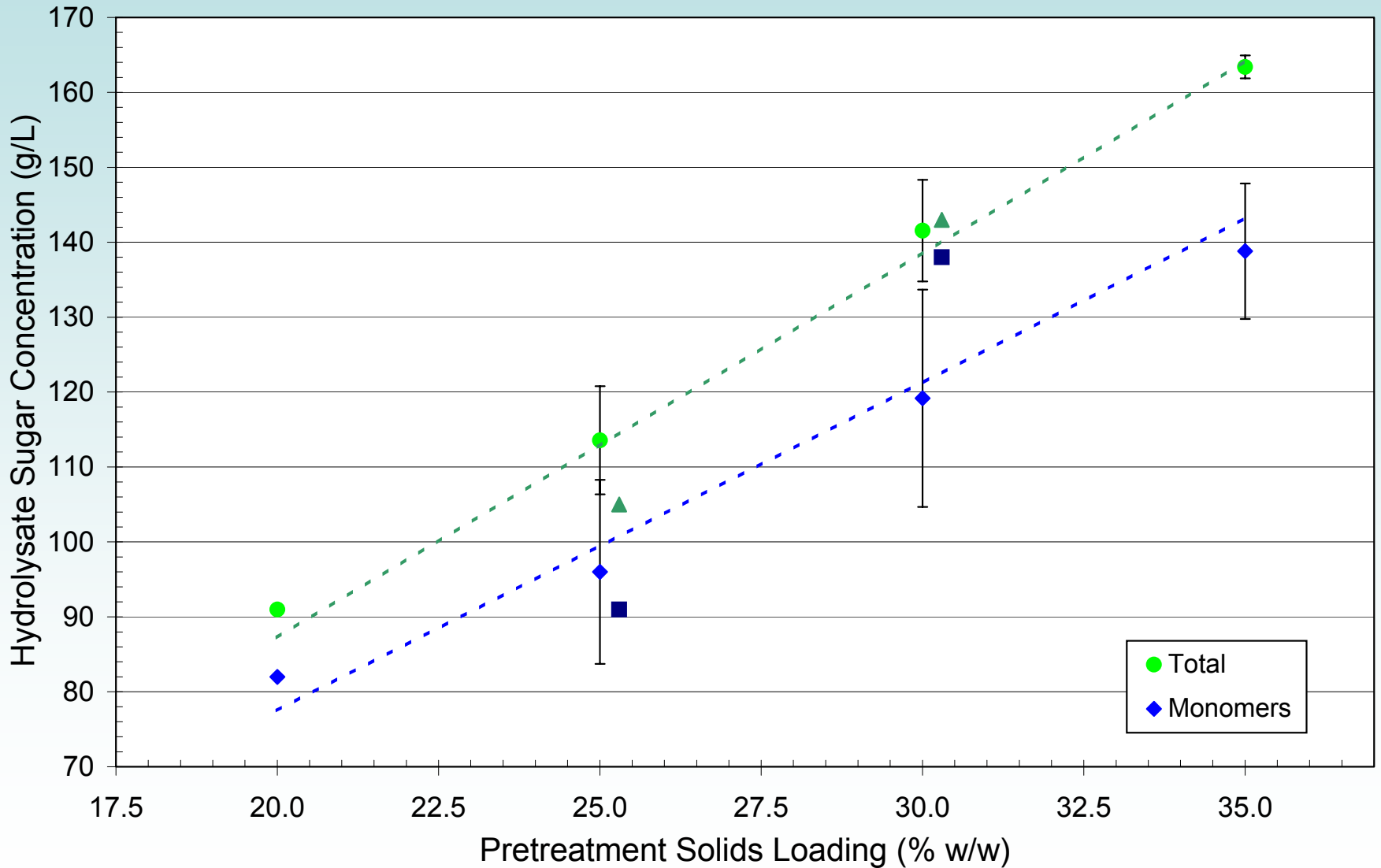
# Sugar Concentration = f(Solids Loading)

Ranges in Total Sugar Concentrations



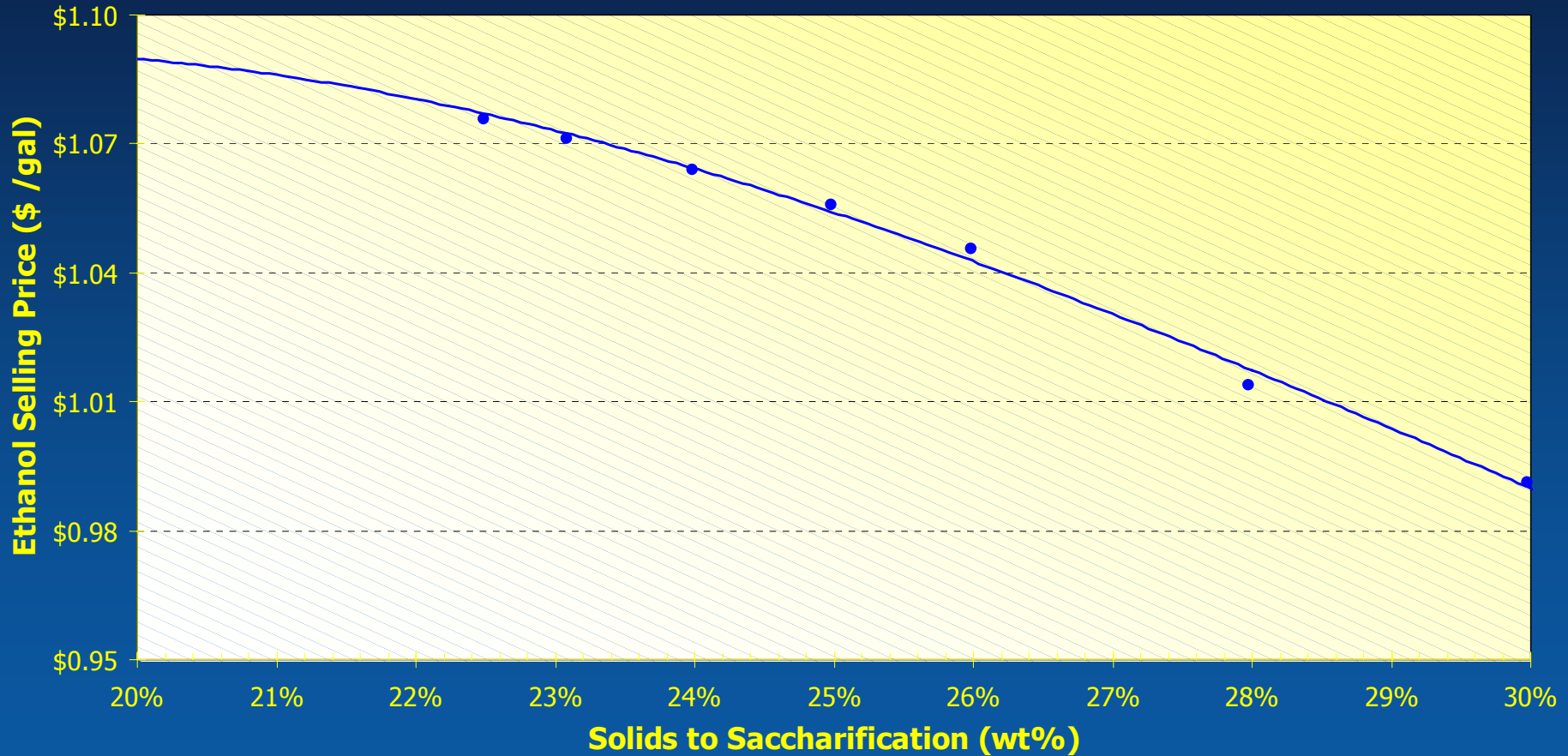
# Sugar Concentration = f(Solids Loading)

Comparison of Monomeric versus Total Sugar Concentrations



# Impact of Saccharification Solids Loading

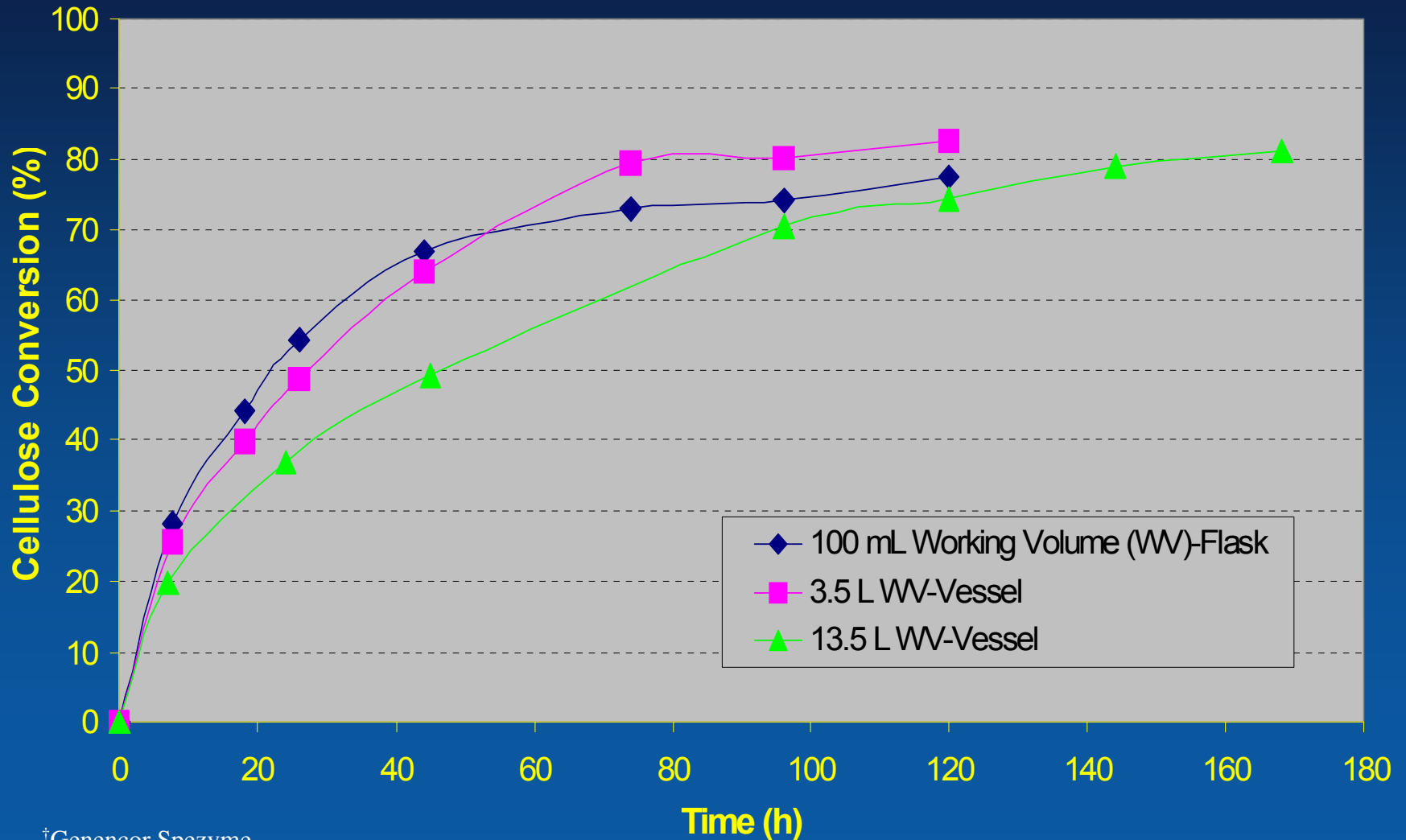
Results of Preliminary Techno-Economic Modeling



# Cellulose Saccharification

## Assessing Potential Scale-up Issues

Pretreated corn stover, 10% solids loading, 20 mg cellulase<sup>†</sup> protein/g cellulose, 45°C



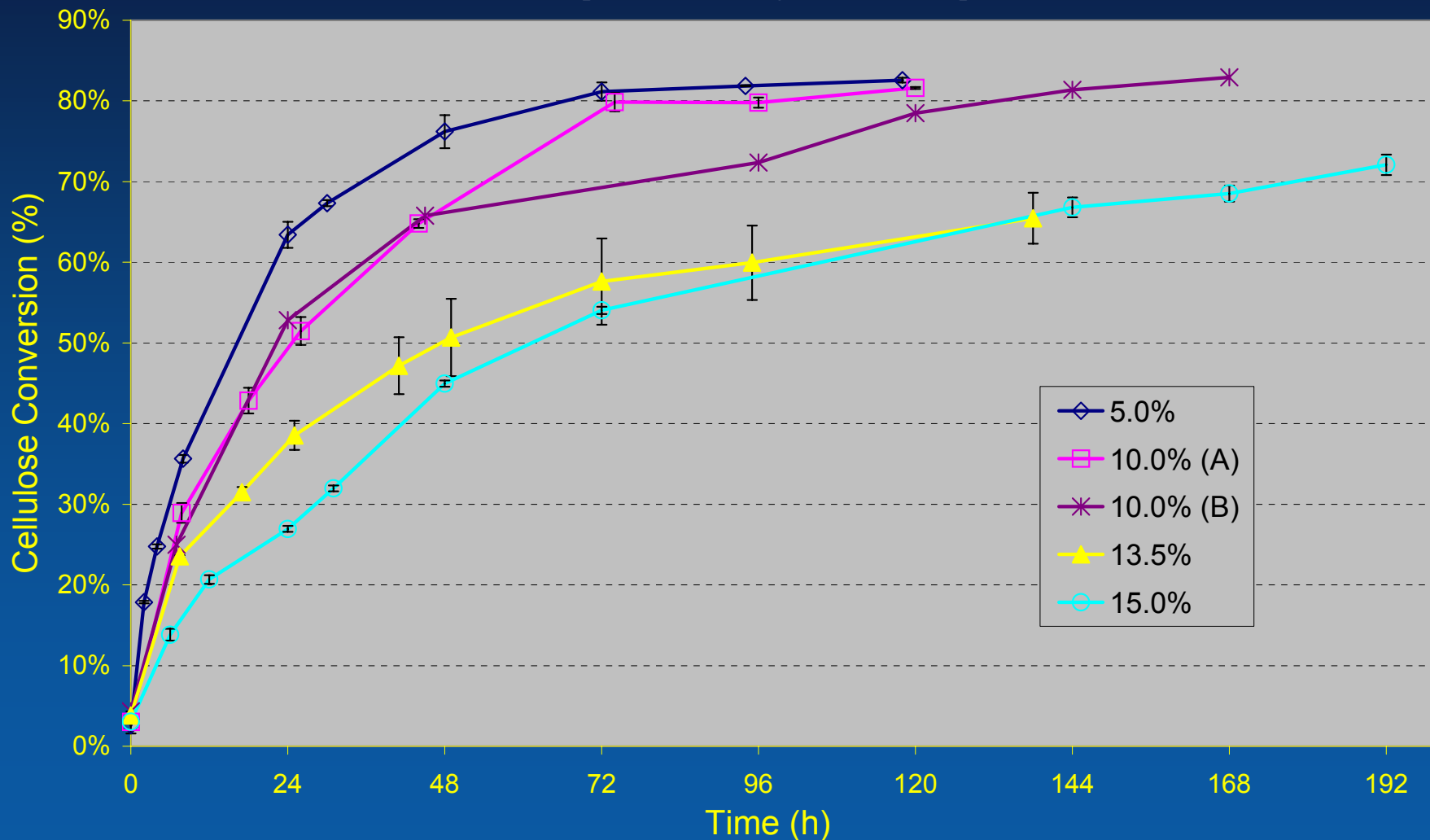
<sup>†</sup>Genencor Spezyme

# Cellulose Saccharification

## Impact of Solids Loading – Preliminary Results

Pretreated corn stover, 20 mg cellulase<sup>†</sup> protein/g cellulose, 45°C

3.5 L working vol, insulated 7-L Bioflo 3000 fermentors fitted with two oversized marine impellers and using modified temperature control



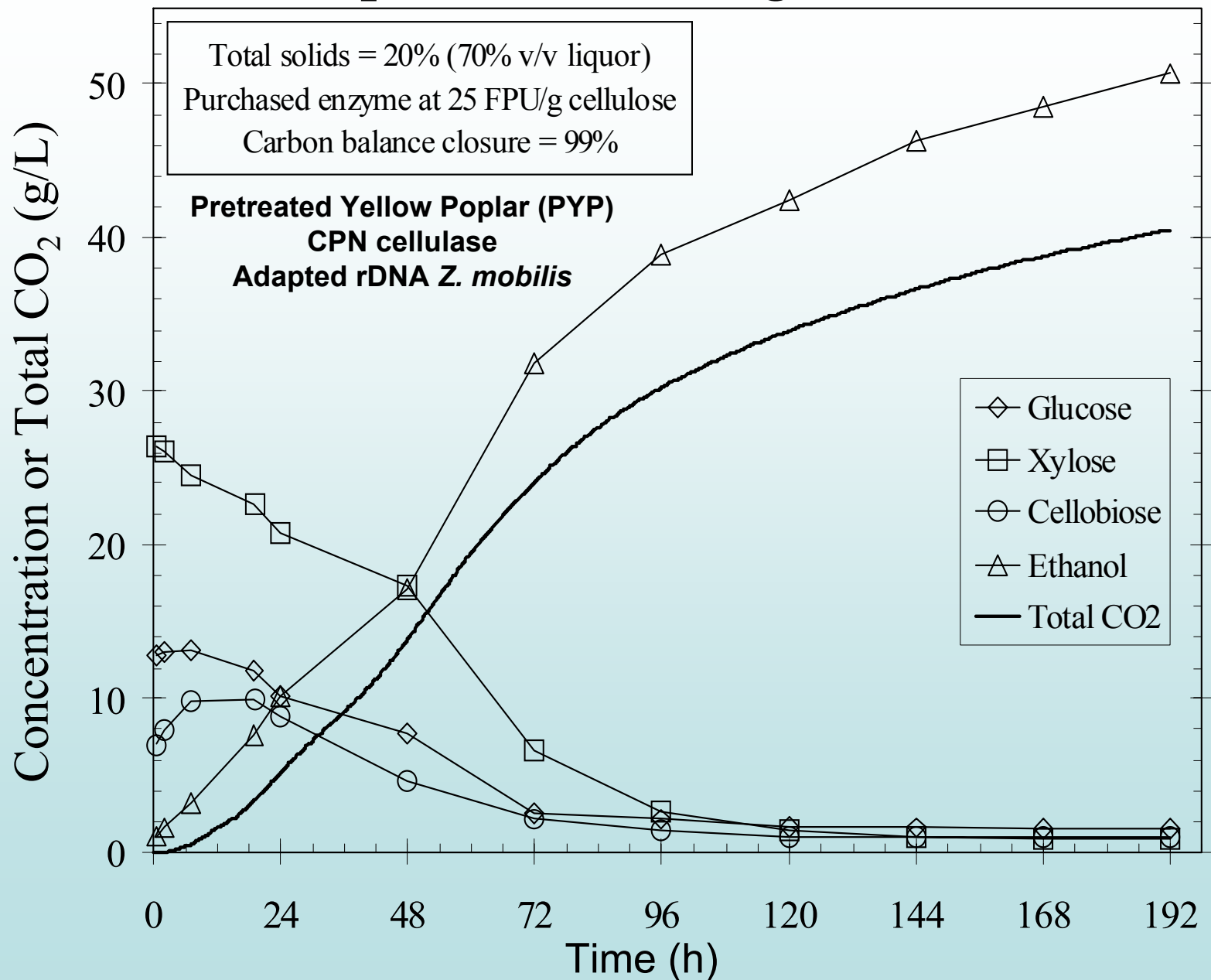
<sup>†</sup>Genencor Spezyme

# Combining Enzymatic Saccharification and Mixed Biomass Sugar Fermentation

- Complex process integration issue influenced by
  - Characteristics of substrate, enzyme(s), and microbe
    - Substrate: What ranges of sugars and toxins are present after pretreatment, what enzyme activities are required to complete saccharification, and how reactive/susceptible is the substrate?
    - Microbe: What sugars can be fermented, and what temperatures and inhibitors tolerated?
    - What Enzyme: How effectively are pretreated solids hydrolyzed, how thermostable are enzymes, and how resistant is the enzyme system to end product inhibition?
  - Many potential substrates, enzyme preparations, and fermentation strain combinations are possible

➤ ***Robust pentose fermentation remains the most critical bottleneck!***

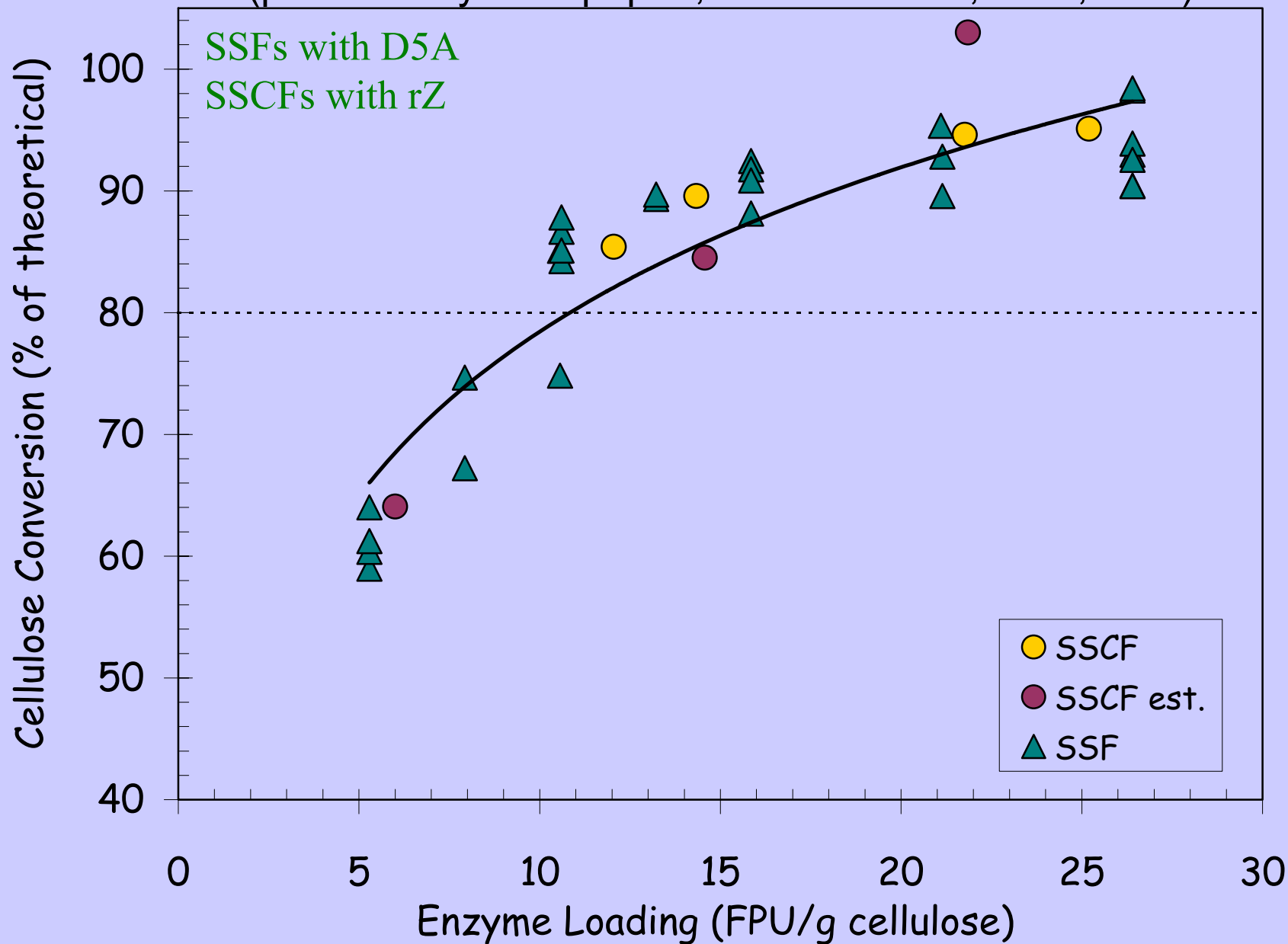
# Mini-pilot Scale Integrated SSCF





# Shakeflask SSF as a Predictor of Integrated SSCF

(pretreated yellow poplar, ~6% cellulose, CPN, 32°C)



# Pilot vs. Bench SSCF

## Amoco CRADA Phase 3 Bench Scale Report 1.8\*

10 FPU CPN (+ 2 IU GA)/g cellulose, LNH-ST, APR Corn Fiber, 20% total solids, 30°C, pH 5

*Cofefermentation of Glucose and Xylose*

253

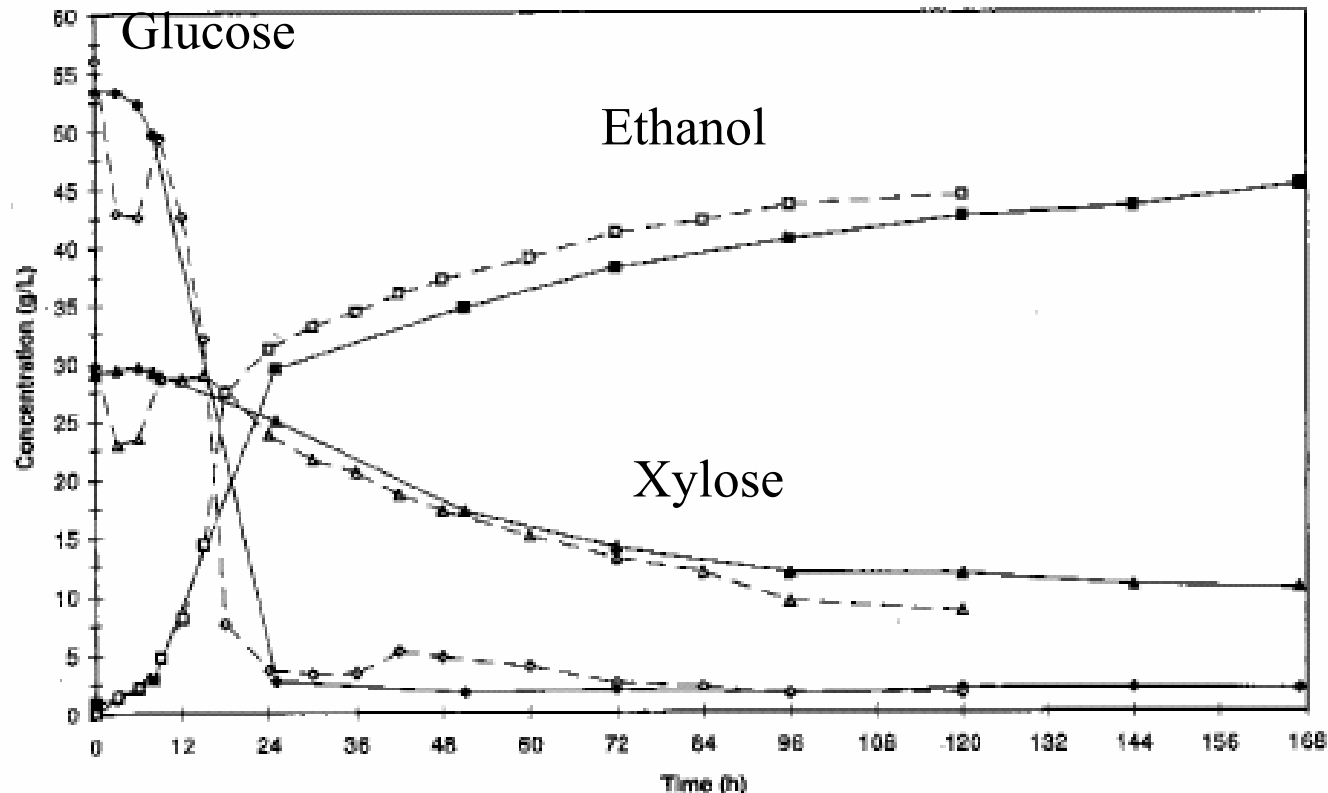


Fig. 4. Comparative study of the bench scale (closed symbols and continuous lines) and pilot scale (open symbols and dashed lines) performance of LNH-ST during the SSCF of pretreated corn biomass (batch 2). The symbols represent the concentrations of glucose (◆, ◇), xylose (▲, △), and ethanol (■, □).

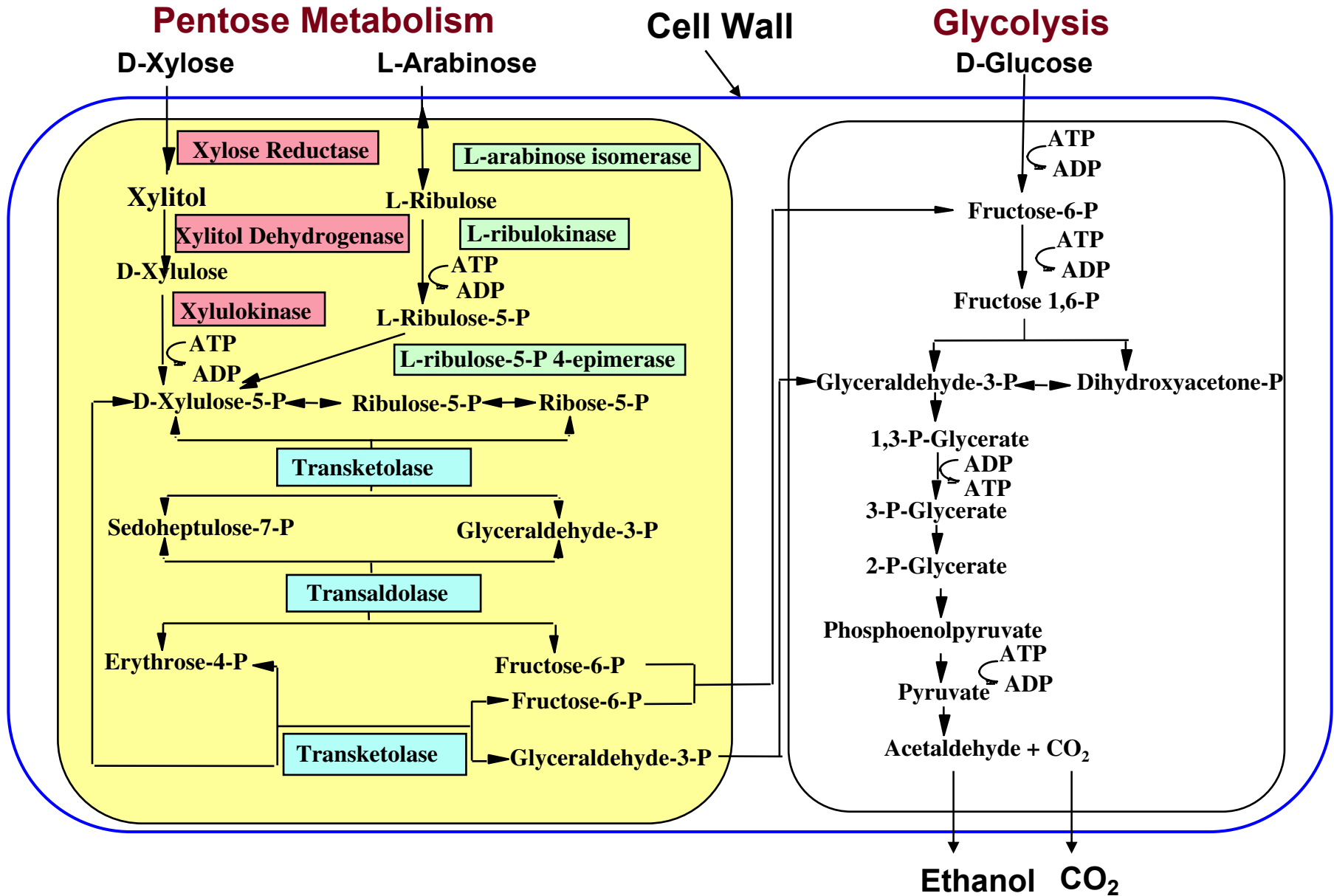
\* Figure from: Toon et al., 1997, *Appl. Biochem. Biotechnol.* 63-65: 243-255.

# Biomass Sugar Fermentation Needs

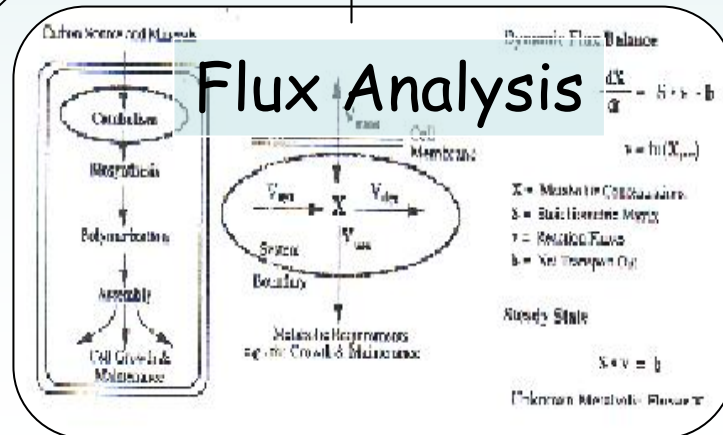
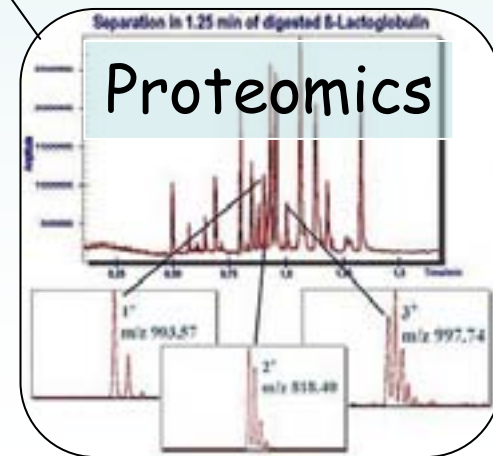
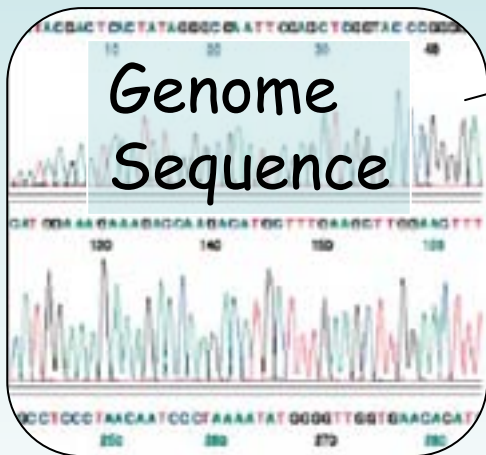
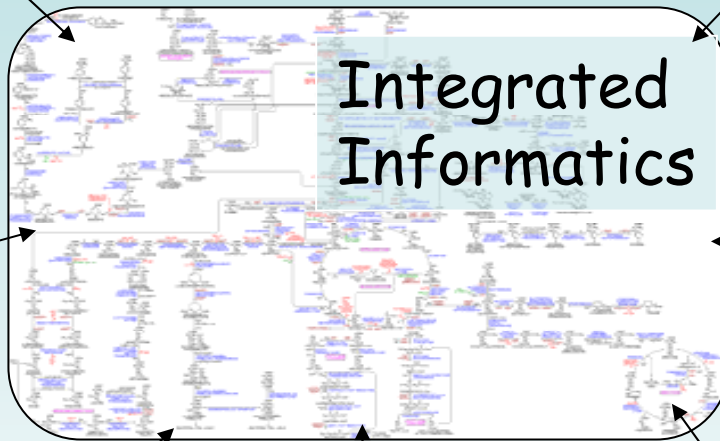
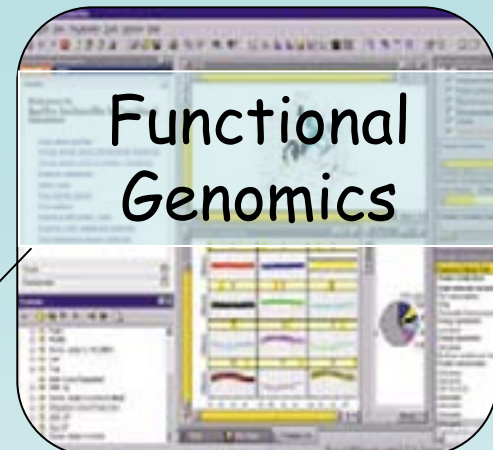
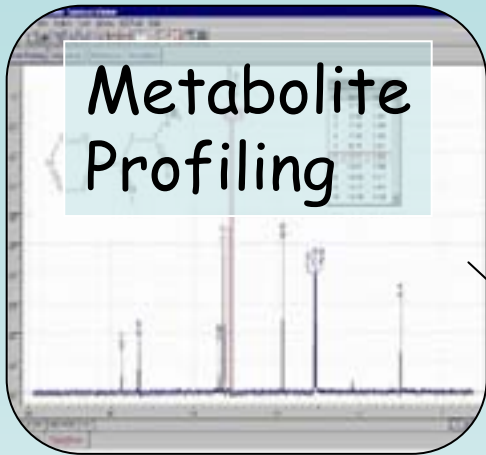
- High Yield Requires Fermenting all Biomass Sugars
  - Glucose, Xylose, Arabinose, Mannose, Galactose
- Resistant to toxic materials/chemicals in hydrolysates
  - Acids, phenolics, salts, sugar oligomers, ...
- Robust, able to out-compete contaminating microbes
  - Temperature, pH
  - High fermentation rates
- Minimum metabolic byproducts

➤ *Metabolic engineering holds the key!*

# Achieving Robust Pentose Fermentation



# Metabolic Eng "Omics" Tool Kit



# Outline

- Biomass Basics
- Overview of Conversion Options
- Details of Enzyme-based Technology
- Biorefining Now and in the Future

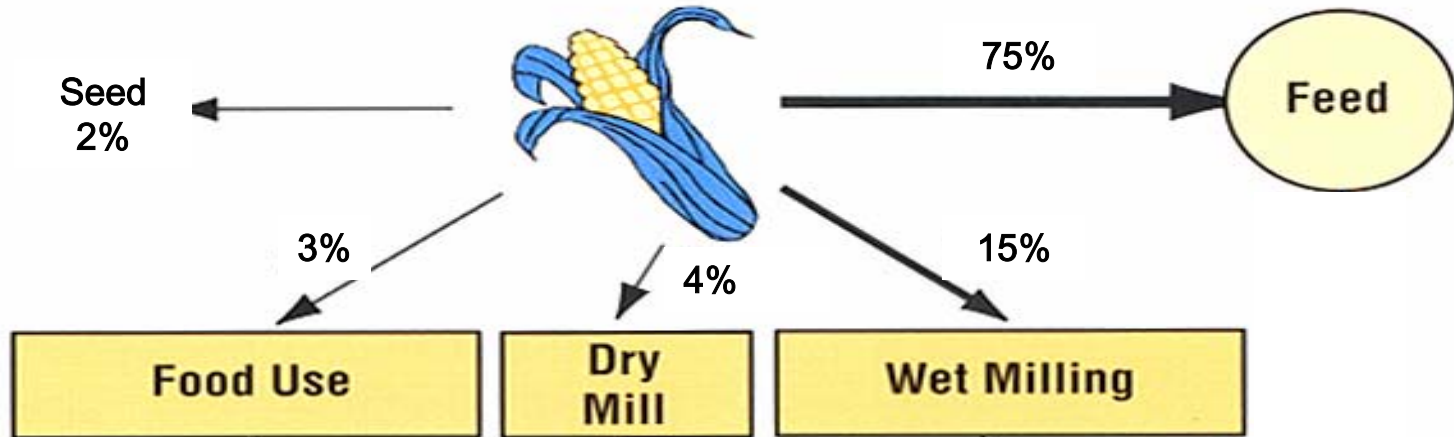
# Today's Sugar Platform Biorefineries

## Examples

- Domestic
  - Corn mills (wet and dry)
  - Paper mills (virgin and recycle)
- International
  - Sugar Mills (cane and beet)
    - Especially Brazil's sugar-ethanol mills



# Today's Corn Grain Biorefineries



## Directly Consumed

- Sweet corn
- Popcorn

## Processed to

- Flours
- Grits
- Bran
- Tortillas
- Chips

## Processed to

- Ethanol
- Feed

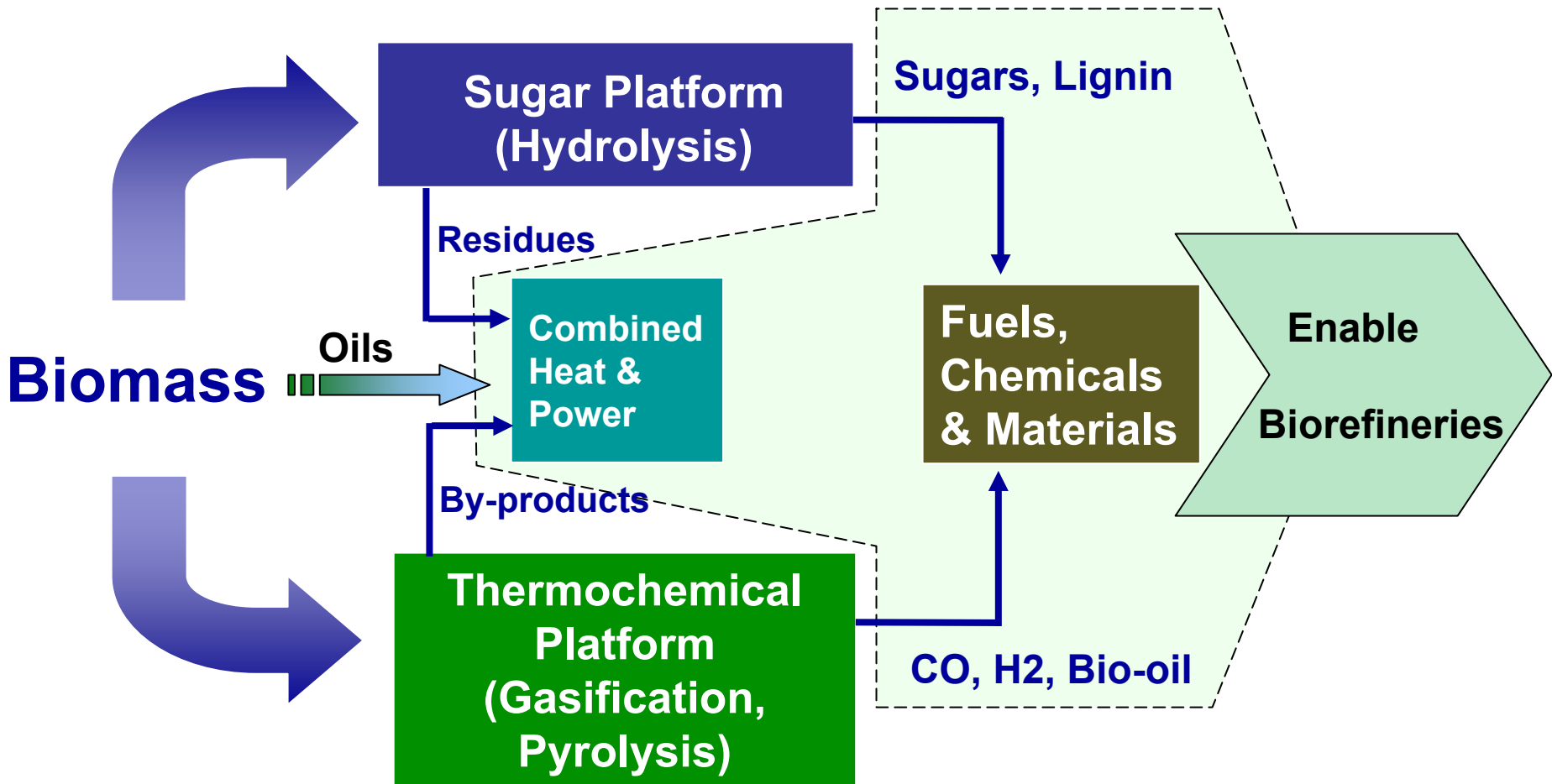
## Processed to

- Oil
  - Gluten
  - Foods
  - Starch
  - Industrial Products
- Starch to Sugar Products**
- Syrups
  - Ethanol
  - Industrial Fermentation Products (many)

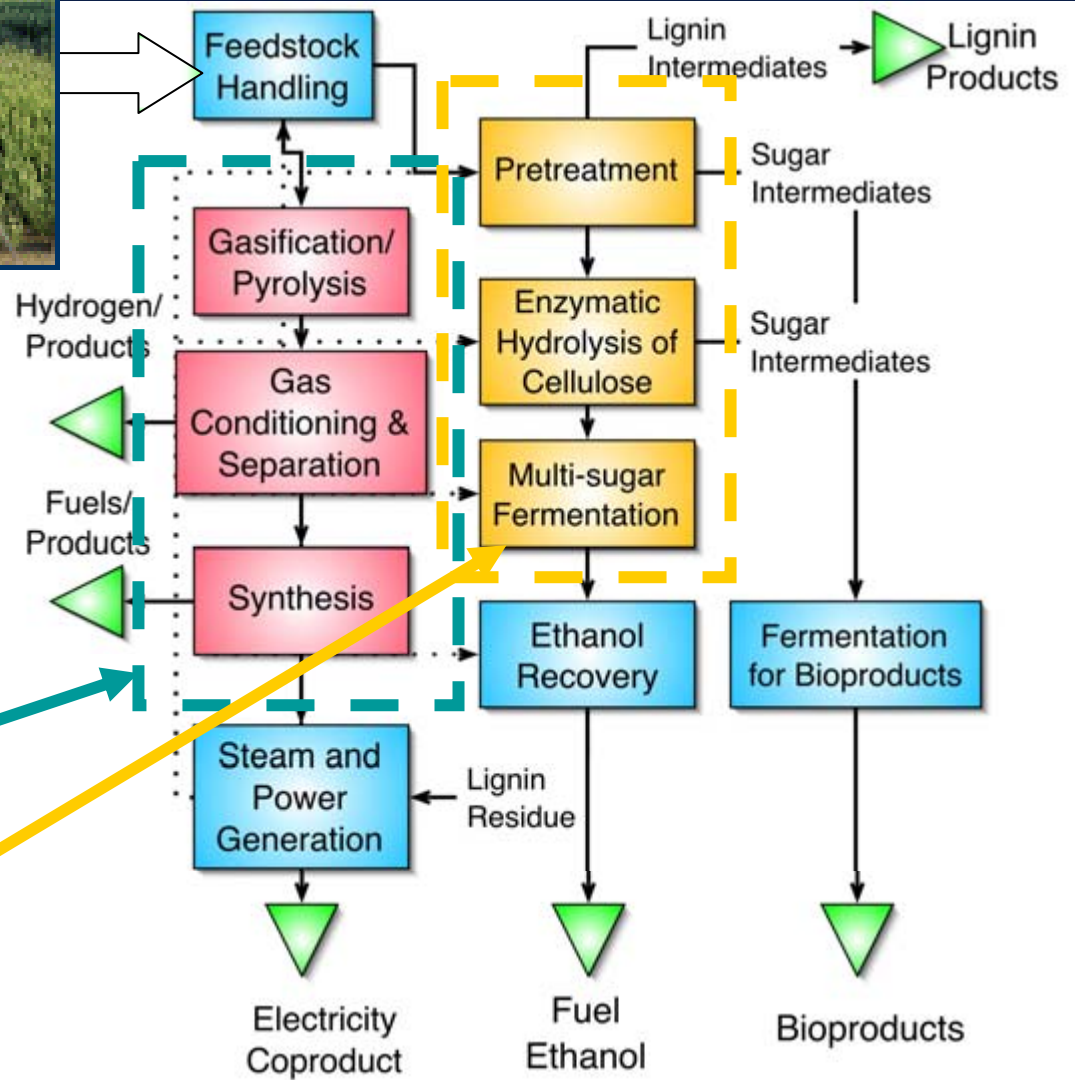
## Emerging products

- polymers & chemicals

# Biomass Conversion Technology “Platforms”



# Cellulosic Biorefinery Vision



An integrated biorefinery will make use of:

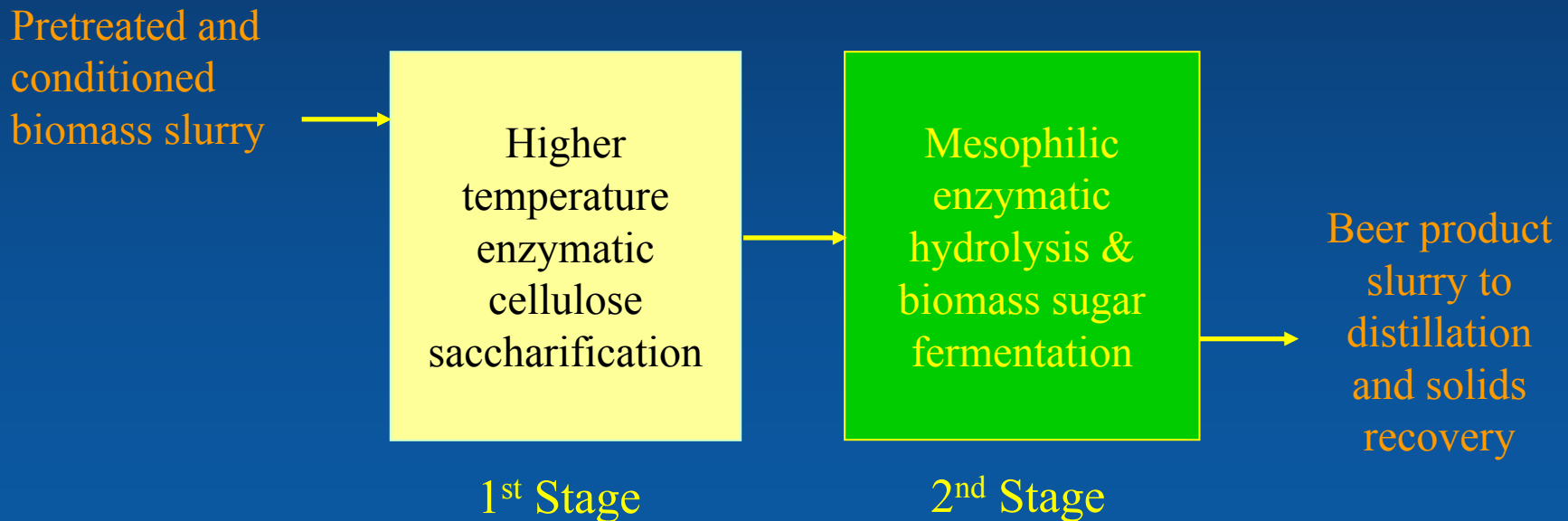
- Thermochemical conversion technology
- Biochemical conversion technology
- Existing technology
  - Available today

# Challenges to Deploying Future Lignocellulosic Biorefineries

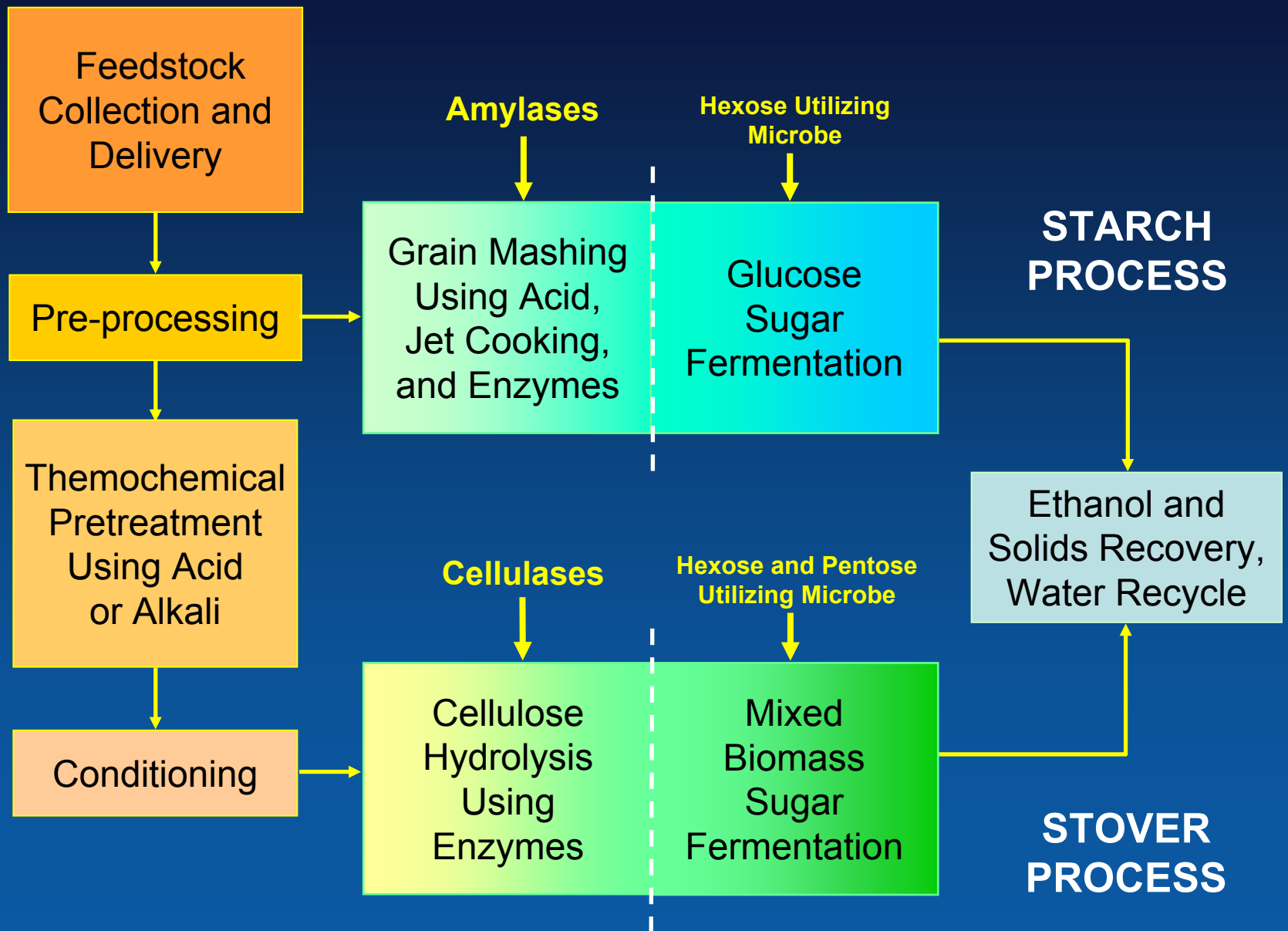
- Demonstrating economic competitiveness in the marketplace
  - Must be able to show compelling economics with acceptable risk **relative to the competition**, i.e., provide a value proposition that can compete with the current industrial sugar platform
- *Example: Compare process economics of an existing corn dry mill versus a hypothetical enzymatic process using corn stover. Both producing ethanol and one coproduct.*

# Probable Commercial Configuration

- Anticipate exploiting cost effective cellulase preparations in a two stage saccharification/fermentation process
  - 1<sup>st</sup> stage: Operate at enzymes'  $T_{opt}$  to exploit thermostability and produce an intermediate sugar stream (consistent with “sugar platform” concept)
  - 2<sup>nd</sup> stage: Inoculate, run in SSF/SSCF mode to achieve high cellulose conversion yield

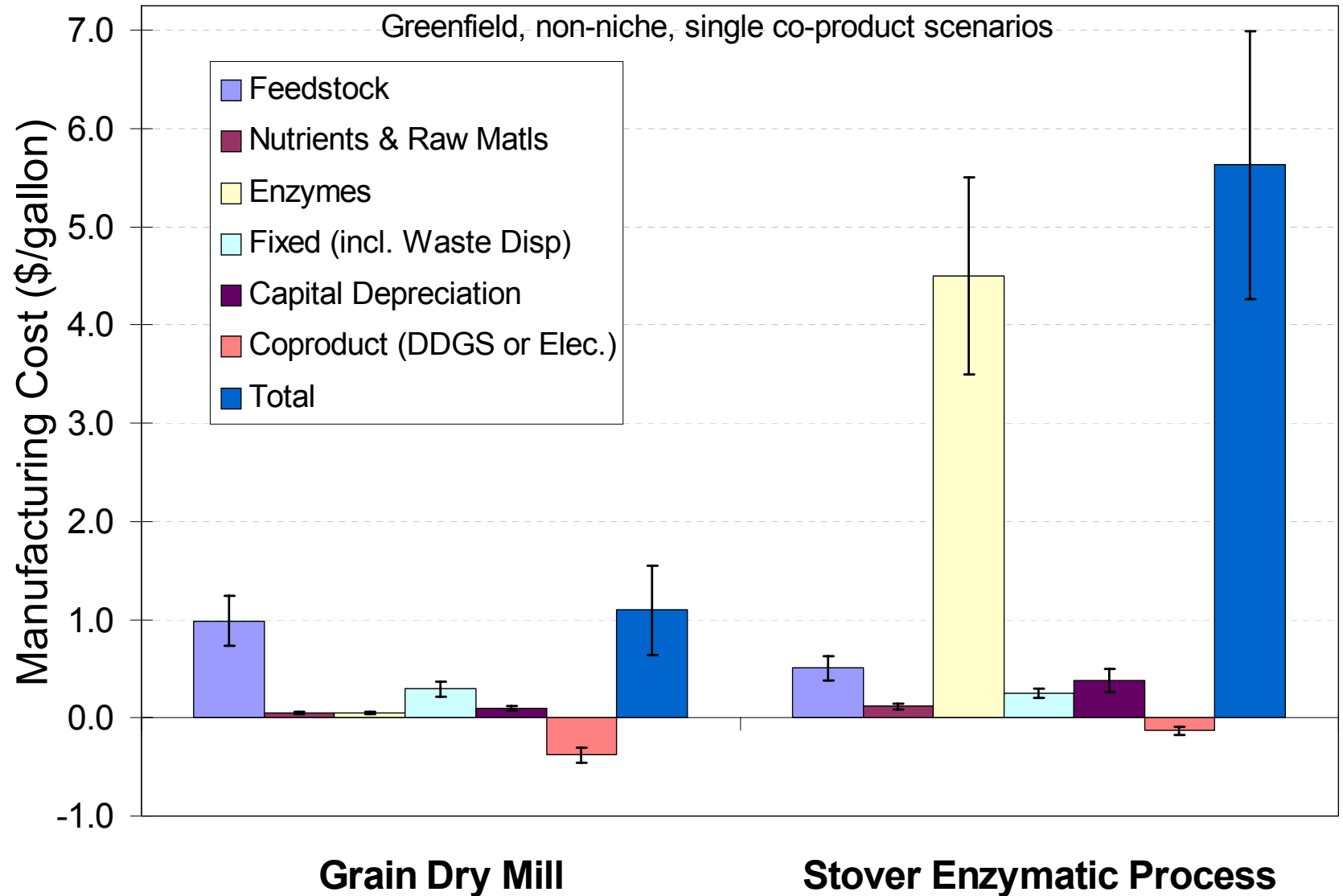


# Conversion Process Steps



# Comparative Economics

Where We Were: Estimated Process Economics as of Late 1990s



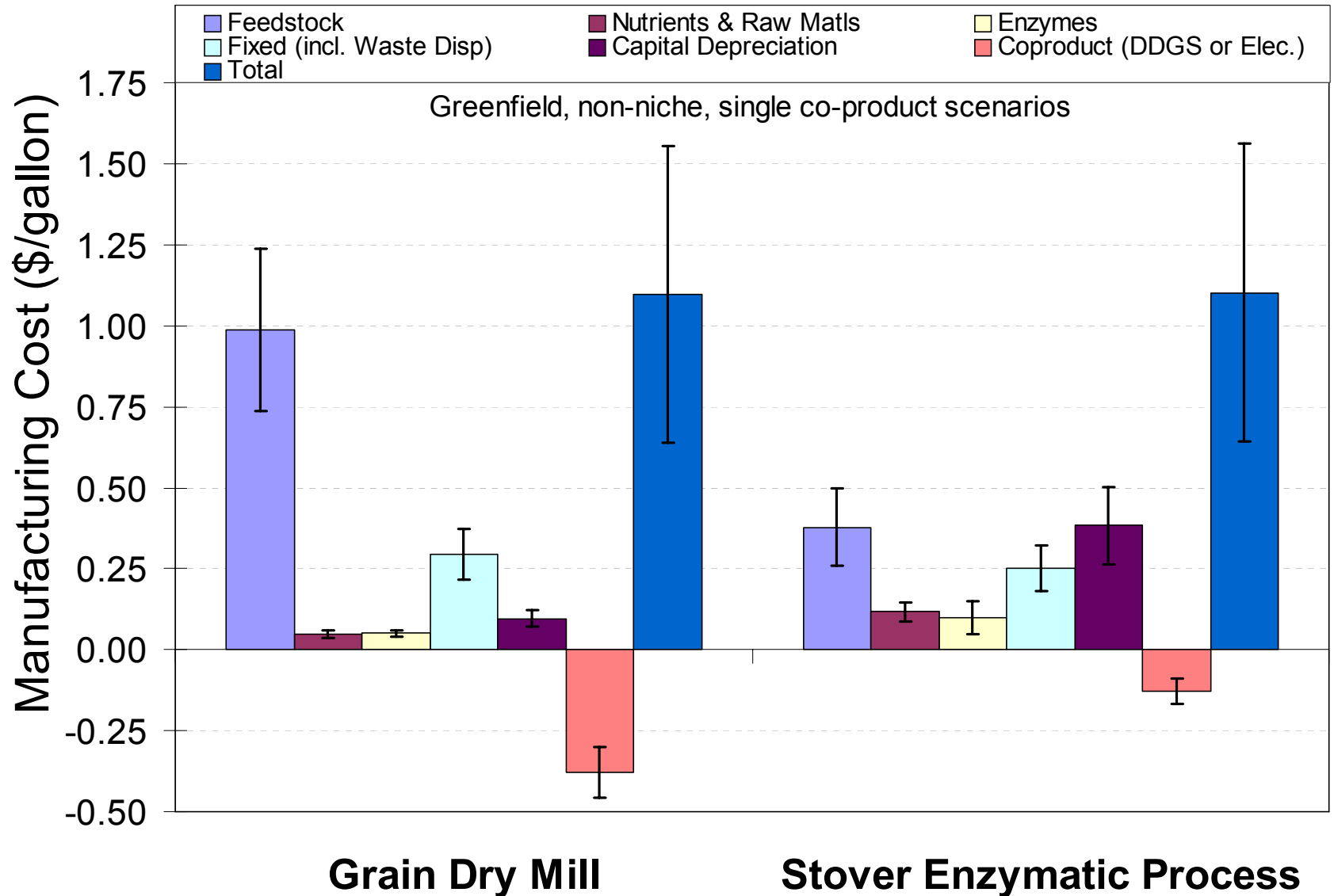
# Key Findings

- Costs driven by
  - Feedstock (grain or stover)
  - Enzymes (stover)
  - Utilities prices (gas and electricity; grain)
  - Capital equipment (stover)
- *Observation of enzyme cost hurdle led USDOE to emphasize cellulase cost reduction RFP that ultimately led to contracts with Genencor and Novozymes.*
- *What will comparative economics look like when cost targets achieved?*



# Target Economics

## Future Goal



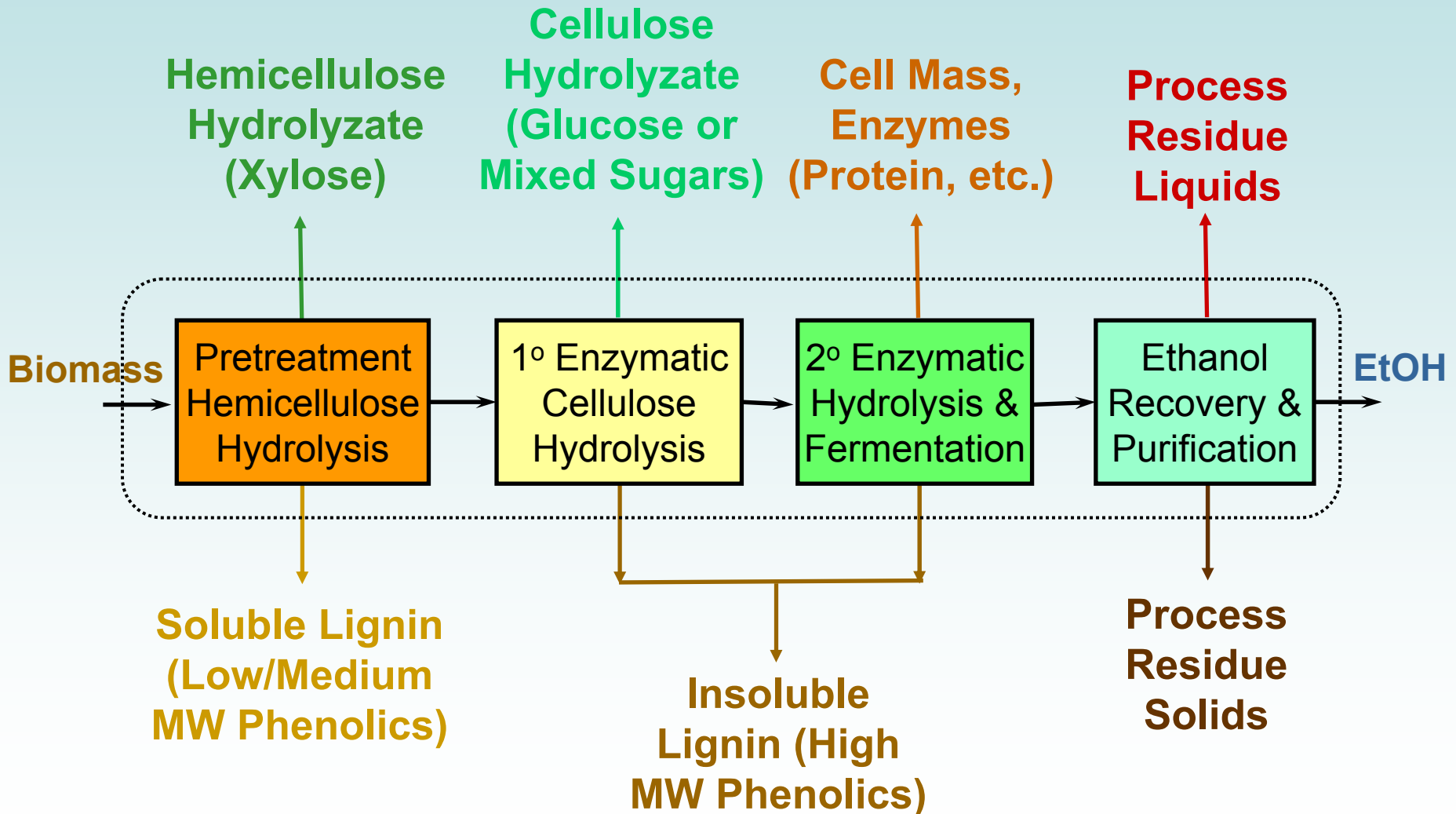
# Opportunities and Challenges

- Lower operating cost
  - Operating cost less enzymes potentially 20-40% lower processing stover
  - Diversifying feedstock options provide hedge against rising grain prices
- Higher capital cost
  - \$2.5-4.0/annual gal for stover vs. \$1.0-1.5 for grain
  - Co-location and co-products can reduce capital burden

# Current Situation

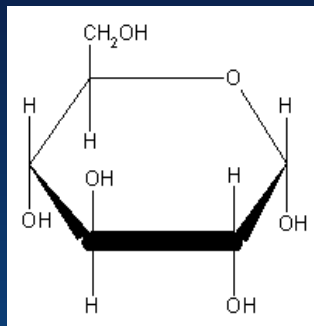
- Technology becoming market competitive
  - Cost of enzymes falling dramatically
  - Process chemistry gaps being elucidated
  - Capital cost decreasing through process intensification
- Deployment risk being reduced
  - Many commercial projects underway
  - Iogen operating demonstration plant in Ottawa, ON (Canada)
  - Engineering of hardier ethanologens progressing
- Societal and environmental benefits being proved
  - First “cradle to grave” Life Cycle Analysis completed

# Potential for Novel Coproducts from Enzymatic Sugar Platform Process

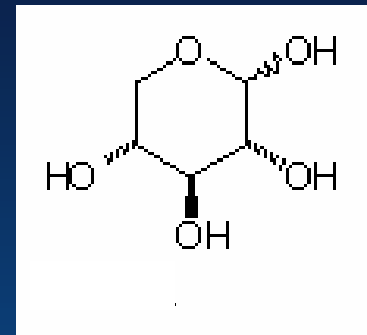
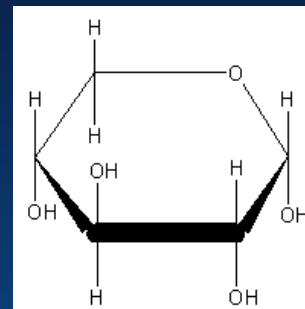
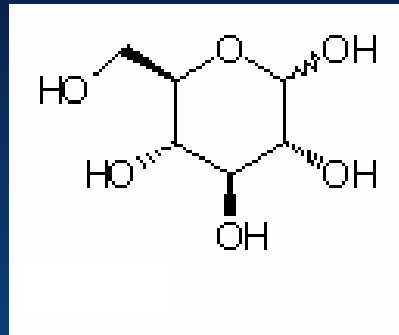


# Potential Opportunities for D-Xylose

(as an alternative to existing sugar products, esp. glucose)



**$\alpha$ -D-Glucose**

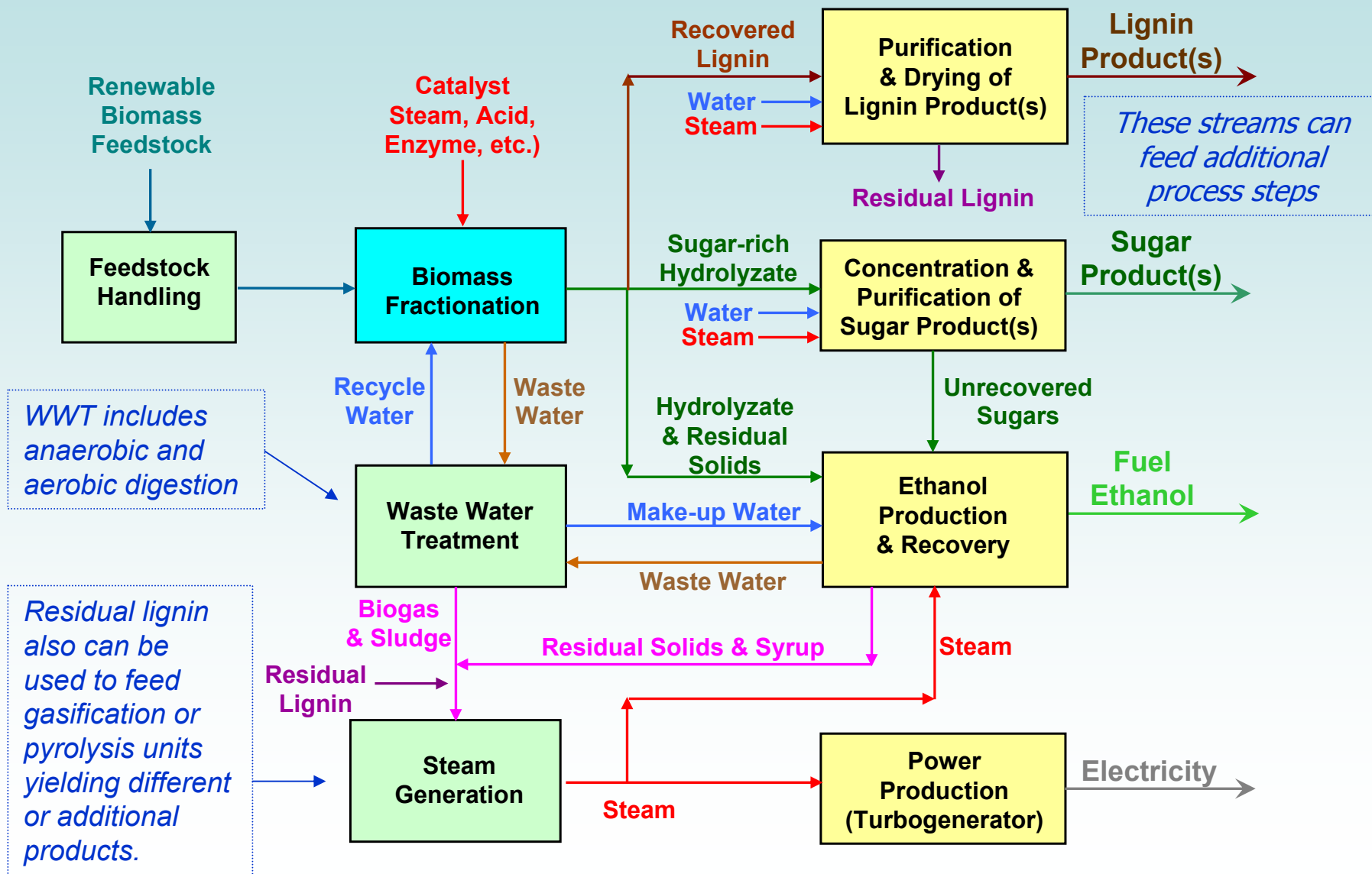


**$\alpha$ -D-Xylose**

- Chiral molecule for specialty products
  - Build off unique structure and properties of xylose, e.g. xylitol
  - Exploit chirality for new product synthesis
- Novel monomer for biomaterials and biopolymers
- Carbon source for fermentation processes
  - Avoid glucose catabolite repression
  - Reduce operational constraints, e.g.,  $\downarrow \mu_{\max}$ ,  $\downarrow \text{OUR}_{\max}$

# Multiproduct Lignocellulose Biorefinery

## Sugar (and Lignin) Platform Example



# Outlook

- Sustainability benefits must be validated
- Great progress being made....
  - Compelling operating costs within reach
  - Commercialization risks diminishing
- ...But more needed to achieve market competitiveness
  - Process(es) must be proved at scale
  - Feedstock supply systems must be developed/validated
- Breakthroughs will spur deployment
  - Robust ethanologens (>10% EtOH on pentoses)
  - Supportive legislation/policies

# Challenges Ahead – Conversion Tech.

Scientific Fundamentals	Engineering Fundamentals	Demonstration and Commercialization
<ul style="list-style-type: none"><li>• Biomass chemistry and physical properties</li><li>• Fractionation</li><li>• Catalysis<ul style="list-style-type: none"><li>• Chemical</li><li>• Biological (enzymes and microorganisms)</li></ul></li><li>• Genetic and protein engineering</li></ul>	<ul style="list-style-type: none"><li>• Process integration</li><li>• Material and energy balances</li><li>• Solids handling and feeding</li><li>• Reactor design</li><li>• Catalyst production</li><li>• Reaction kinetics</li><li>• Separation technology</li><li>• Materials of construction</li><li>• Control systems and automation</li></ul>	<ul style="list-style-type: none"><li>• Decrease financial risk (in the context of energy price fluctuations)</li><li>• Process knowledge at large scale</li><li>• Lower capital and operating costs</li><li>• Reduce environmental risk (minimize waste)</li><li>• <b><u>Integrate systems for fuels, chemicals, materials, and power for optimum product slate</u></b></li></ul>

Increasing costs and industry involvement





# Alternative Fuels User Facility (AFUF)

- Unique modern user facility *developed to support biomass and bioprocess R&D*
  - Completed in 1994
  - 10,000 ft<sup>2</sup> Process Demonstration Unit
  - 6,000 ft<sup>2</sup> supporting bench scale laboratories
- Mission:
  - Enable commercial development partners
  - Facilitate rapid identification of economically attractive biomass/bioprocessing opportunities
  - Develop, test and validate bioconversion processes at bench, minipilot and pilot scales

10,000 ft<sup>2</sup> Integrated  
Process Development  
Unit (PDU)

6,000 ft<sup>2</sup> bench scale  
process development  
& support laboratories



# Alternative Fuels User Facility (AFUF) Process Development Unit

A fully integrated biomass to ethanol plant

- Processes one ton biomass per day
- Extensive pre-treatment equipment options
- Batch & continuous fermentation



# Testing Capabilities at the AFUF

- Integrated Process Development Unit (PDU)
  - Designed to process one (1) ton dry biomass per day
    - *This is the smallest scale at which continuous high solids pretreatment and liquor conditioning can be performed*
  - Major components include:
    - Sunds Hydrolyzer vertical pretreatment reactor
    - AST continuous column system for liquor conditioning
    - Four (4) 9000 L fermentors
    - Supporting equipment
      - Feedstock handling
      - Seed production
      - Distillation (ethanol stripping)
      - Various S/L separations devices
      - Etc.

# AFUF Testing Capabilities, cont'd

- Minipilot systems for biomass pretreatment and integrated bioprocess testing
  - *smallest scale for performing batch high solids pretreatment and continuous high solids bioprocessing*
  - Major components include several smaller pretreatment systems (3-4 L scales) and a variety of highly configurable bioprocessing systems (10-100 L scales)
- Extensive small scale bench systems for batch screening of prospective conversion processes
- Together, these capabilities enable high quality validation of batch, fed-batch and continuous bioprocesses prior to scaling up to more costly pilot scale
  - Assess performance of continuous processes at high solids (biomass) concentrations (>20% total solids, >15% insoluble solids)
  - Produce accurate performance data supported by reliable carbon mass balance closures (100% ±5%)



# Microbial Fermentation Examples

- Microorganisms:
  - Bacteria, yeast and fungi
    - *Zymomonas mobilis*, *Escherichia coli*
    - *Saccharomyces cerevisiae*, *Pichia stipitis*
    - *Trichoderma reesei*, *Aspergillus niger*
- Processes:
  - EtOH fermentation ( $\pm$  enzymatic hydrolysis)
  - Protein (e.g., hydrolase production)
  - Valued-added products from xylose
- Experimental systems:
  - Test tube through 9000-L fermentors
  - With or without solids (slurries)
  - Batch, fed-batch, or continuous
  - Anaerobic, microaerophilic, or aerobic



# Outline

- Biomass Basics
- Overview of Conversion Options
- Details of Enzyme-based Technology
- Biorefining Now and in the Future

➤ *Wrap Up*

# Additional Information



- EERE Biomass Program

- <http://www.eere.energy.gov/biomass/>

- ✓ Multi-year Technical Plan (MYTP)
    - ✓ Biomass feedstocks, sugars platform, and products R&D
    - ✓ Process engineering and life cycle analysis (LCA)
    - ✓ Capabilities, facilities and expertise

- NREL Biomass Research

- <http://www.nrel.gov/biomass/>

- ✓ Capabilities, staff, projects
    - ✓ Energy analysis and LCA tools
    - ✓ Publications database

- Joint USDOE-USDA Biomass R&D Initiative

- [www.bioproducts-bioenergy.gov](http://www.bioproducts-bioenergy.gov)

- ✓ Status/archives detailing initiative strategies and recent high-level progress, including RFPs issued and funds/projects awarded
    - ✓ Biomass “Fact Sheets” for each state in the US (see publications)



# Final Thought...

“...fossil fuels are a one-time gift that lifted us up from subsistence agriculture and eventually should lead us to a future based on renewable resources”

Kenneth Deffeyes, *Hubbert's Peak*, 2001

# Thank You

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