NREL National Renewable Energy Laboratory

A national laboratory of the U.S. Department of Energy
Office of Energy Efficiency & Renewable Energy

Innovation for Our Energy Future

Biotechnological Routes to Biomass Conversion

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National Renewable Energy Laboratory

DOE/NASULGC Biomass & Solar Energy Workshops
August 3-4, 2004

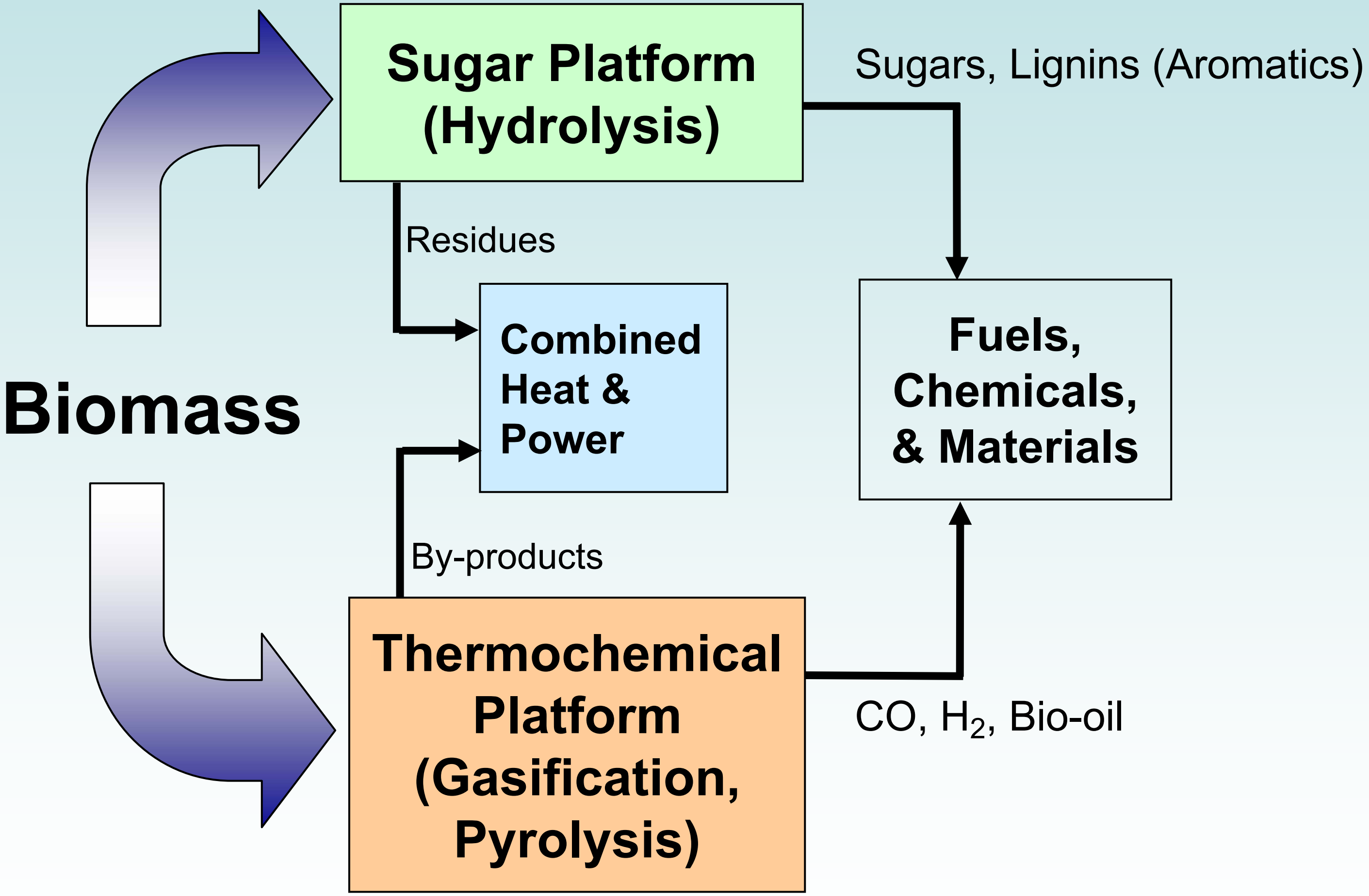
NREL is operated by Midwest Research Institute • Battelle

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"



Sugar Platform (Hydrolysis)

Sugars, Lignins (Aromatics)

Residues

Combined Heat & Power

Fuels, Chemicals, & Materials

Biomass

By-products

Thermochemical Platform (Gasification, Pyrolysis)

CO, H_2, Bio-oil

Outline

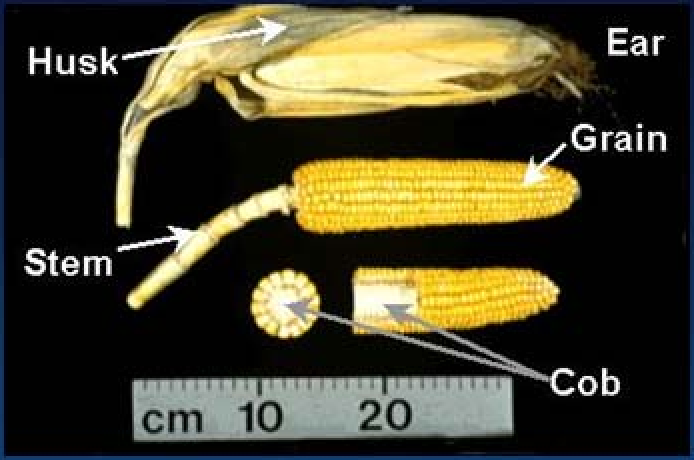
- Biomass Basics
- Overview of Conversion Options
- Details of Enzyme-based Technology
- Biorefining Now and in the Future

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.

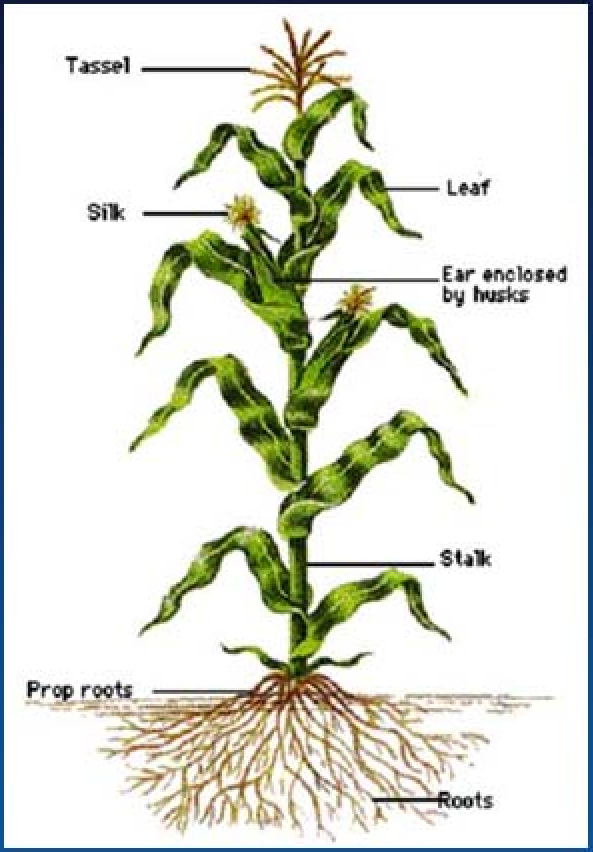
- *Emphasis of today’s presentation will be conversion of **lignocellulosic** biomass*
  - *Comparison to illustrate the differences between starchy and fibrous feedstocks: corn grain versus corn stover*

Corn Grain vs. Corn Stover



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

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



GRAIN

http://arnica.csustan.edu/key/corn.jpg

STOVER

Biomass Basics

- Grain contains
  - ≥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

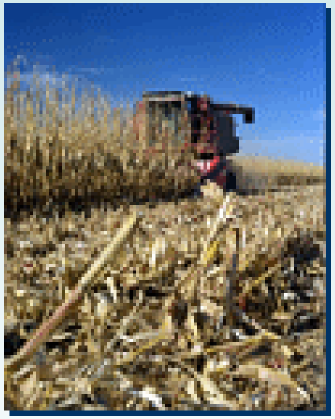
Composition: Grain vs. Stover

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

Biomass Resources and Key Issues

Wood Residues

Sawdust
Wood waste
Pulp mill wastes



Agricultural Residues

Corn stover
Rice hulls
Sugarcane bagasse
Animal waste

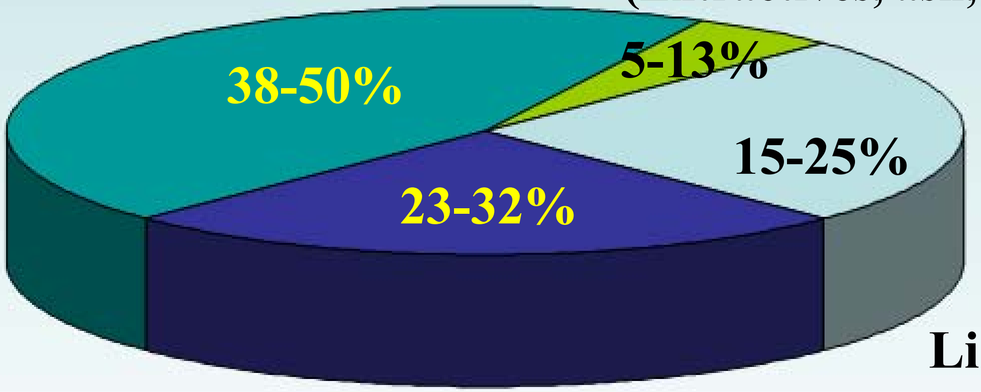
Energy Crops

Switchgrass
Hybrid poplar
Willow

- Quality
  - Composition
  - Ease of Conversion
- Cost
  - Production
  - Collection and Transportation
  - Quantity Available
- Sustainability
  - Land, Air and Water Resources

Biomass Composition

Hardwoods



Cellulose

(Glucose sugar)

38-50%

Other

(Extractives, ash, etc.)

5-13%

15-25%

Lignin

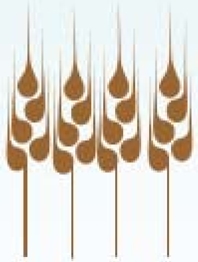
(Phenylpropyl-based)

23-32%

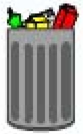
Hemicellulose

(Pentose sugars)

Grasses



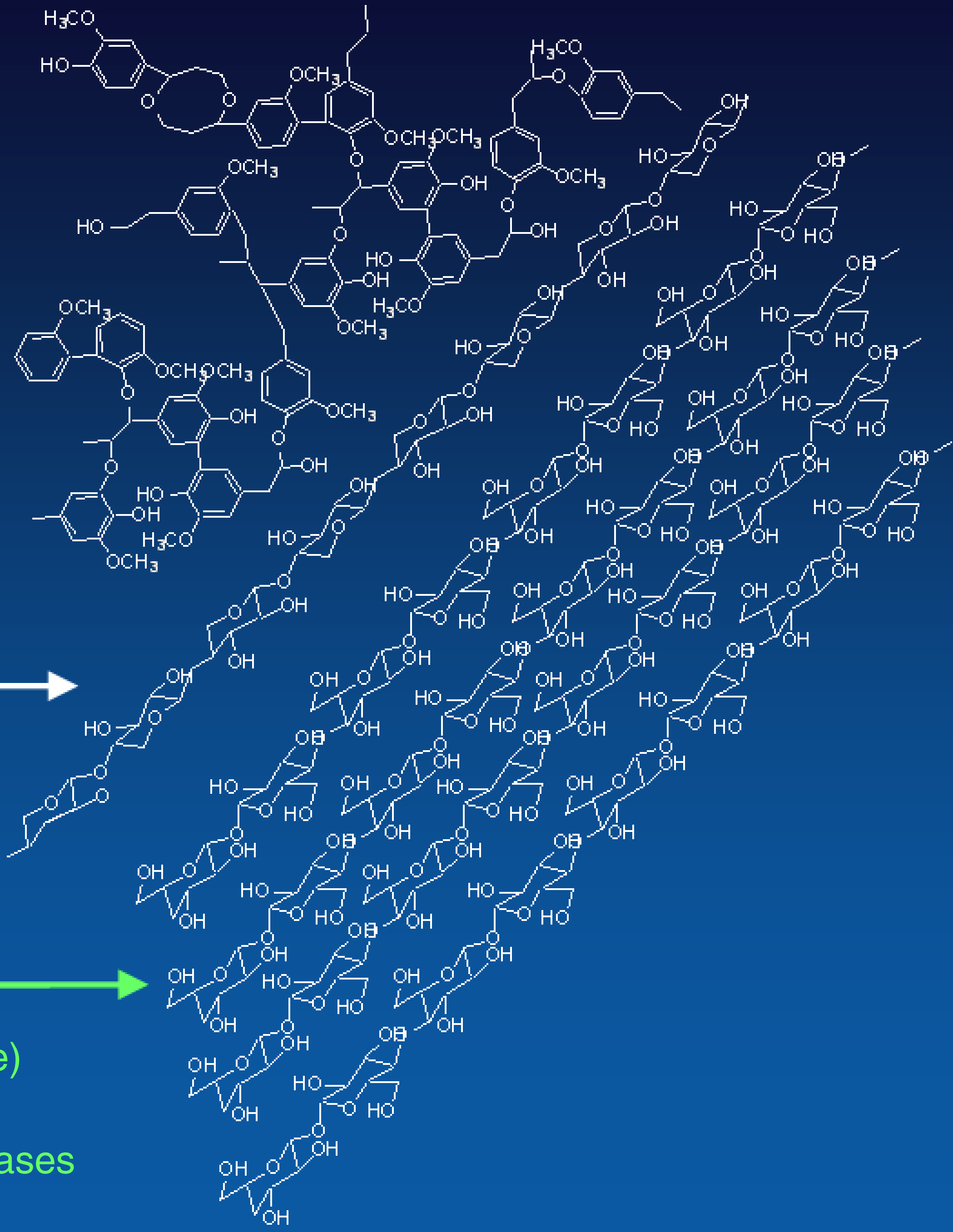
Crop residues



MSW

Softwoods

Major Plant Cell Wall Components



Lignin: 10-25%

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

Hemicellulose: 15-30%

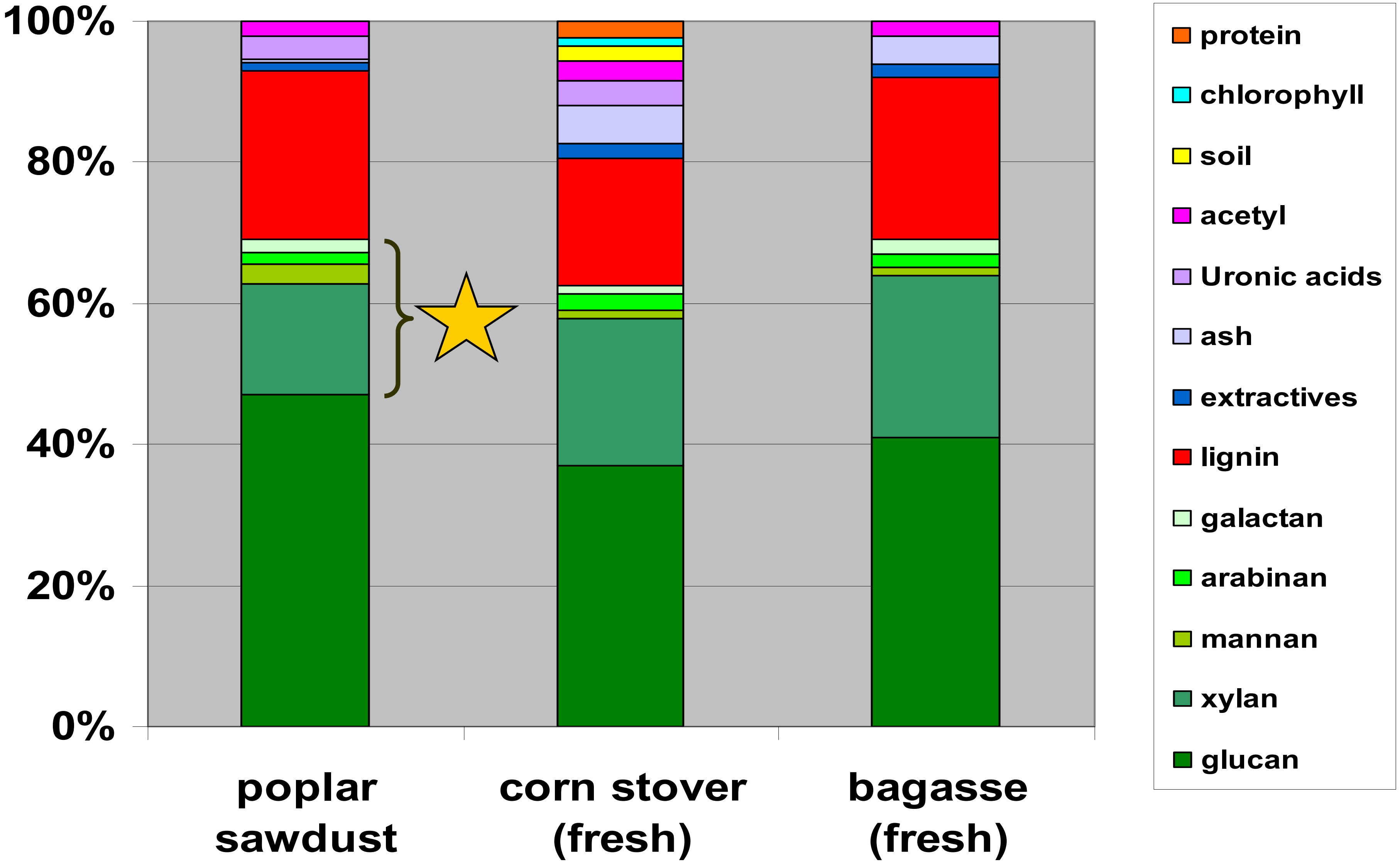
- Heteropolymer of pentoses and hexoses
- Variably substituted (acetyl, uronics)
- More easily depolymerized

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

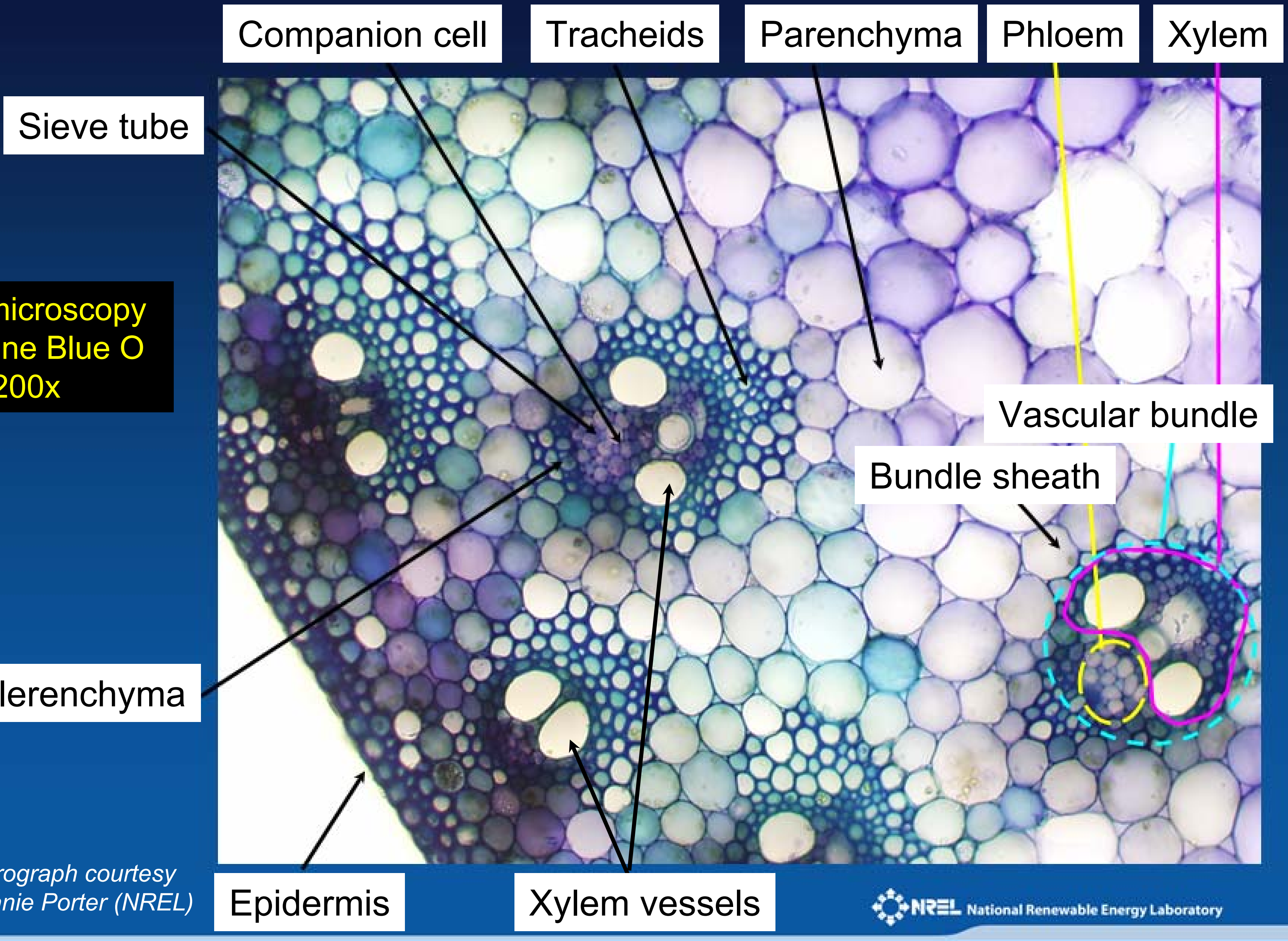
TEM
Tecnai G^2 Quanta 400 FEG

SEM
Quanta 400 FEG

AFM
MultiMode PicoForce

NSOM
AURORA-3

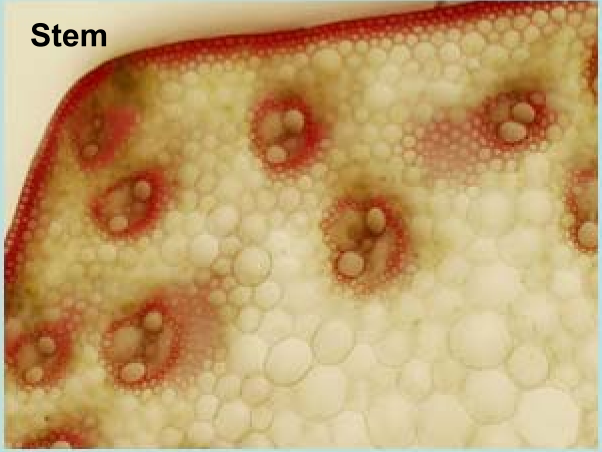
Heterogeneity Across a Single Corn Stem*

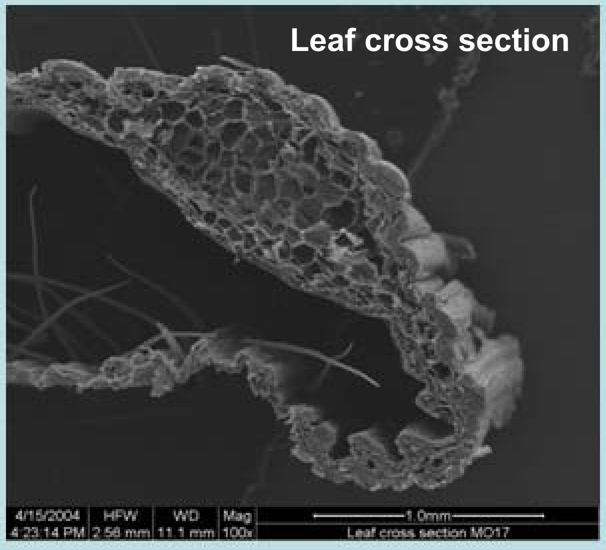


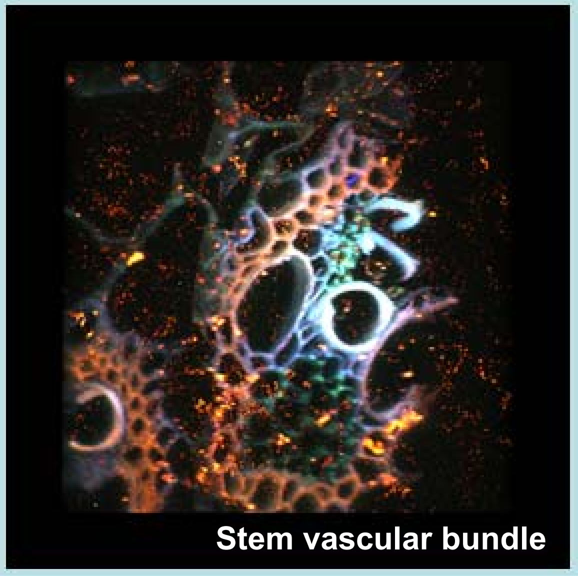
Light microscopy
Toluidine Blue O
200x

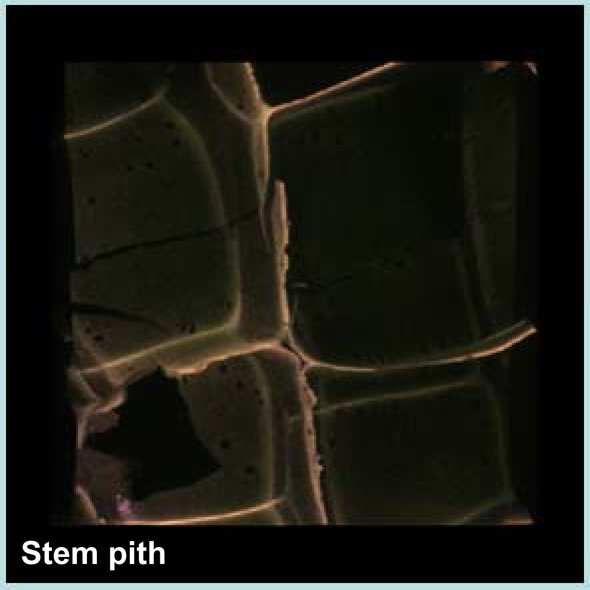
**Photomicrograph courtesy of Stephanie Porter (NREL)*

Structural Complexity at Many Scales*


White light, 100x

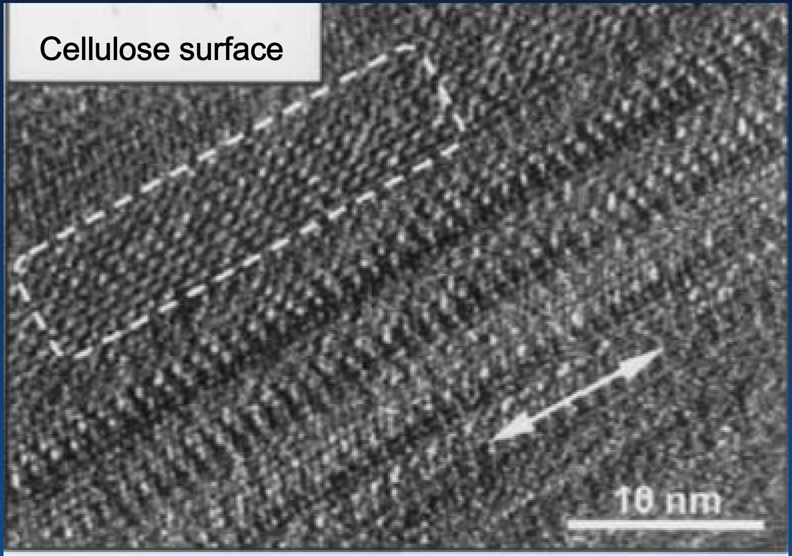

SEM, 100x


UV Fluorescence, 600x


Confocal, 1000x

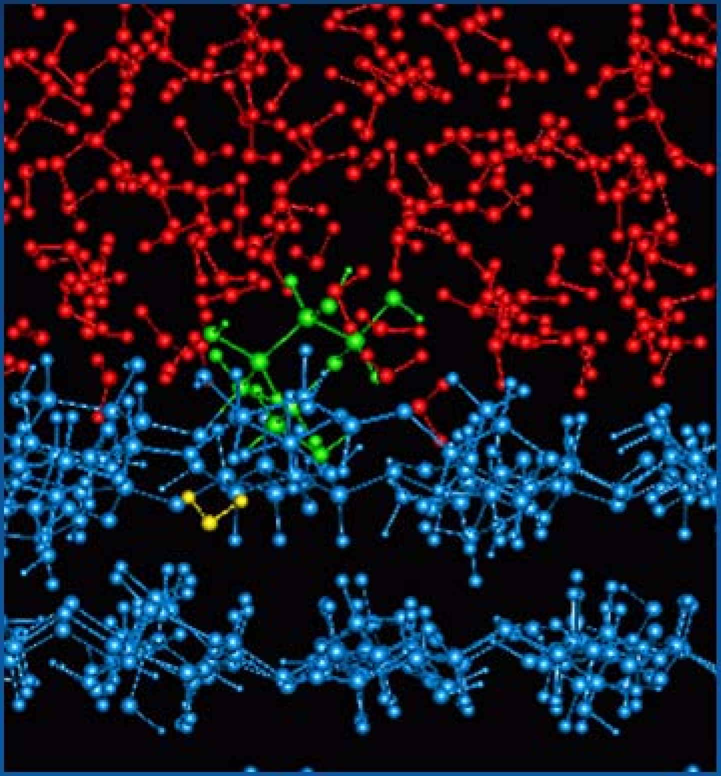
*Images courtesy of S. Porter (NREL)

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

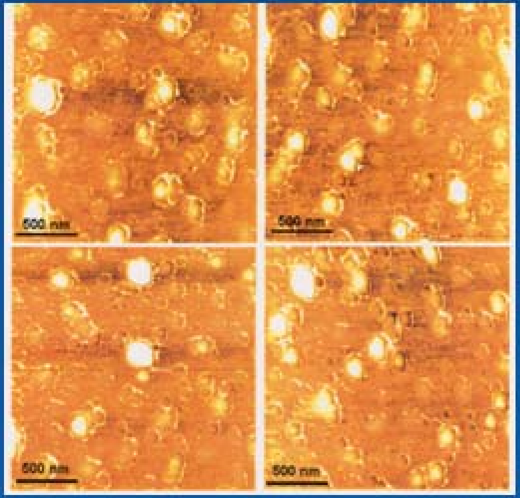


Monitor cellulose surfaces during pretreatment and enzymatic hydrolysis

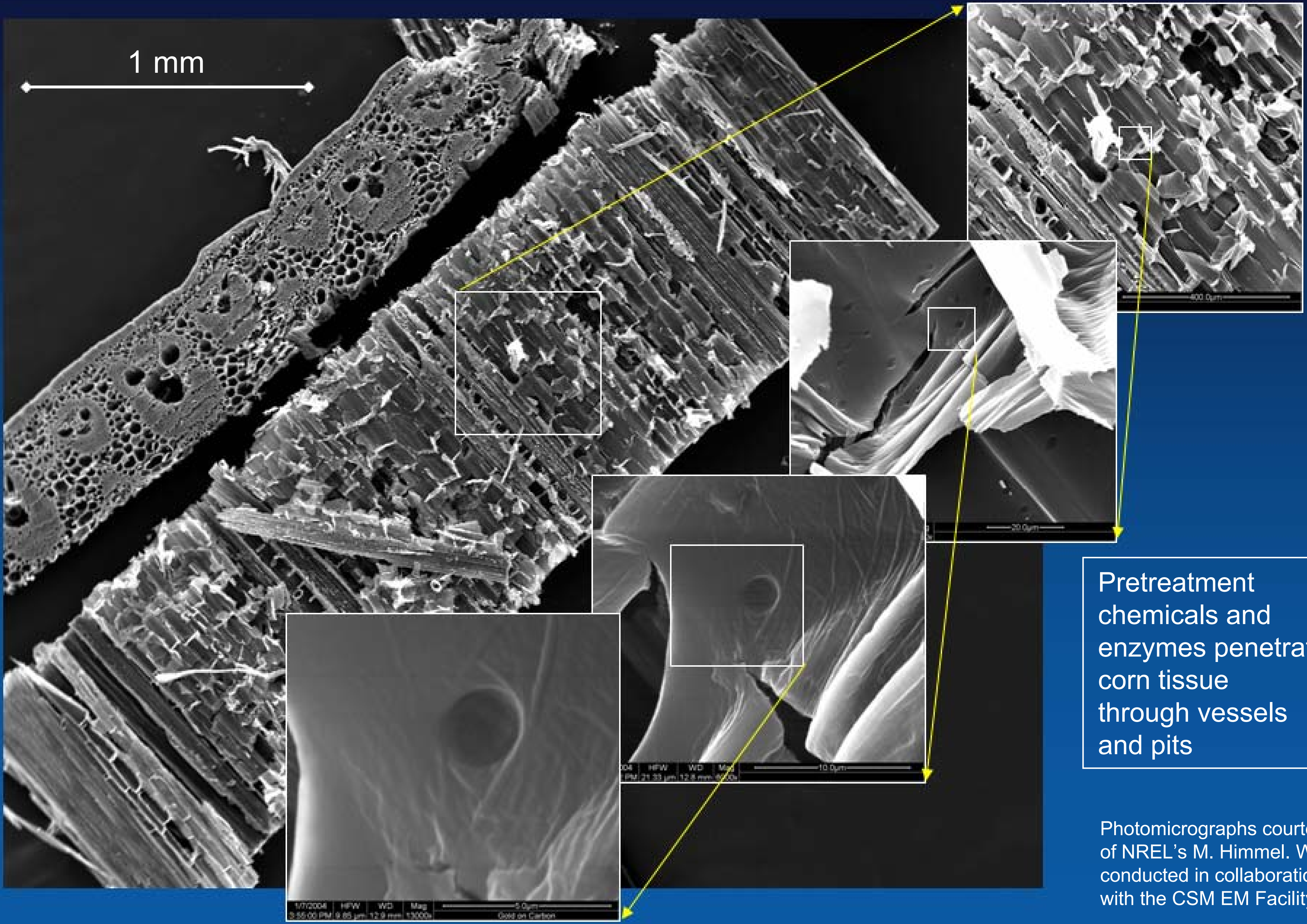
Test molecular models



Visualize changes to biomass surfaces caused by various pretreatment processes



SEM of Corn Stems – How small are pits?

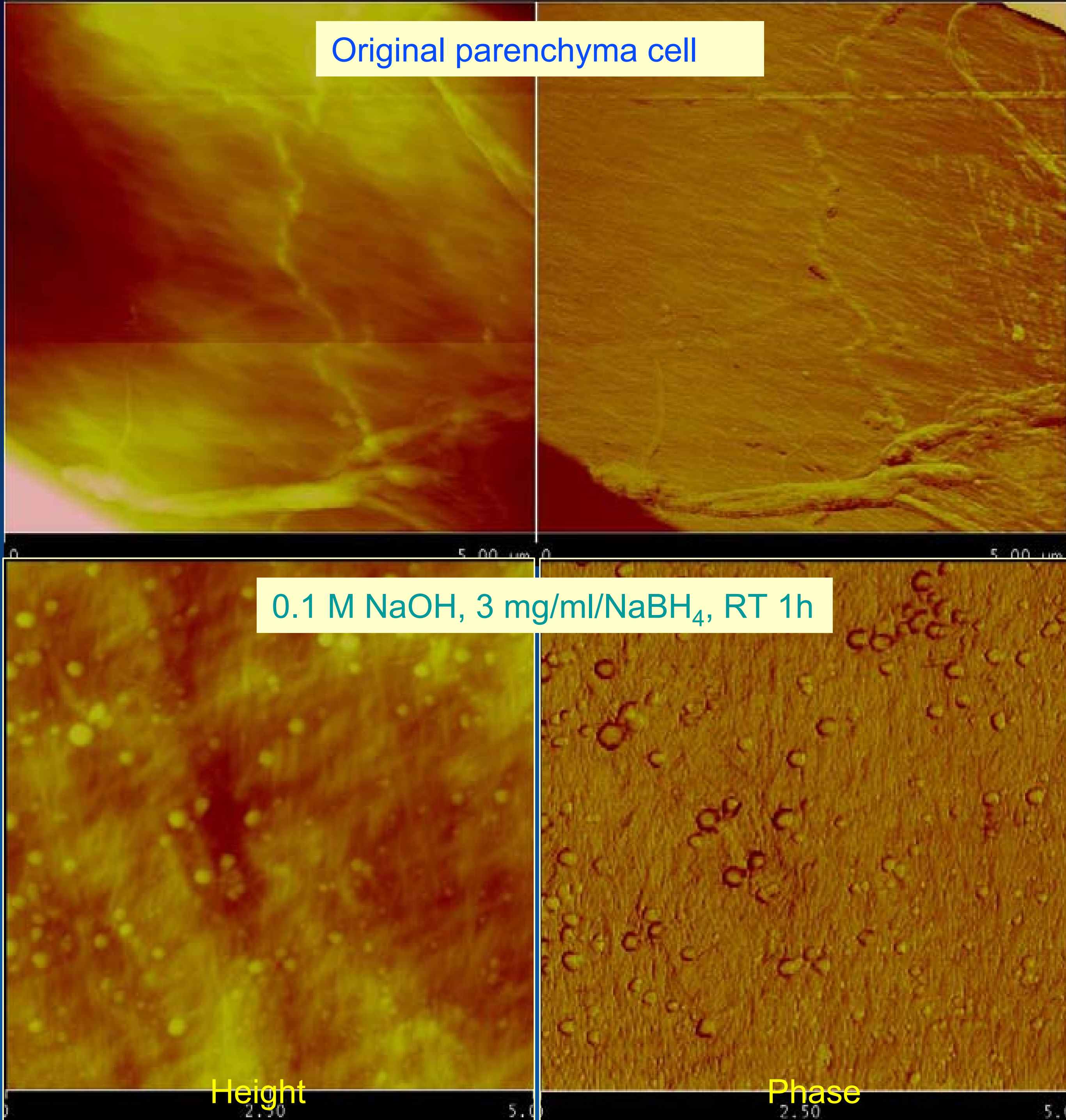


Pretreatment chemicals and enzymes penetrate corn tissue through vessels and pits

Photomicrographs courtesy of NREL’s M. Himmel. Work conducted in collaboration with the CSM EM Facility.

AFM pith parenchyma cell cell-wall structure

Tapping mode
Scan size: 5x5µm



Original parenchyma cell

0.1 M NaOH, 3 mg/ml/NaBH$_4$, RT 1h

Outline

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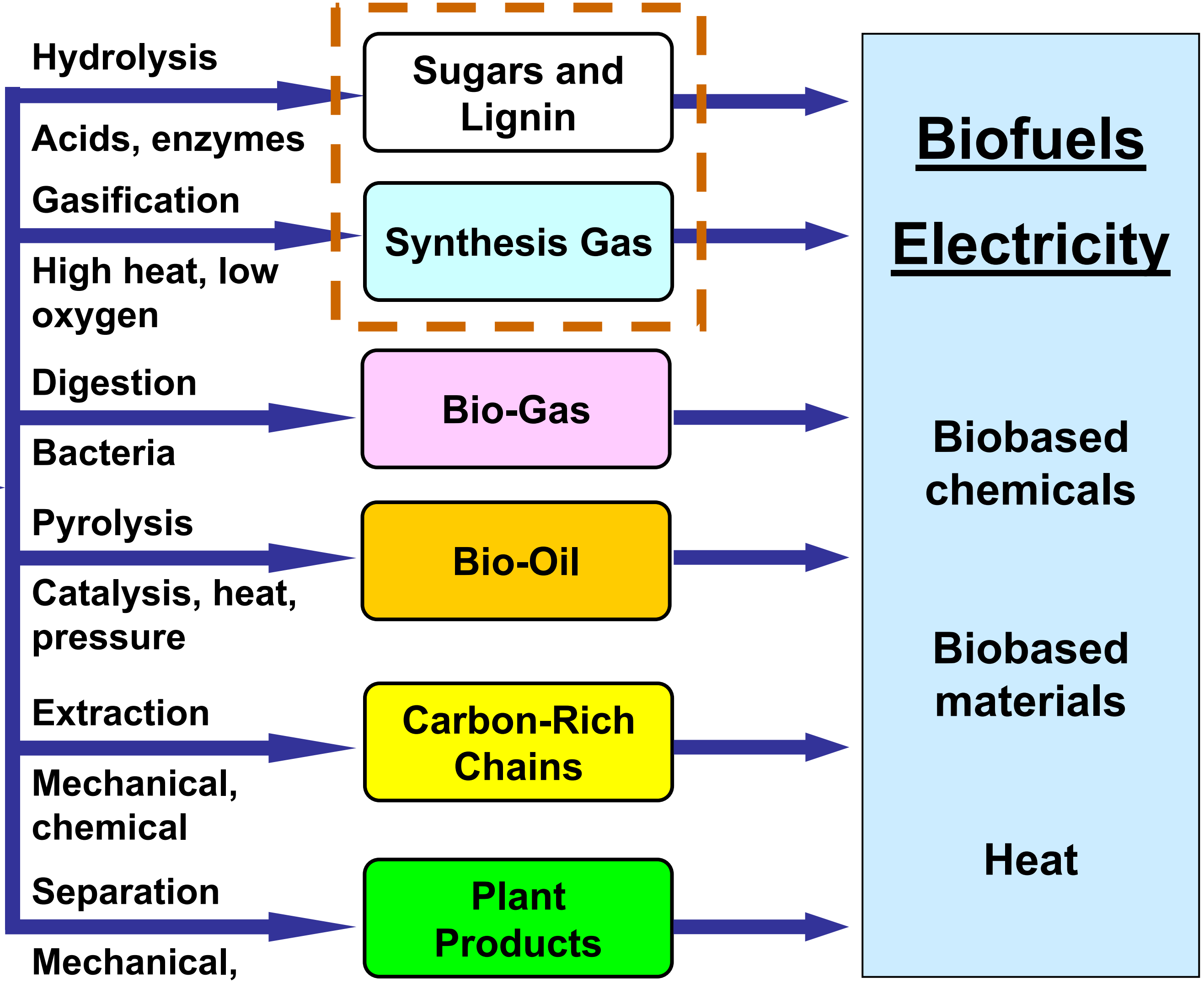
- Biorefining Now and in the Future

Biomass Energy Options

Feedstock production, collection, handling & preparation



Hydrolysis

Acids, enzymes

Sugars and Lignin

Gasification

High heat, low oxygen

Synthesis Gas

Digestion

Bacteria

Bio-Gas

Pyrolysis

Catalysis, heat, pressure

Bio-Oil

Extraction

Mechanical, chemical

Carbon-Rich Chains

Separation

Mechanical, chemical

Plant Products

Biofuels

Electricity

Biobased chemicals

Biobased materials

Heat

Biomass Conversion (or Fractionation)

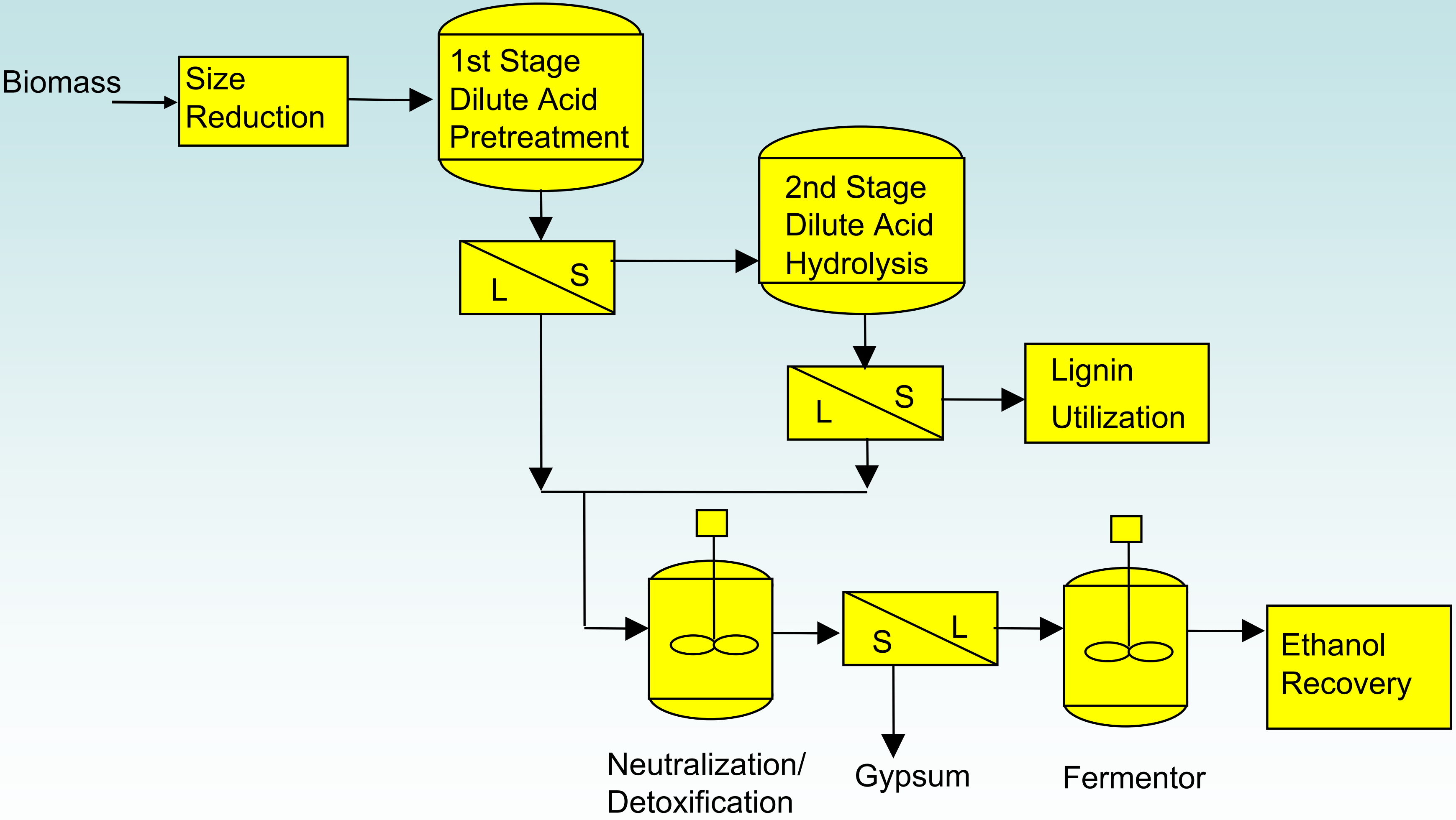
- Approaches
  - Mechanical
    - e.g., milling, comminution, decompression
  - Thermal
    - e.g., hot water, steam, heat
  - Chemical
    - e.g., acids, alkalis, solvents
  - Biological
    - e.g., cellulases, hemicellulases, ligninases

➢ *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

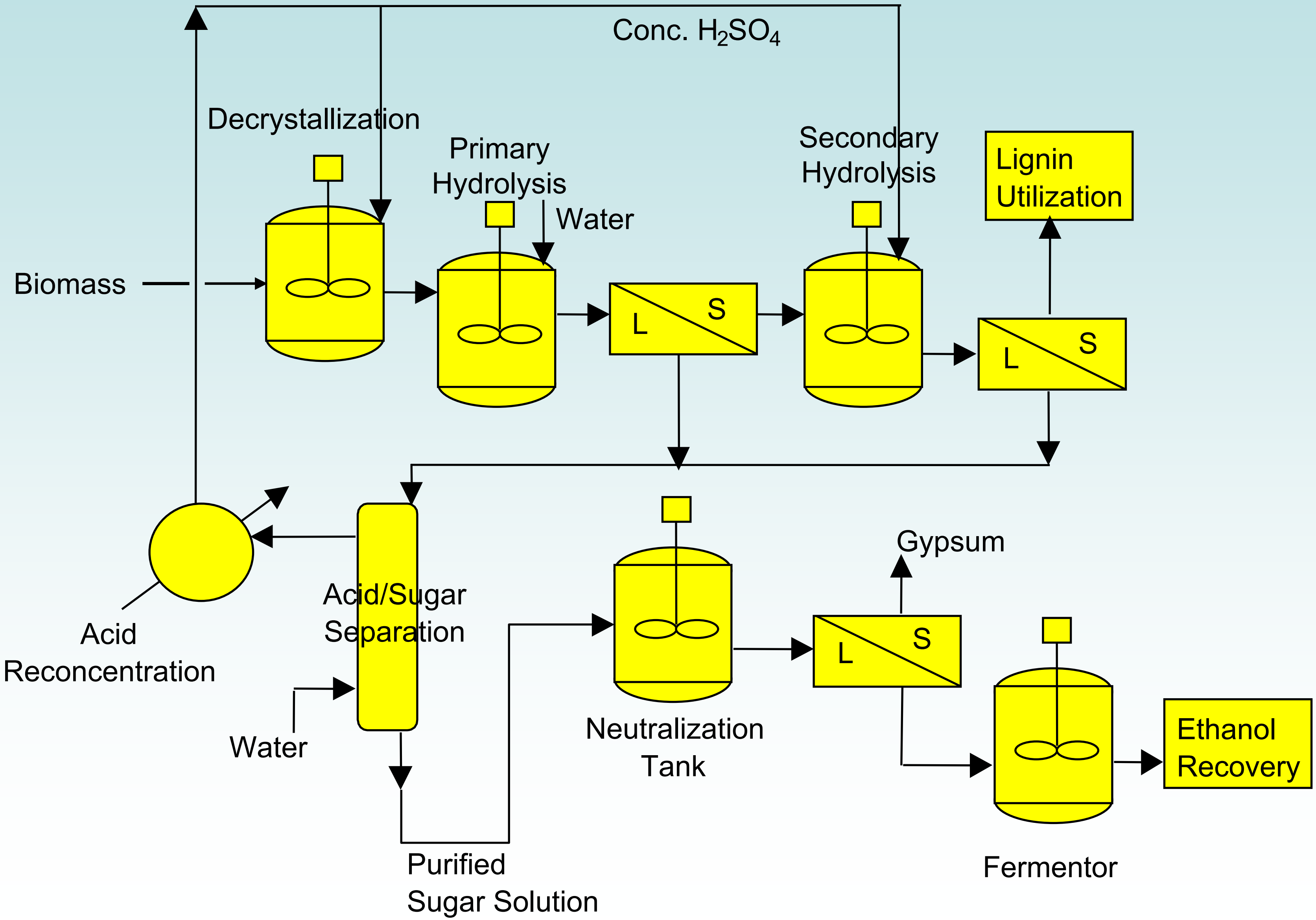
Two-Stage Dilute Acid Process



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

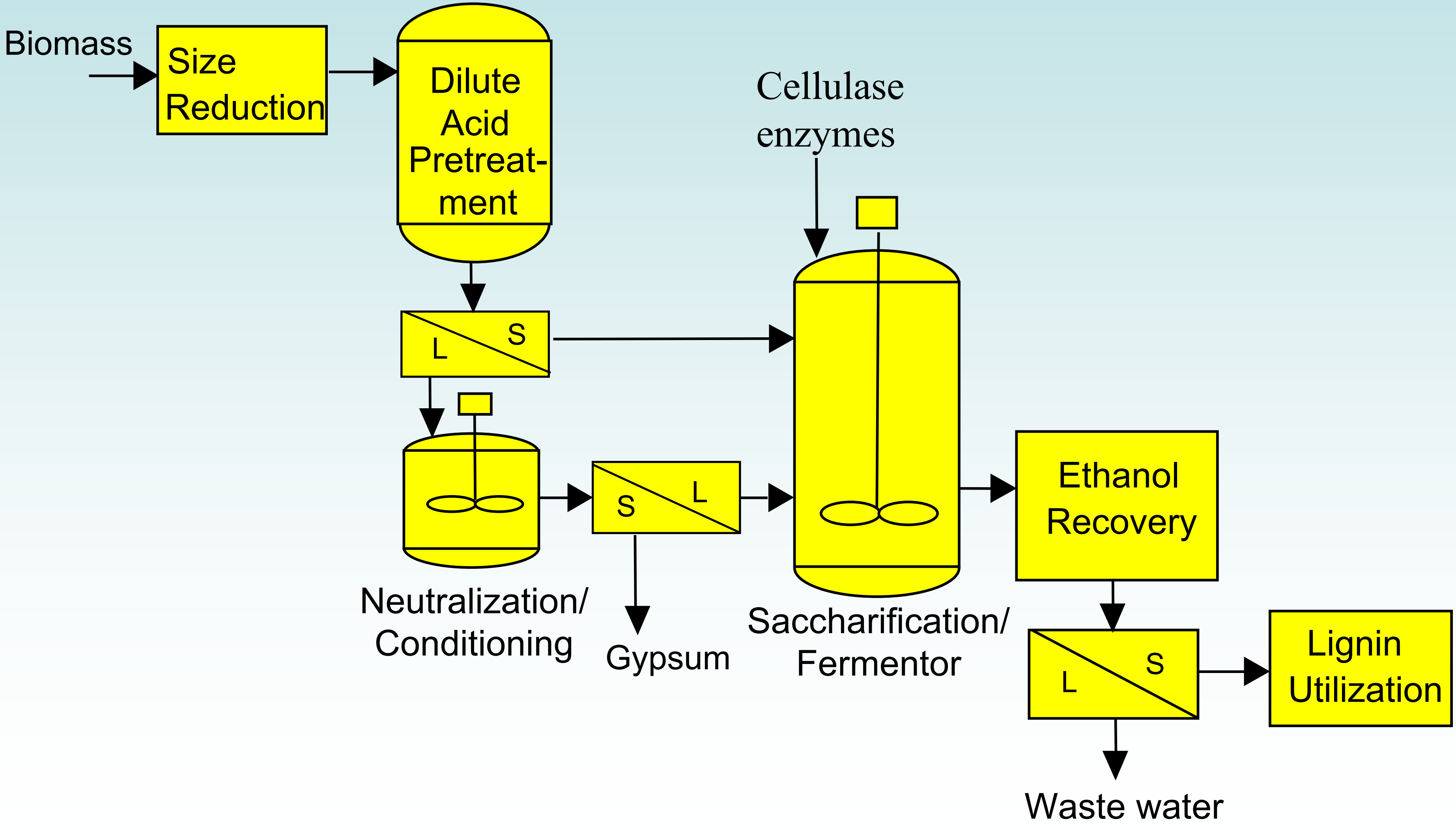
Concentrated Acid Process



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



Biomass

Size Reduction

Dilute Acid Pretreat-ment

Cellulase enzymes

Neutralization/ Conditioning

Gypsum

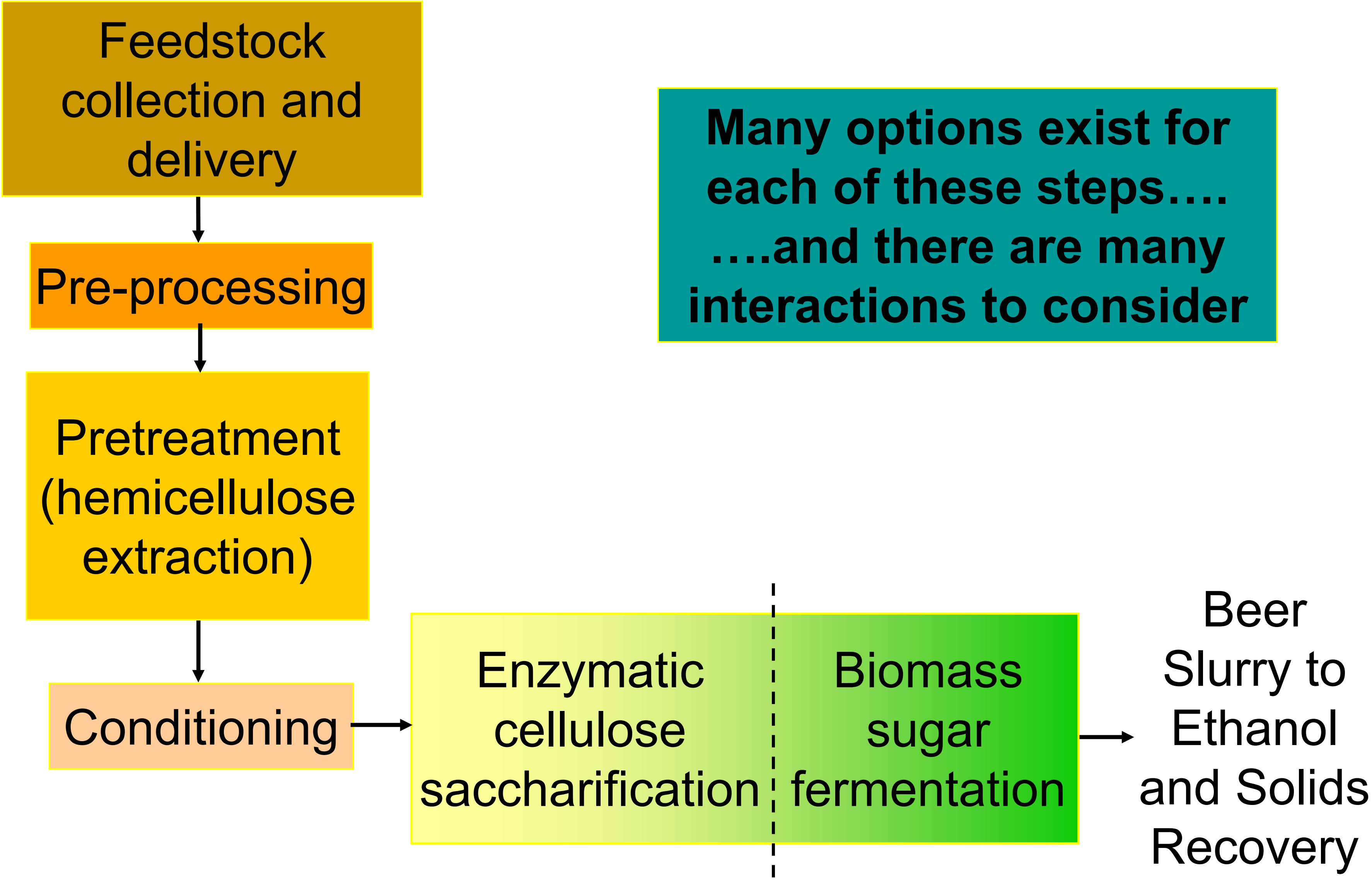
Saccharification/ Fermentor

Ethanol Recovery

Lignin Utilization

Waste water

Evolving Enzymatic Process



Feedstock collection and delivery

Pre-processing

Pretreatment (hemicellulose extraction)

Conditioning

Enzymatic cellulose saccharification

Biomass sugar fermentation

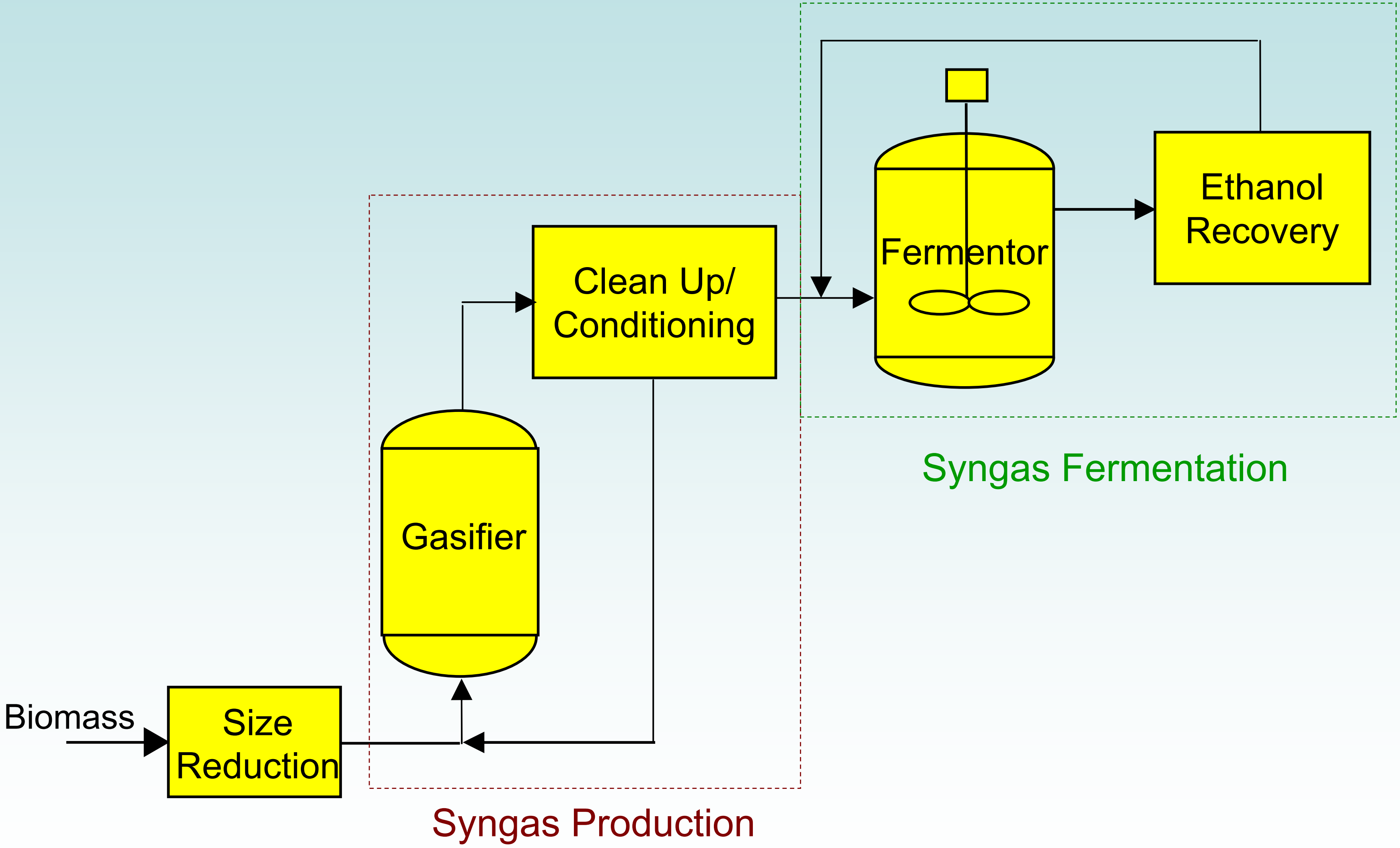
Beer Slurry to Ethanol and Solids Recovery

Many options exist for each of these steps….
….and there are many interactions to consider

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_2 and H_2 to ethanol

$$6\,CO + 3\,H_2O \longrightarrow C_2H_5OH + 4\,CO_2$$

$$6H_2 + 2\,CO_2 \longrightarrow C_2H_5OH + 3\,H_2O$$

- 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
    - 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*

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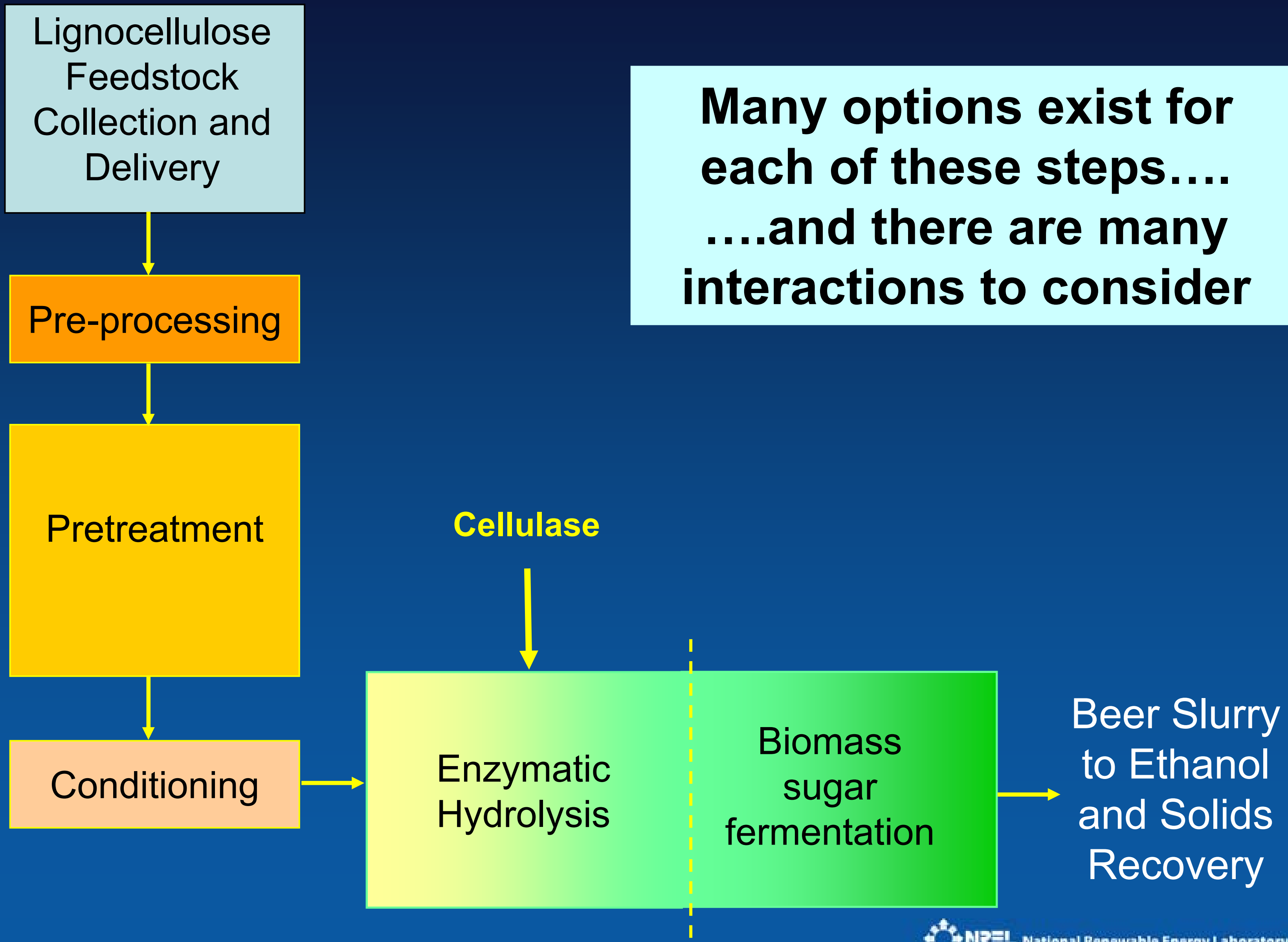
Enzymatic Process for Producing Ethanol



Lignocellulose Feedstock Collection and Delivery

Pre-processing

Pretreatment

Conditioning

Cellulase

Enzymatic Hydrolysis

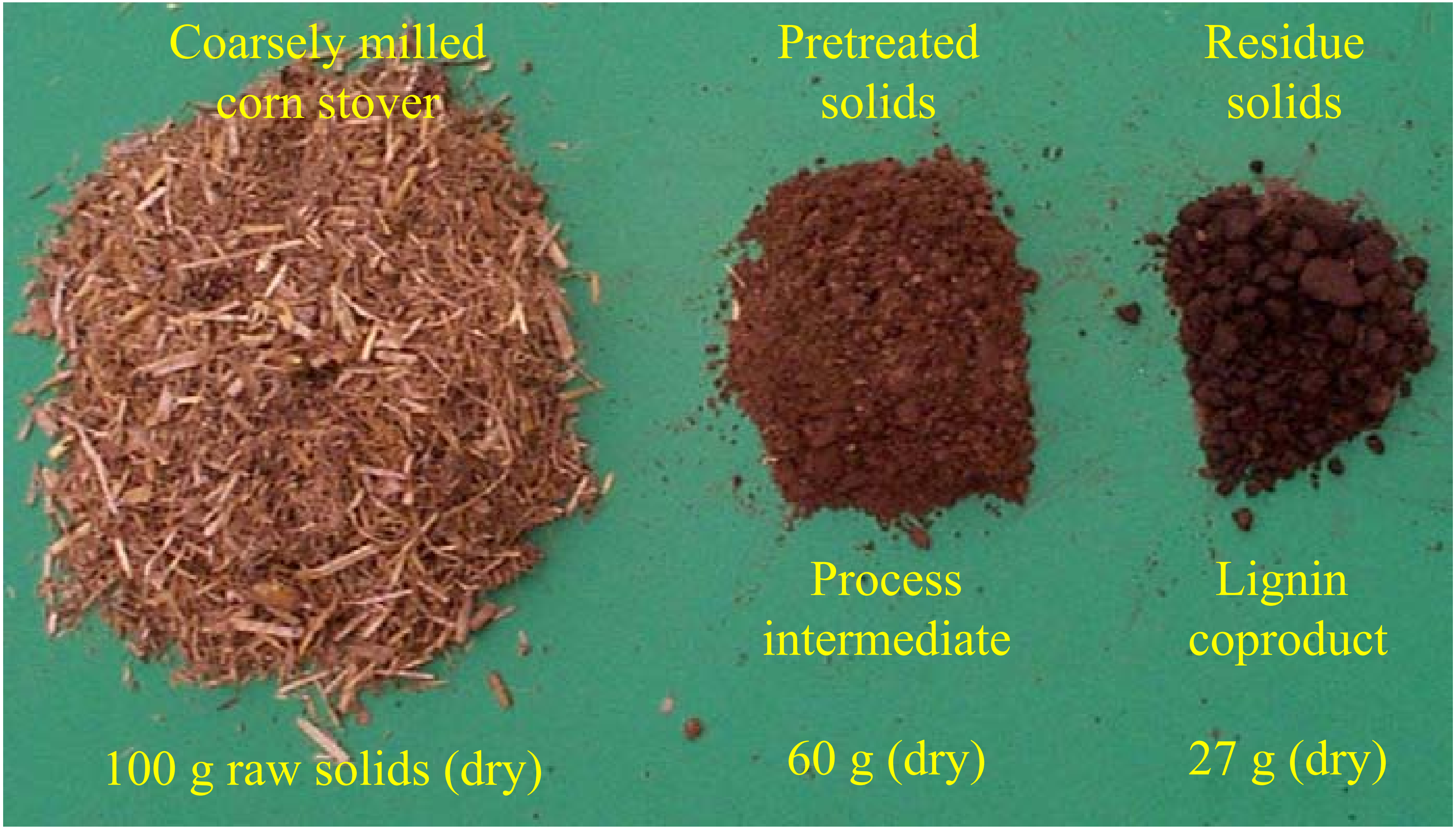
Biomass sugar fermentation

Beer Slurry to Ethanol and Solids Recovery

Many options exist for each of these steps….

….and there are many interactions to consider

Conversion is Technically Feasible…

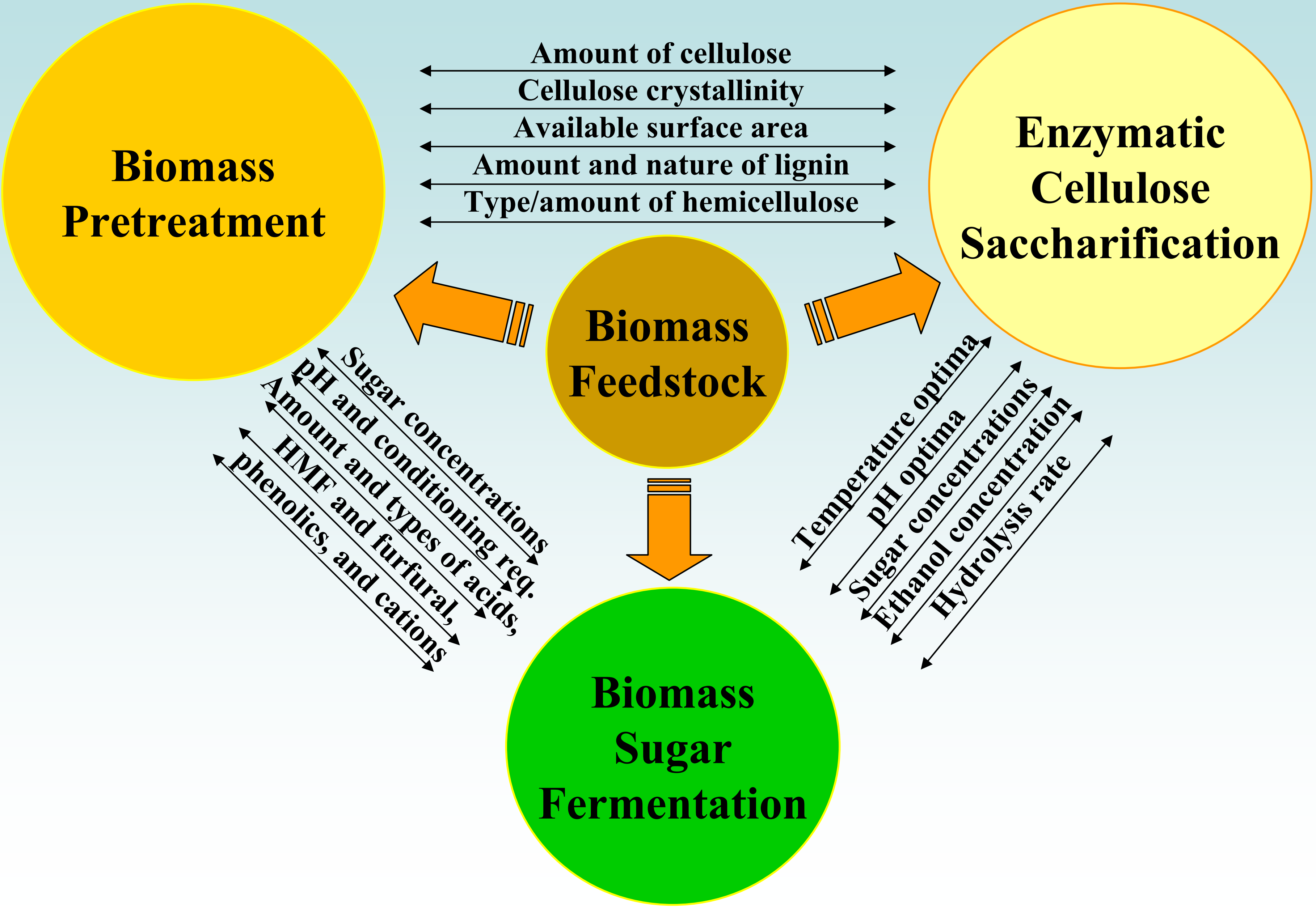


…the Challenge is Making it Economical!

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



Biomass Pretreatment

Amount of cellulose
Cellulose crystallinity
Available surface area
Amount and nature of lignin
Type/amount of hemicellulose

Enzymatic Cellulose Saccharification

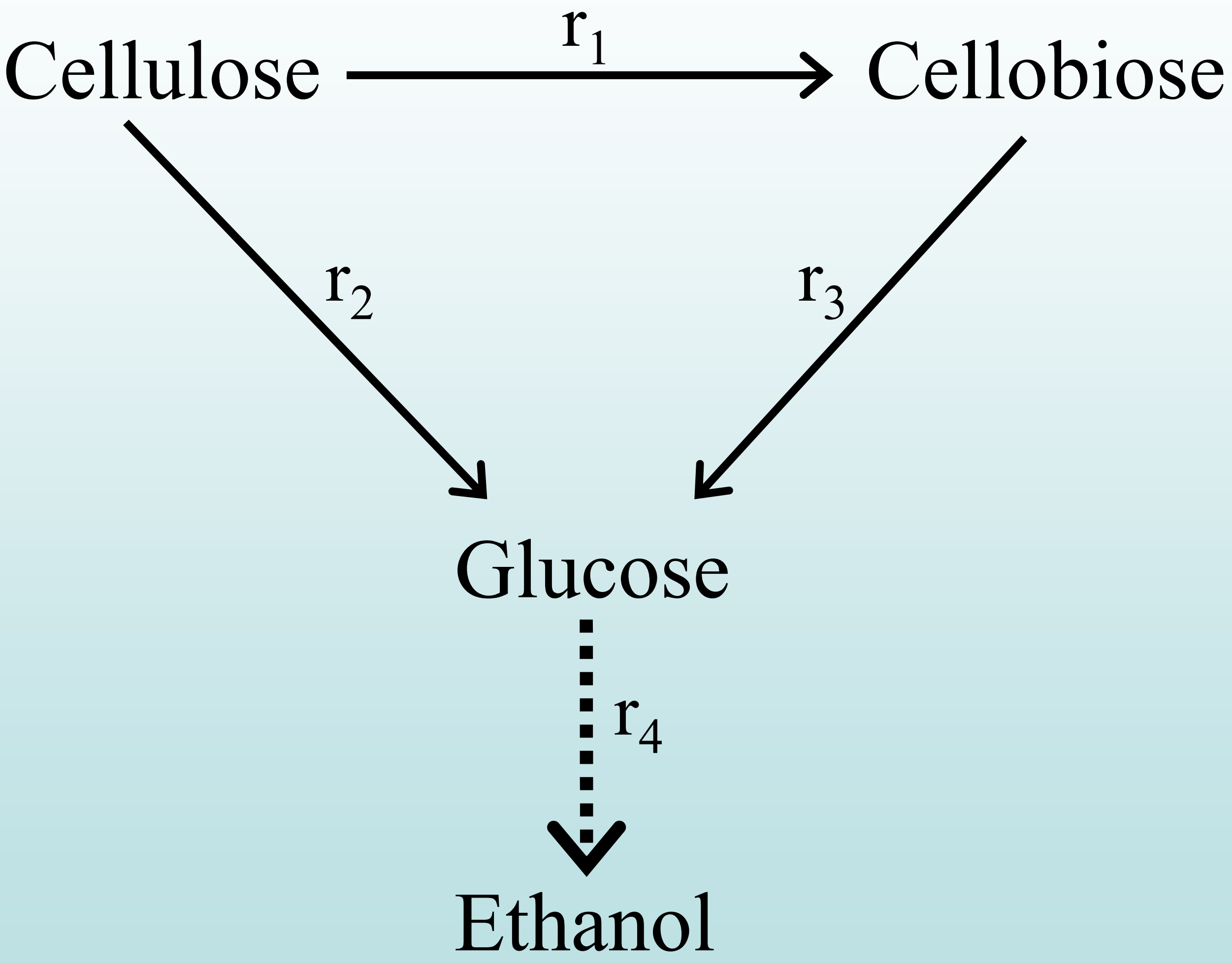
Biomass Feedstock

Sugar concentrations
pH and conditioning req.
Amount and types of acids,
HMF and furfural,
phenolics, and cations

Temperature optima
pH optima
Sugar concentrations
Ethanol concentration
Hydrolysis rate

Biomass Sugar Fermentation

Cellulose Conversion in SSF



Cellulose

r_1

Cellobiose

r_2

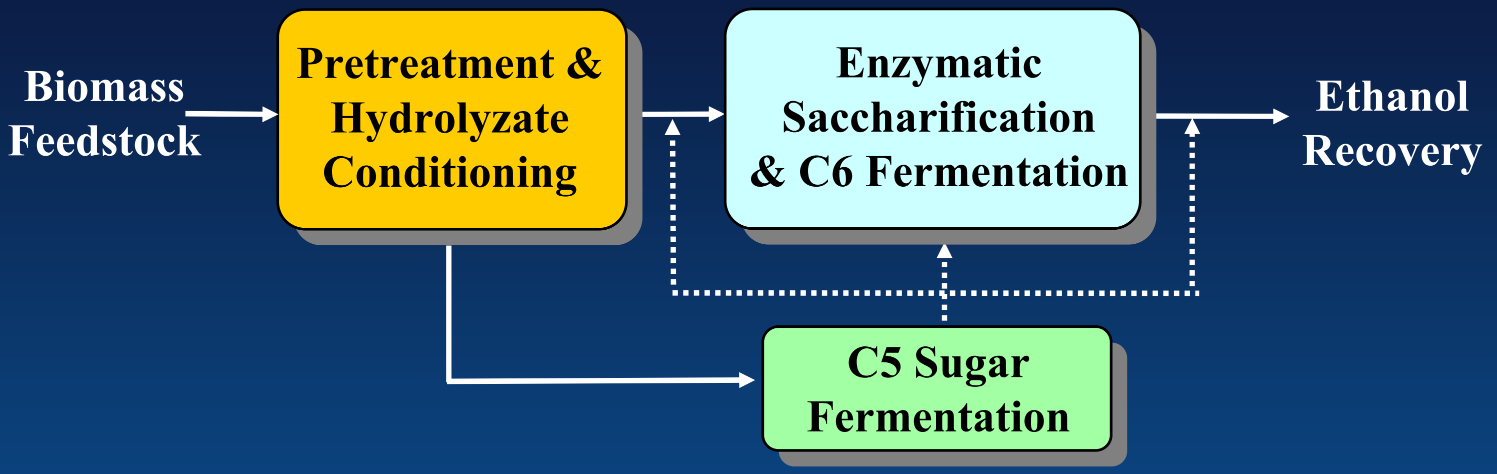
r_3

Glucose

r_4

Ethanol

Enzymatic Hydrolysis Configurations Using Simultaneous Saccharification & Fermentation



Biomass Feedstock

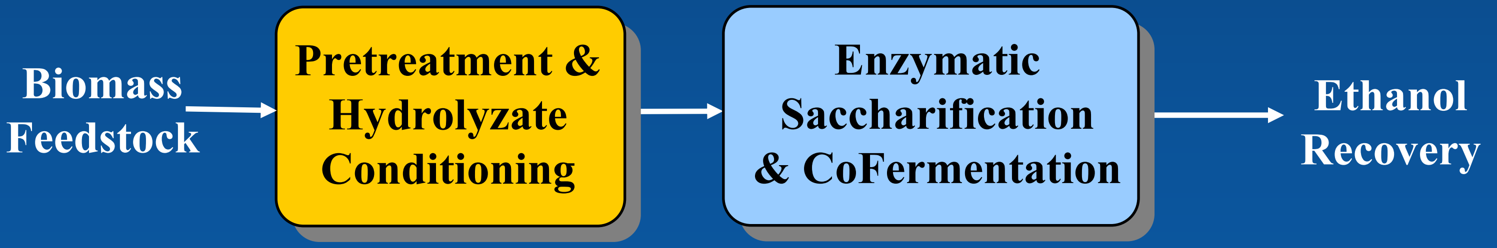
Pretreatment & Hydrolyzate Conditioning

Enzymatic Saccharification & C6 Fermentation

Ethanol Recovery

C5 Sugar Fermentation

Separate C5 and C6 Sugar Fermentation (SSF or SSCF)



Biomass Feedstock

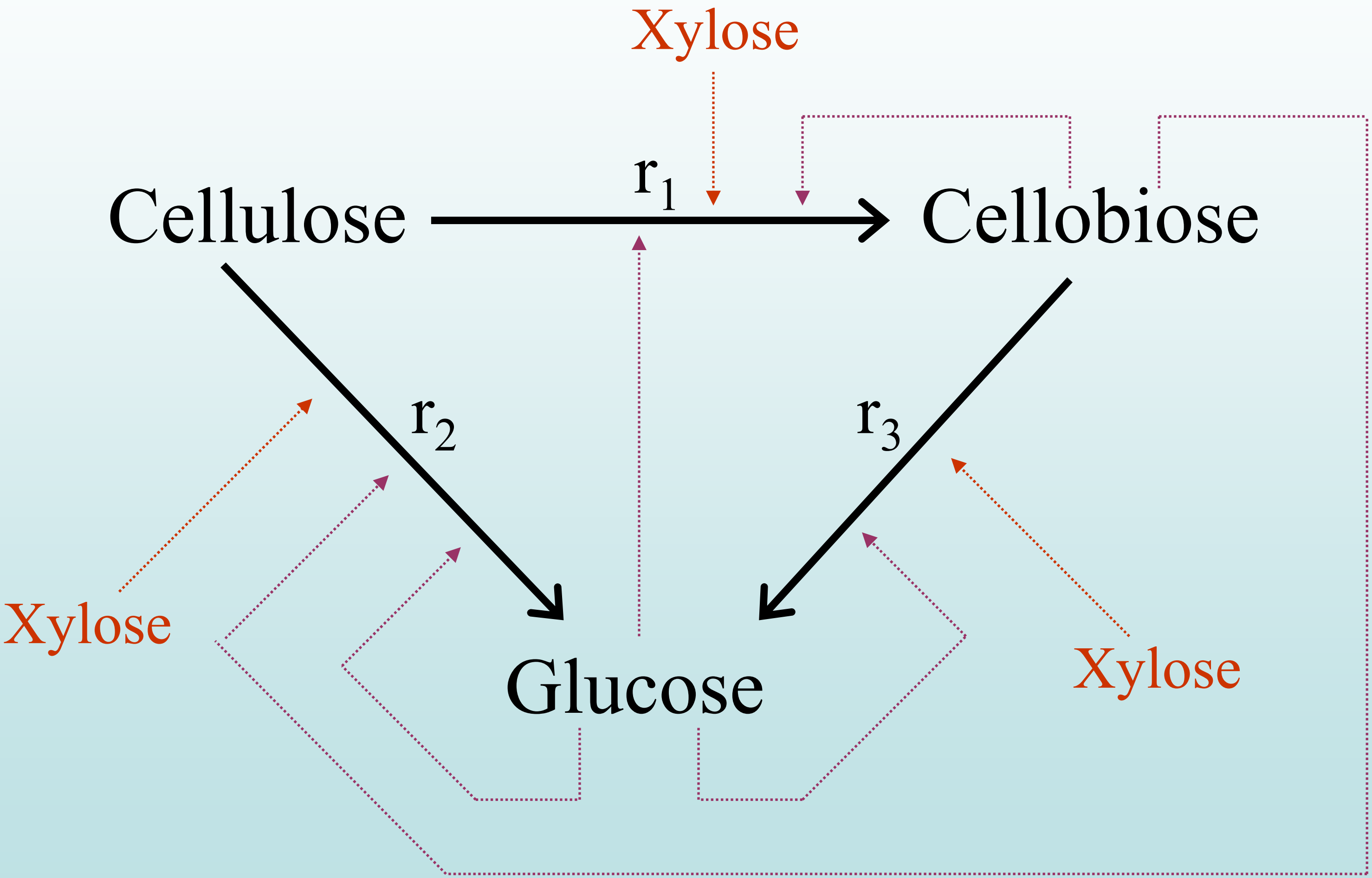
Pretreatment & Hydrolyzate Conditioning

Enzymatic Saccharification & CoFermentation

Ethanol Recovery

SSF with Combined C5 and C6 Sugar CoFermentation (SSCF)

Cellulose Conversion in SHF



Xylose

r_1

Cellulose

Cellobiose

r_2

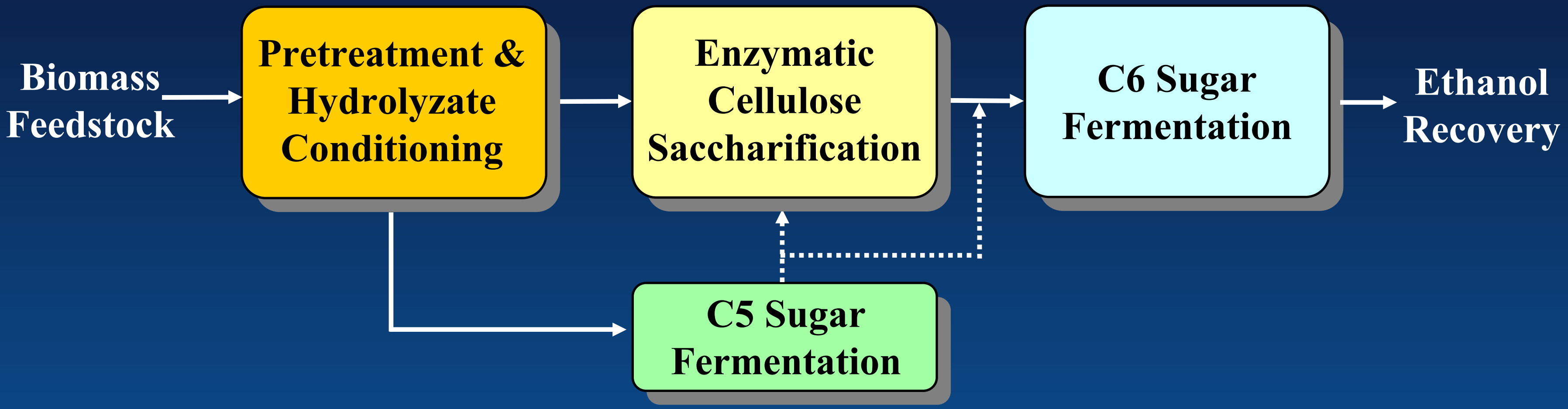
r_3

Xylose

Glucose

Xylose

Process Configurations Based on Sequential Hydrolysis and Fermentation



Biomass Feedstock

Pretreatment & Hydrolyzate Conditioning

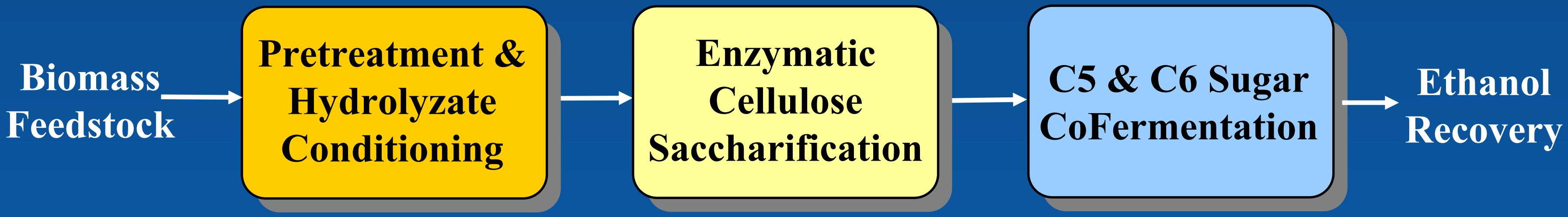
Enzymatic Cellulose Saccharification

C6 Sugar Fermentation

Ethanol Recovery

C5 Sugar Fermentation

SHF with Separate C5 and C6 Sugar Fermentation



Biomass Feedstock

Pretreatment & Hydrolyzate Conditioning

Enzymatic Cellulose Saccharification

C5 & C6 Sugar CoFermentation

Ethanol Recovery

SHF with Combined C5 and C6 Sugar Fermentation

Comparing the Attributes of SSF and SHF Process Configurations

Simultaneous (SSF/SSCF)

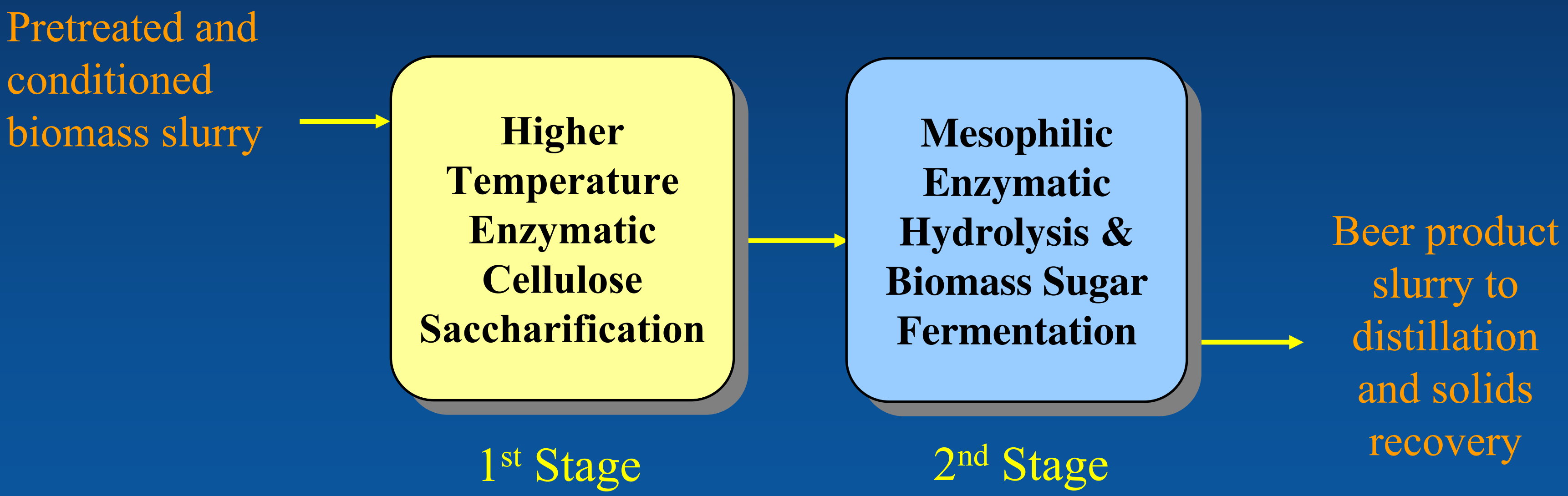
- Minimize enzyme inhibition by accumulating sugars
- Achieve high cellulose conversion yields
- Reduce process complexity via “one step” approach
- Increase pentose utilization and fermentative strain robustness through sustained production and co-utilization of glucose
- Minimize the potential for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



Pretreated and conditioned biomass slurry

Higher Temperature Enzymatic Cellulose Saccharification

1st Stage

Mesophilic Enzymatic Hydrolysis & Biomass Sugar Fermentation

2nd Stage

Beer product slurry to distillation and solids recovery

Hybrid Hydrolysis and Fermentation (HHF)

Technical Barriers

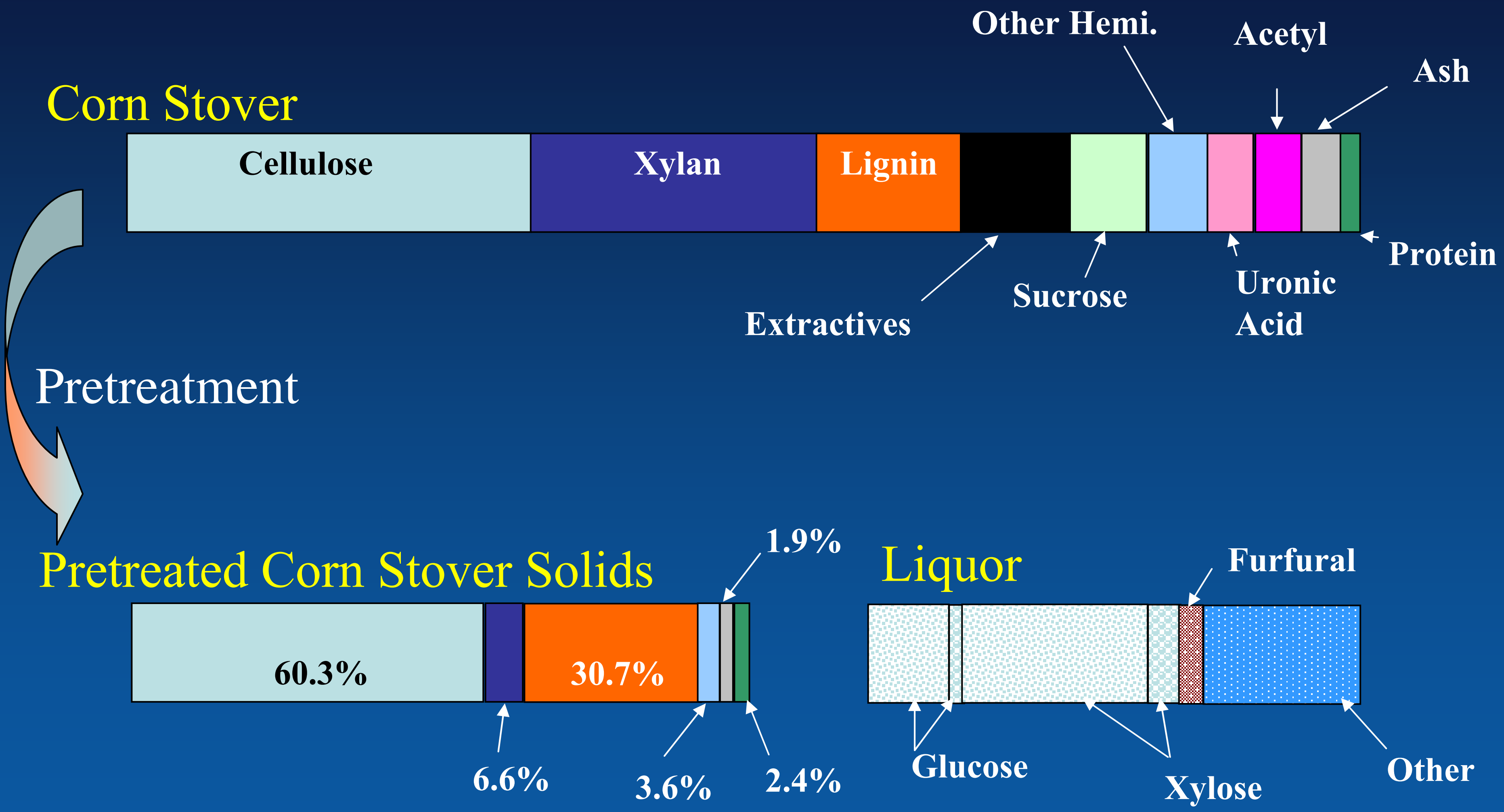
- Feedstock Valuation and Delivery
  - Analytical methods/sensors
  - Supply systems
  - Soil sustainability
- Biomass Recalcitrance to Conversion
  - Pretreatment
  - Enzymatic hydrolysis
  - Pentose fermentation
- Process Integration
  - Solids handling
  - Interactions
  - ➢ ***Process chemistry***

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

Cellulose

Xylan

Lignin

Other Hemi.

Acetyl

Ash

Extractives

Sucrose

Uronic Acid

Protein

Pretreatment

Pretreated Corn Stover Solids

60.3%

30.7%

1.9%

6.6%

3.6%

2.4%

Liquor

Furfural

Glucose

Xylose

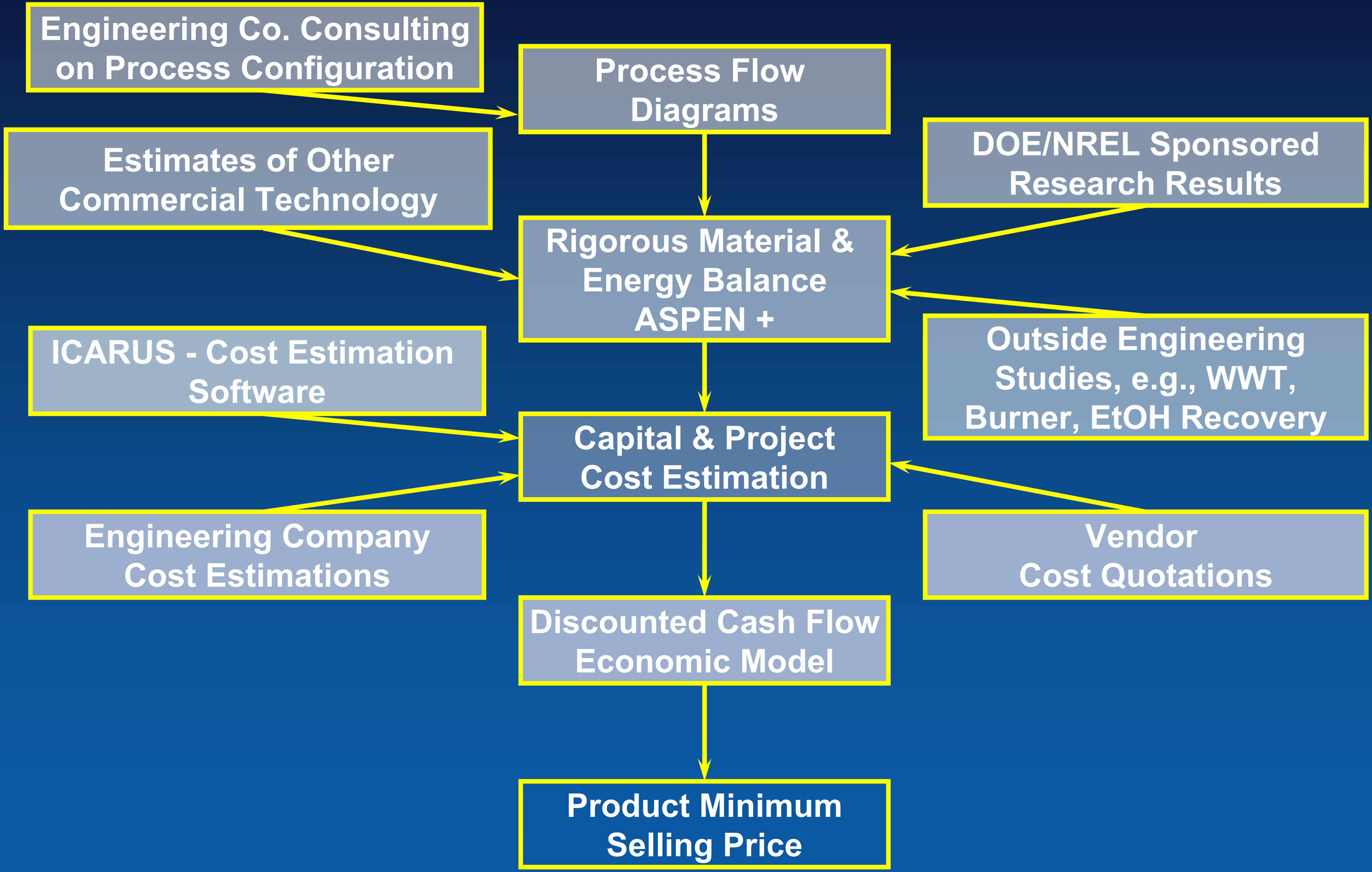
Other

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



Engineering Co. Consulting on Process Configuration

Process Flow Diagrams

Estimates of Other Commercial Technology

DOE/NREL Sponsored Research Results

Rigorous Material & Energy Balance ASPEN +

Outside Engineering Studies, e.g., WWT, Burner, EtOH Recovery

ICARUS - Cost Estimation Software

Capital & Project Cost Estimation

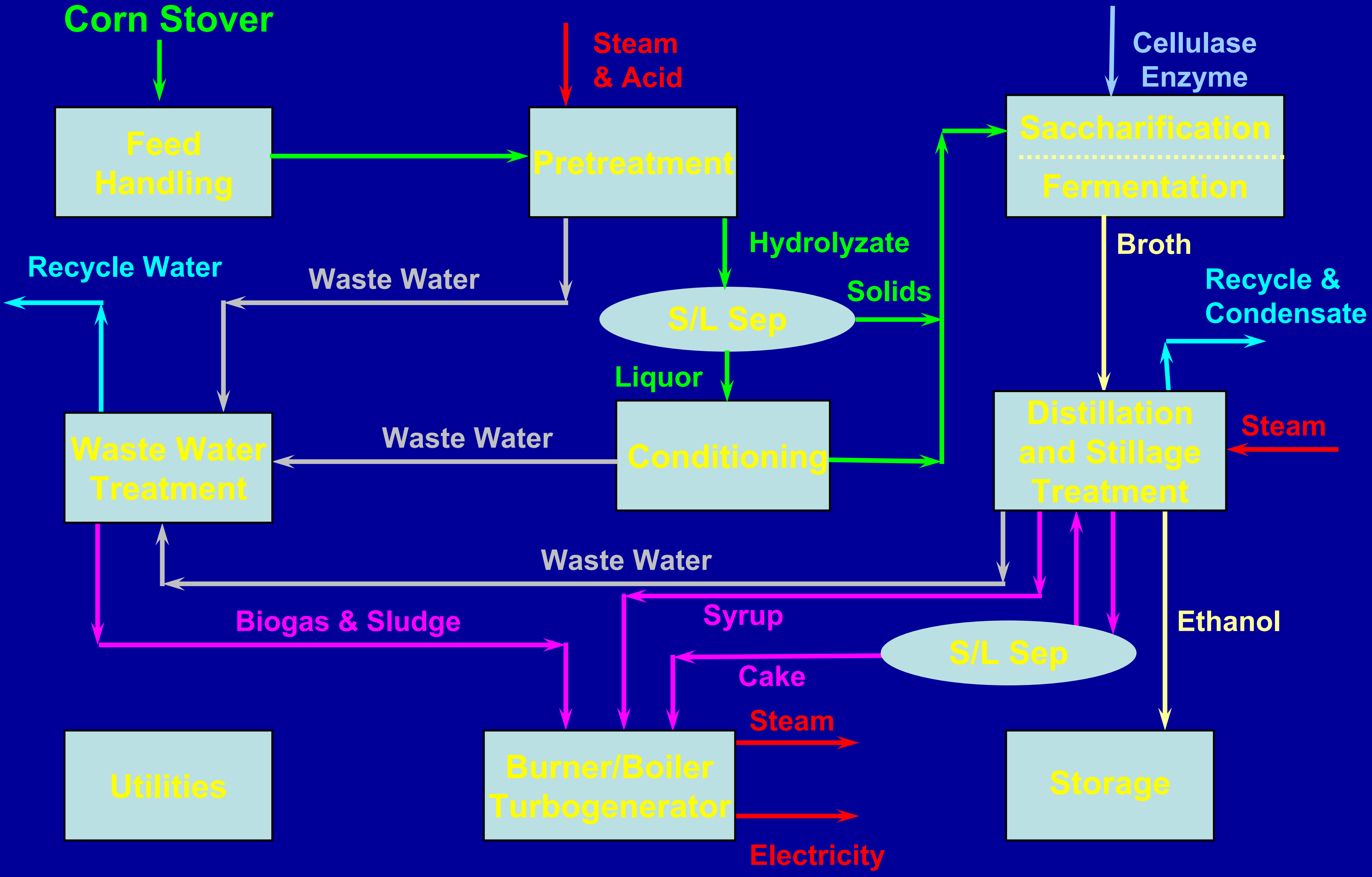
Engineering Company Cost Estimations

Vendor Cost Quotations

Discounted Cash Flow Economic Model

Product Minimum Selling Price

Developing Inclusive Cost Estimates

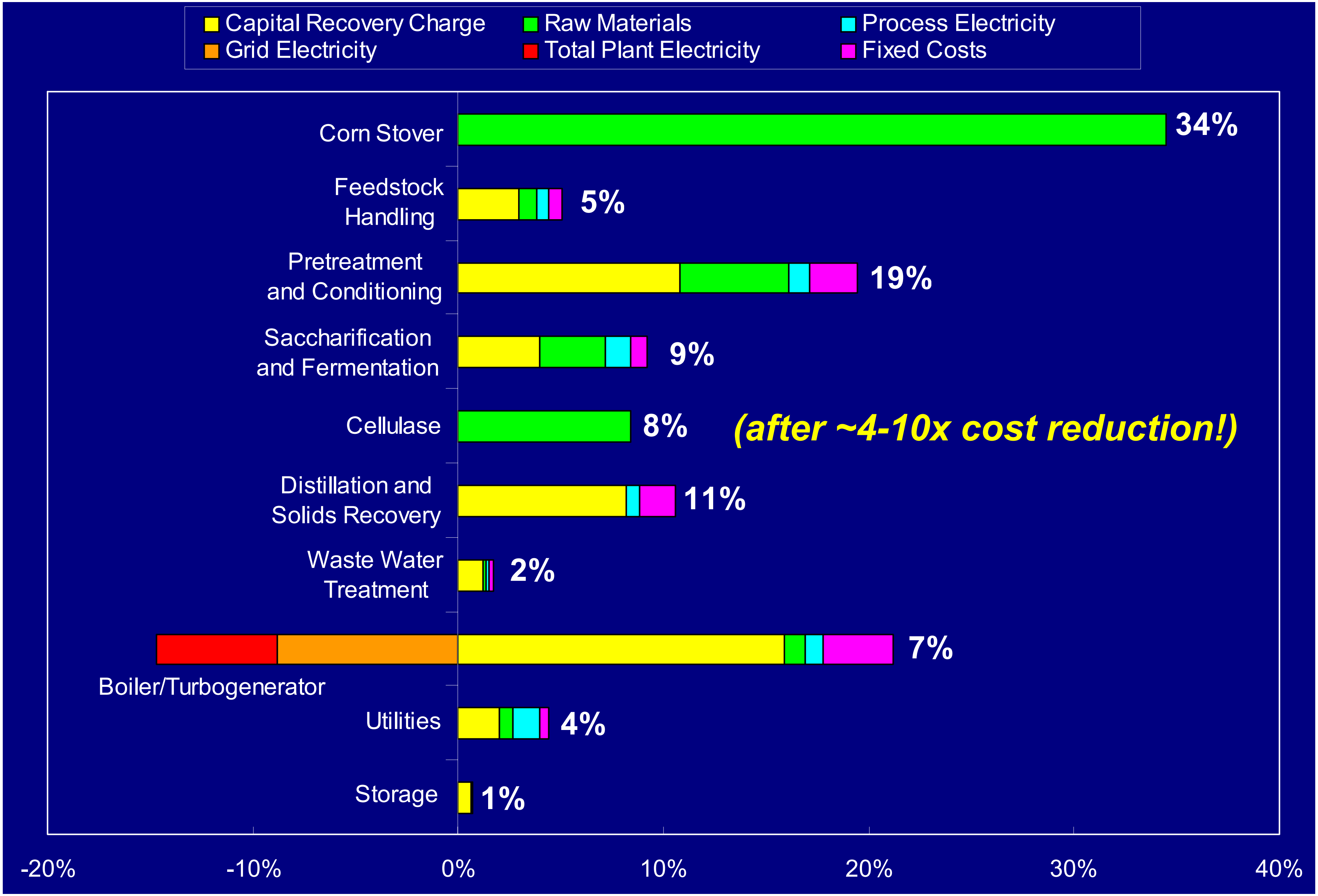


Projected Economics – Example

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

Economic Parameter (Units, $1999)	Value
Min. Ethanol Selling Price ($/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

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

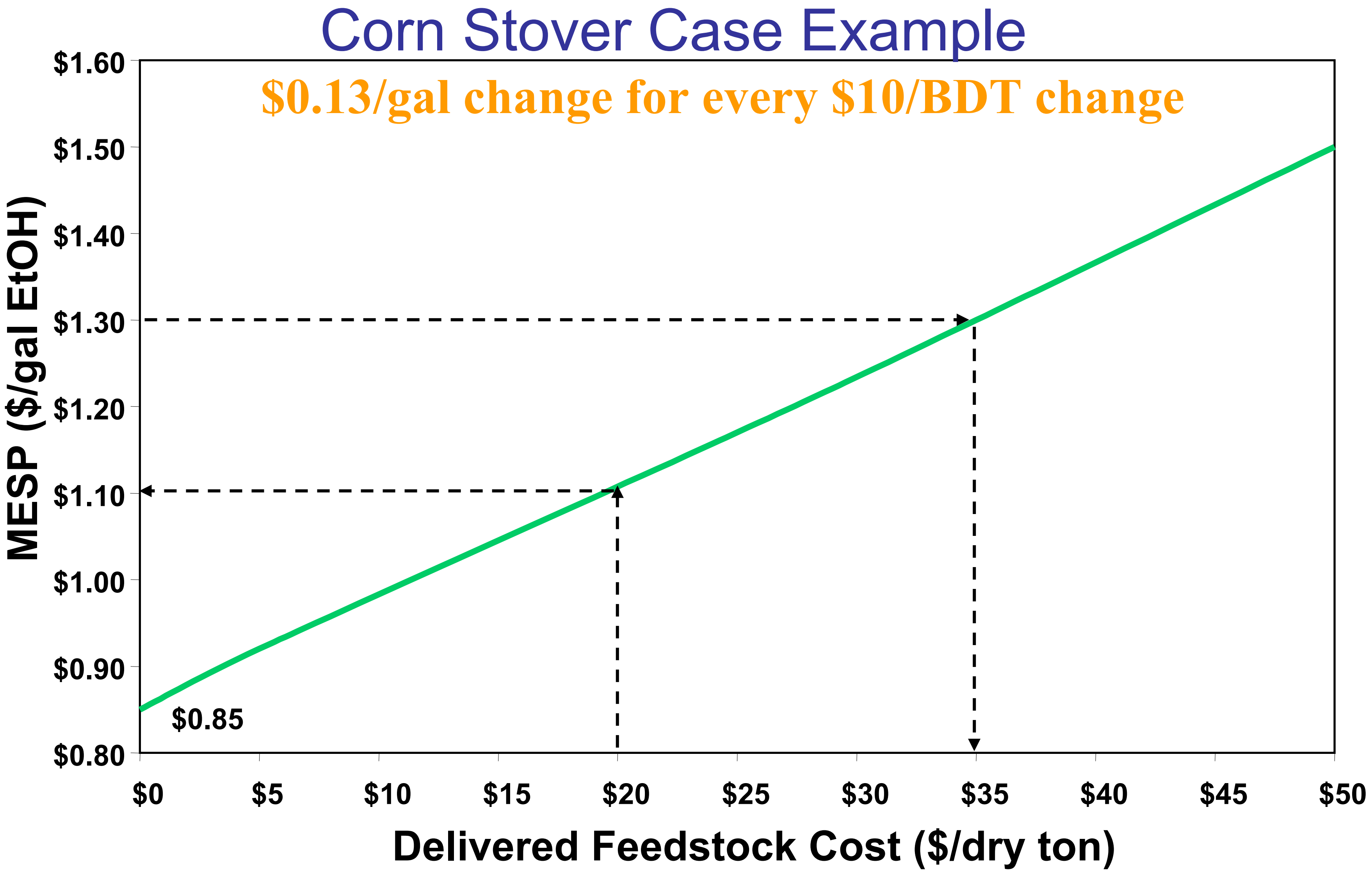
Economic Parameter (Units, $1999)	**Process Case**	**Dry-mill Co-location**	**Coal-fired Power Plant Co-location**
MESP ($/gal)	**$1.30**	**$1.23**	**$1.18**
EtOH Production (MM gal/yr)	60	30 / 30	60
EtOH Yield (gal/dry ton stover)	77.5	77.5	77.5
(gal/bushel corn)		2.85	
TPI ($ MM)	**$200**	**$109 / $70**	**$130**
TPI per Annual Gallon ($/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)

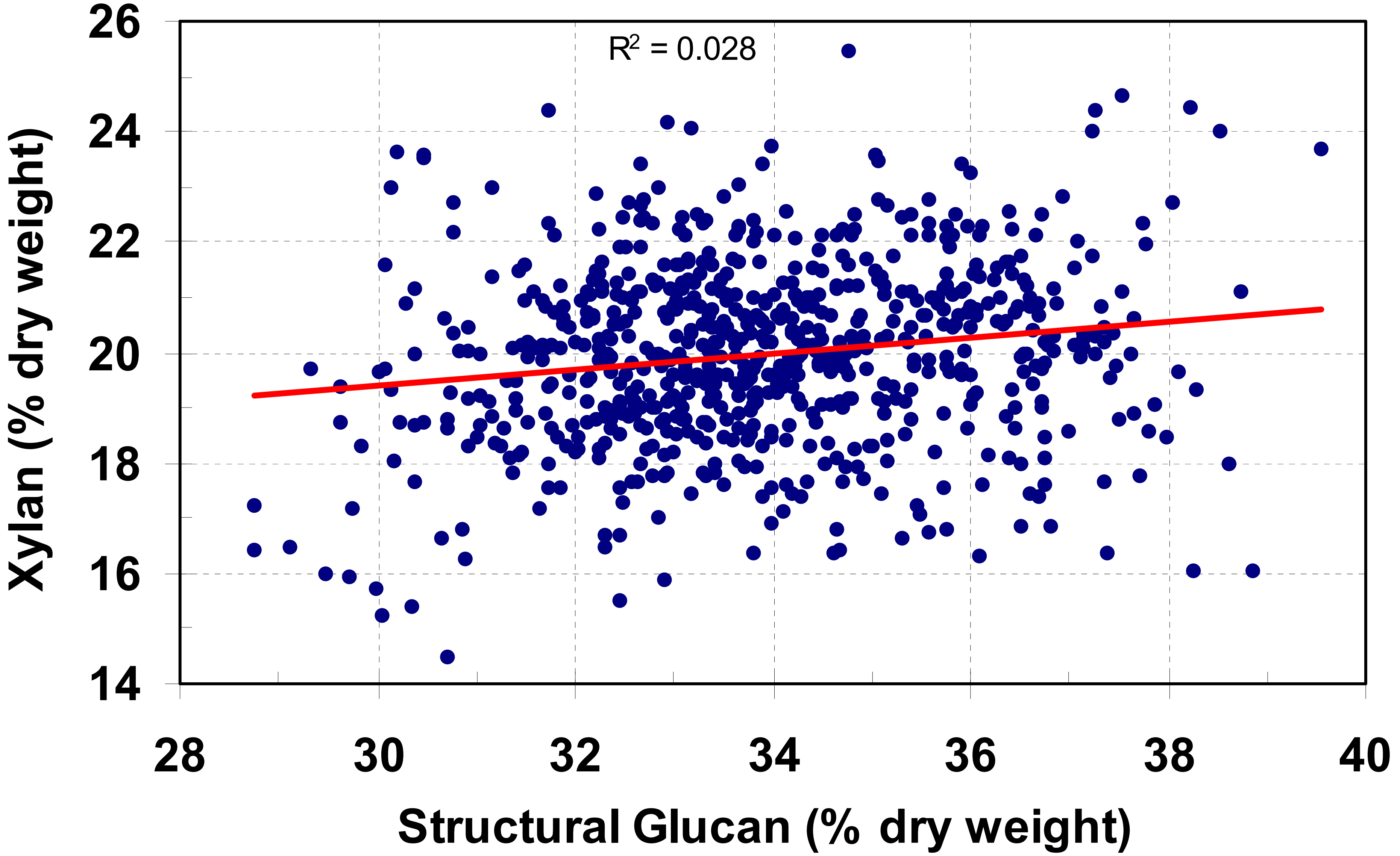
⇒ *Application of NIRS shows that significant knowledge gaps remain about the magnitude and sources of feedstock compositional variability*

Impact of Reducing Feedstock Cost

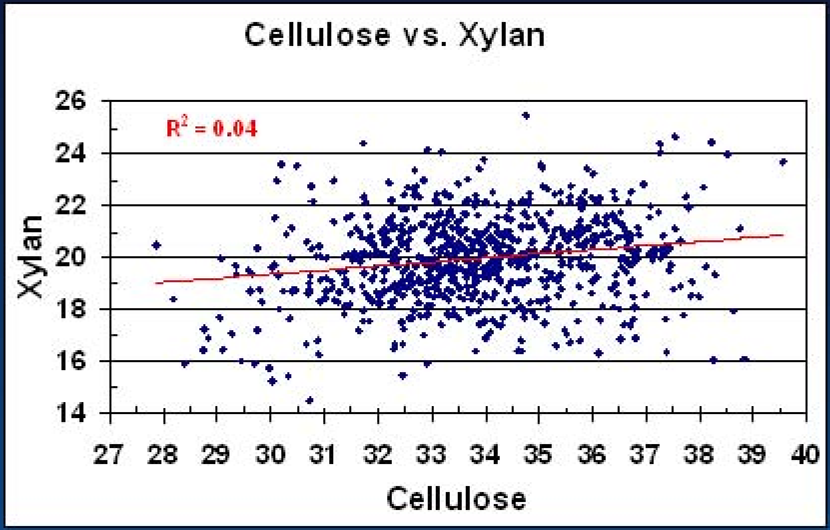


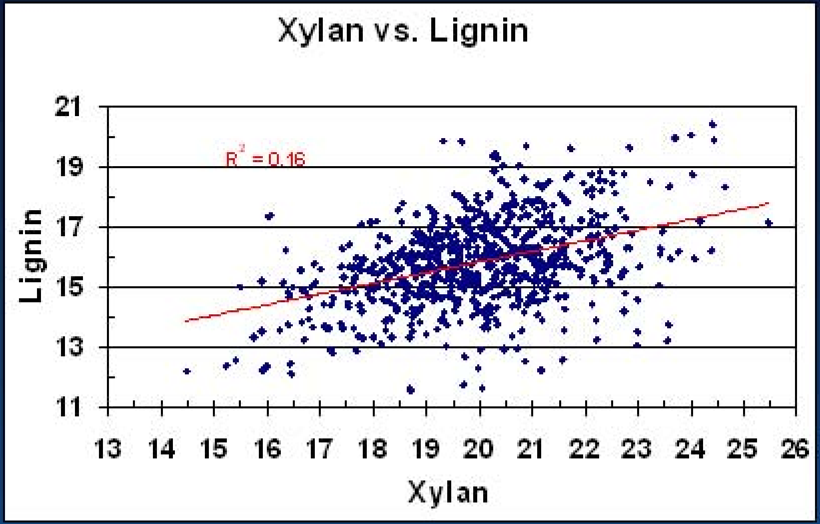
Substantial Feedstock Variability

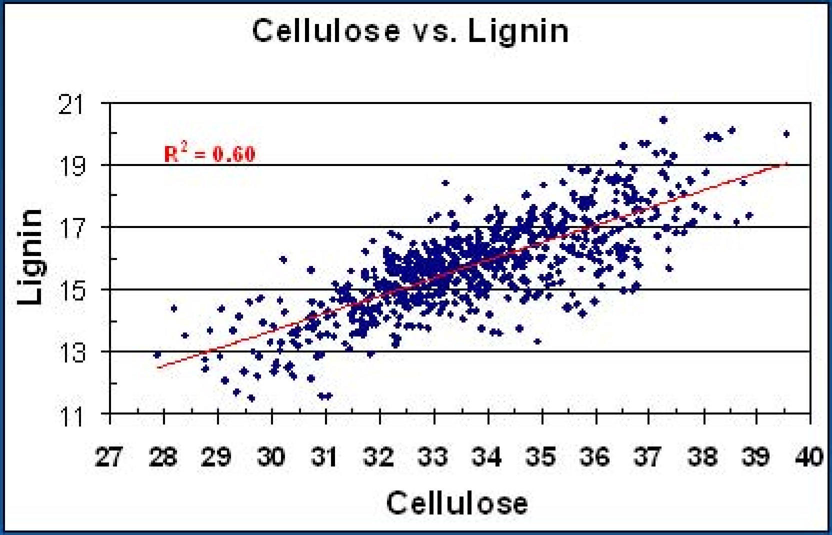
NIR Composition of 731 corn stover samples from the 2001 harvest

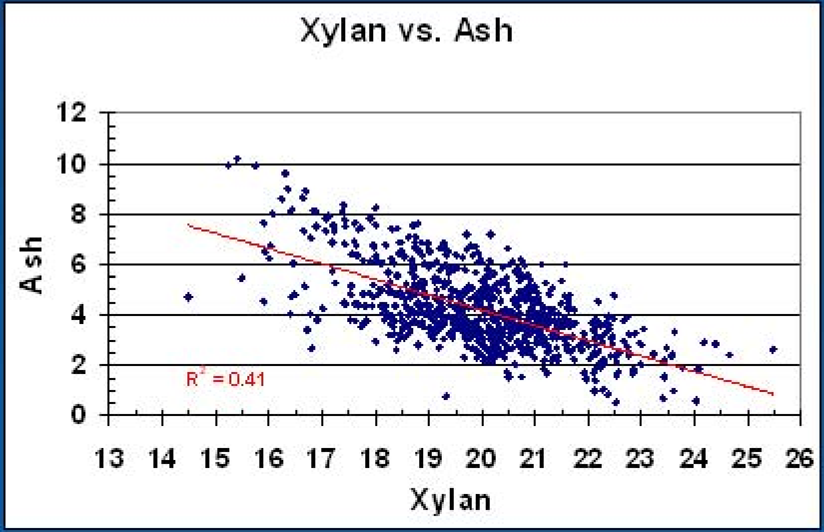


Corn Stover Variability


Cellulose vs. Xylan


Xylan vs. Lignin


Cellulose vs. Lignin

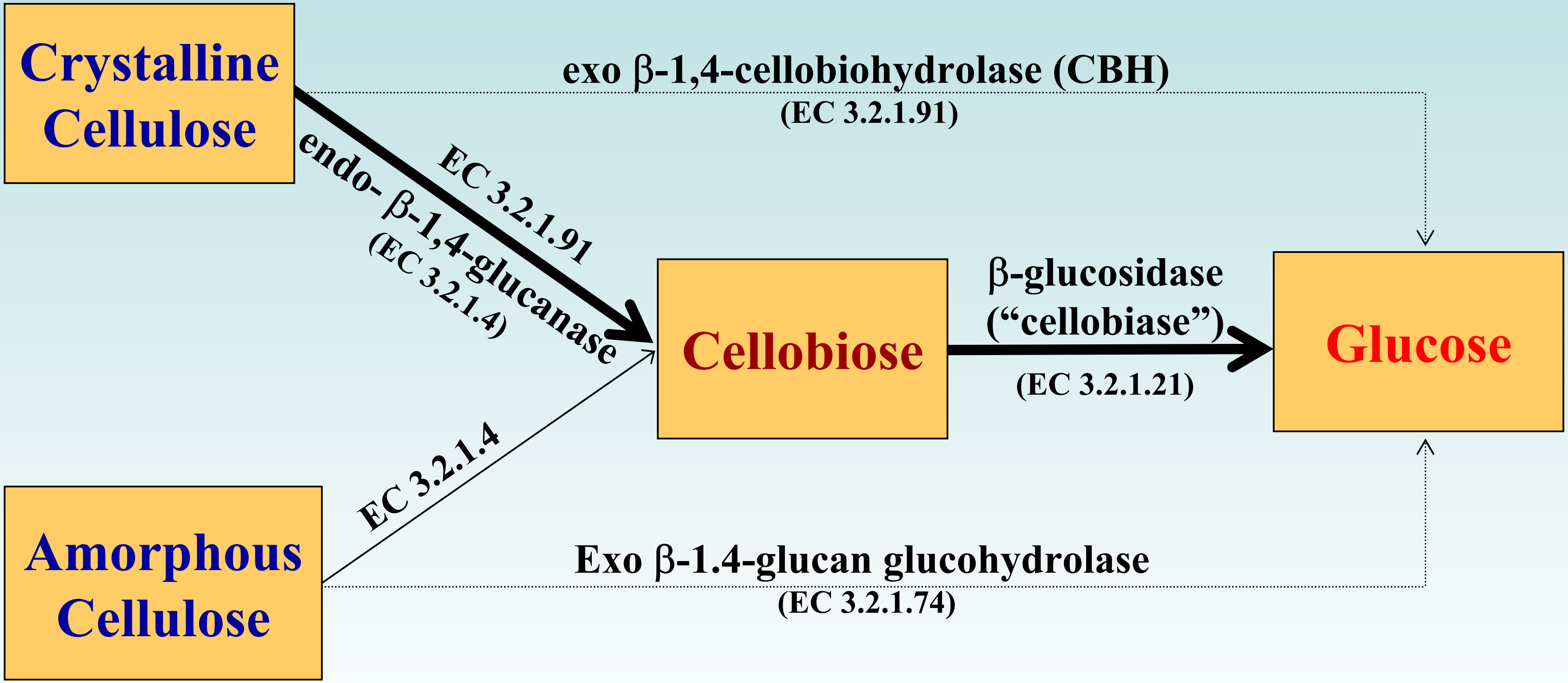

Xylan vs. Ash

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



Crystalline Cellulose

exo β-1,4-cellobiohydrolase (CBH)
(EC 3.2.1.91)

EC 3.2.1.91

endo- β-1,4-glucanase
(EC 3.2.1.4)

Cellobiose

β-glucosidase
(“cellobiase”)
(EC 3.2.1.21)

Glucose

EC 3.2.1.4

Amorphous Cellulose

Exo β-1.4-glucan glucohydrolase
(EC 3.2.1.74)

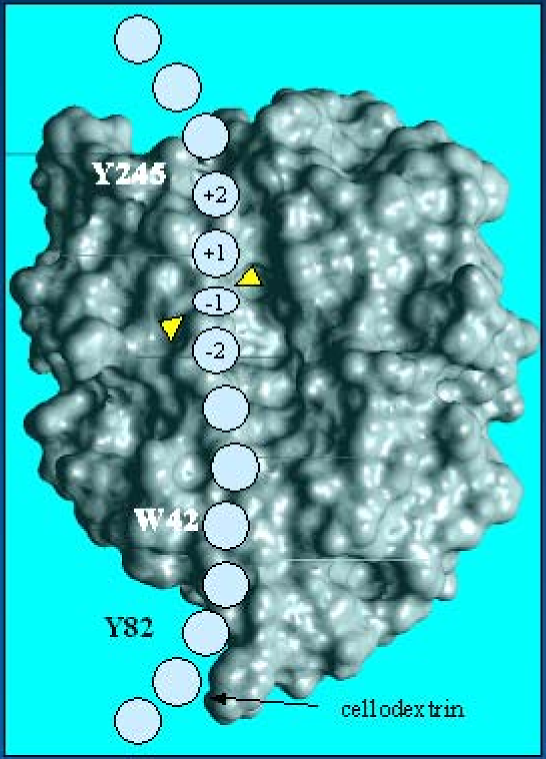
Bold Main Hydrolysis Reactions Proceed via

“Endo” → “Exo” → “β-G”

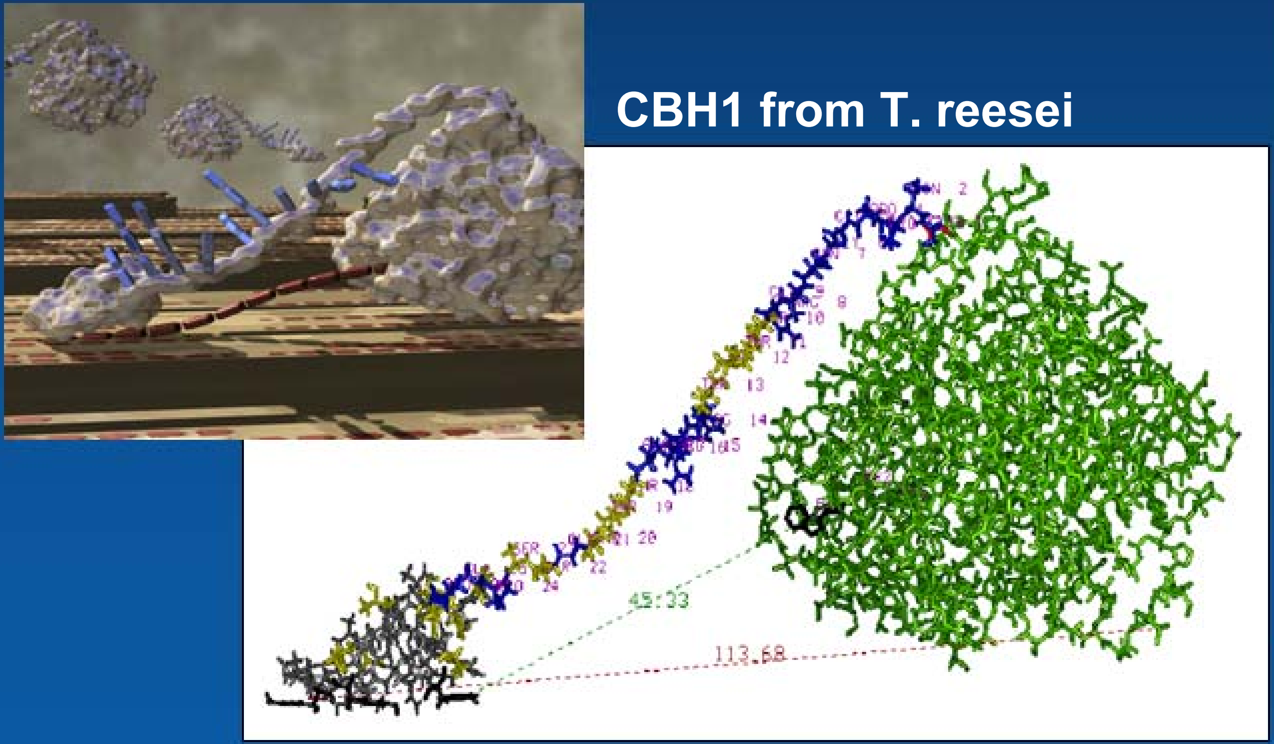
NREL's Enzymatic Hydrolysis Partnerships

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

E1 from *A. cellulotiticus*



CBH1 from T. reesei



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*

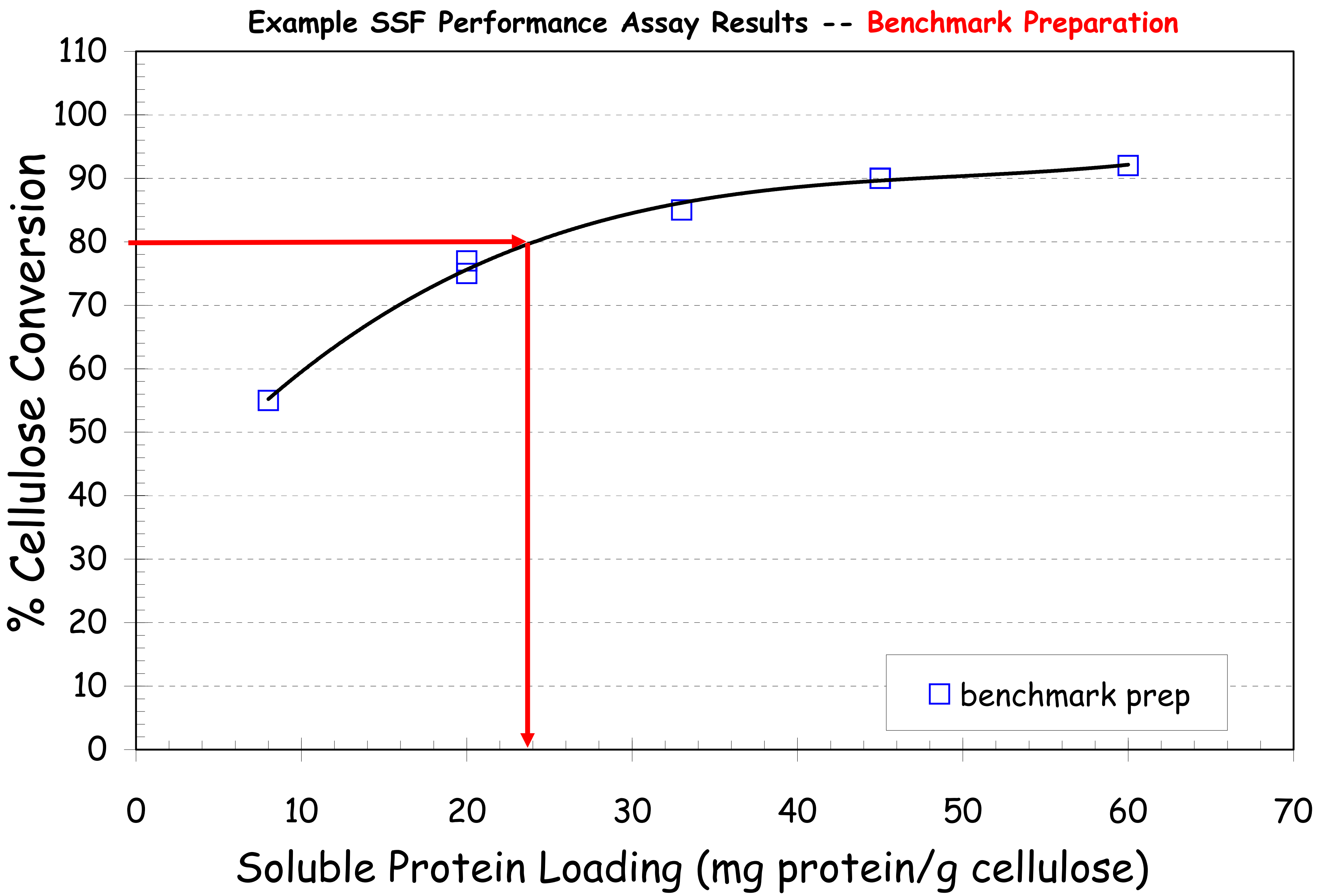
Approach

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

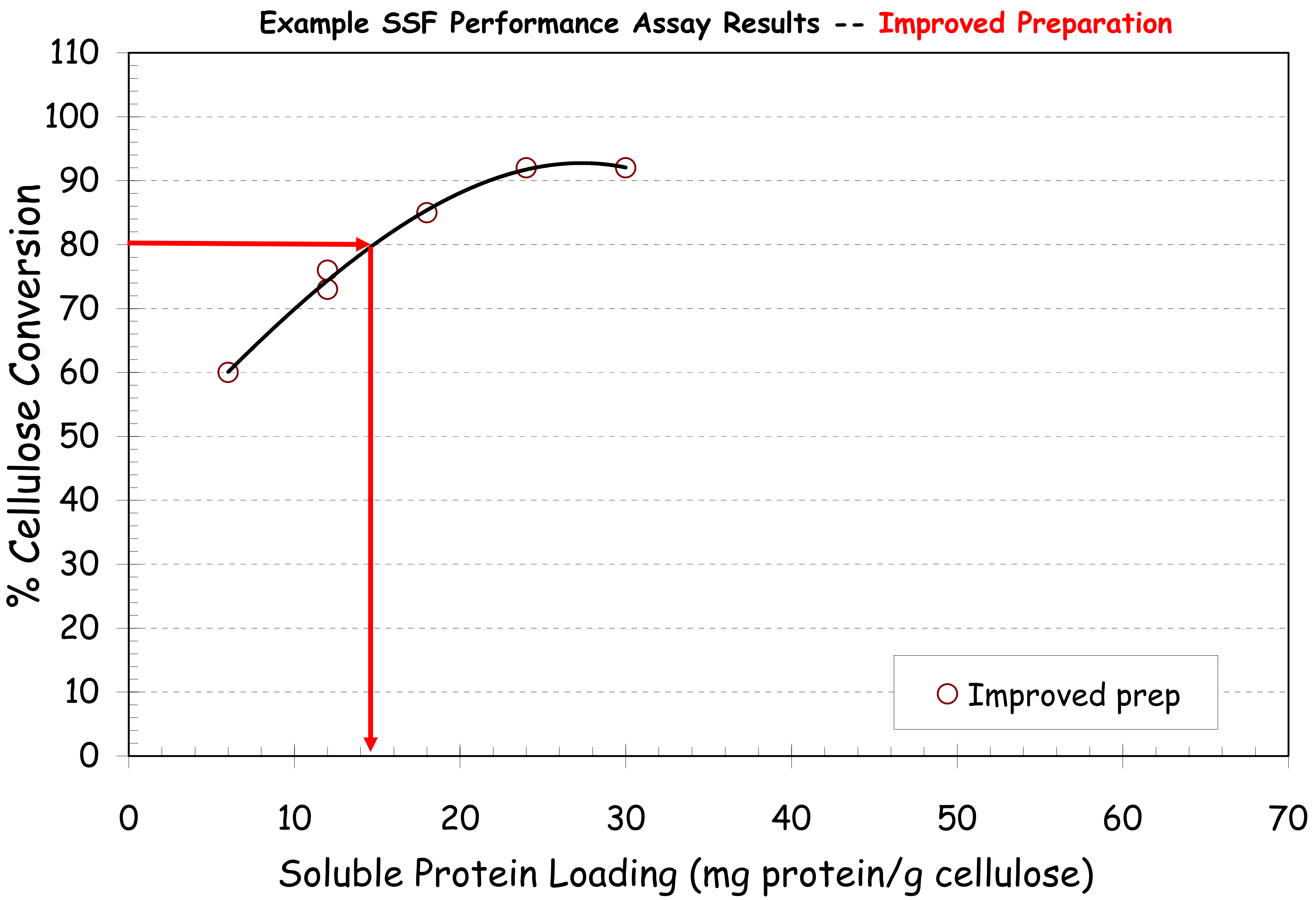
$$C_E = \frac{E_P E_L}{B_N Y}$$

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

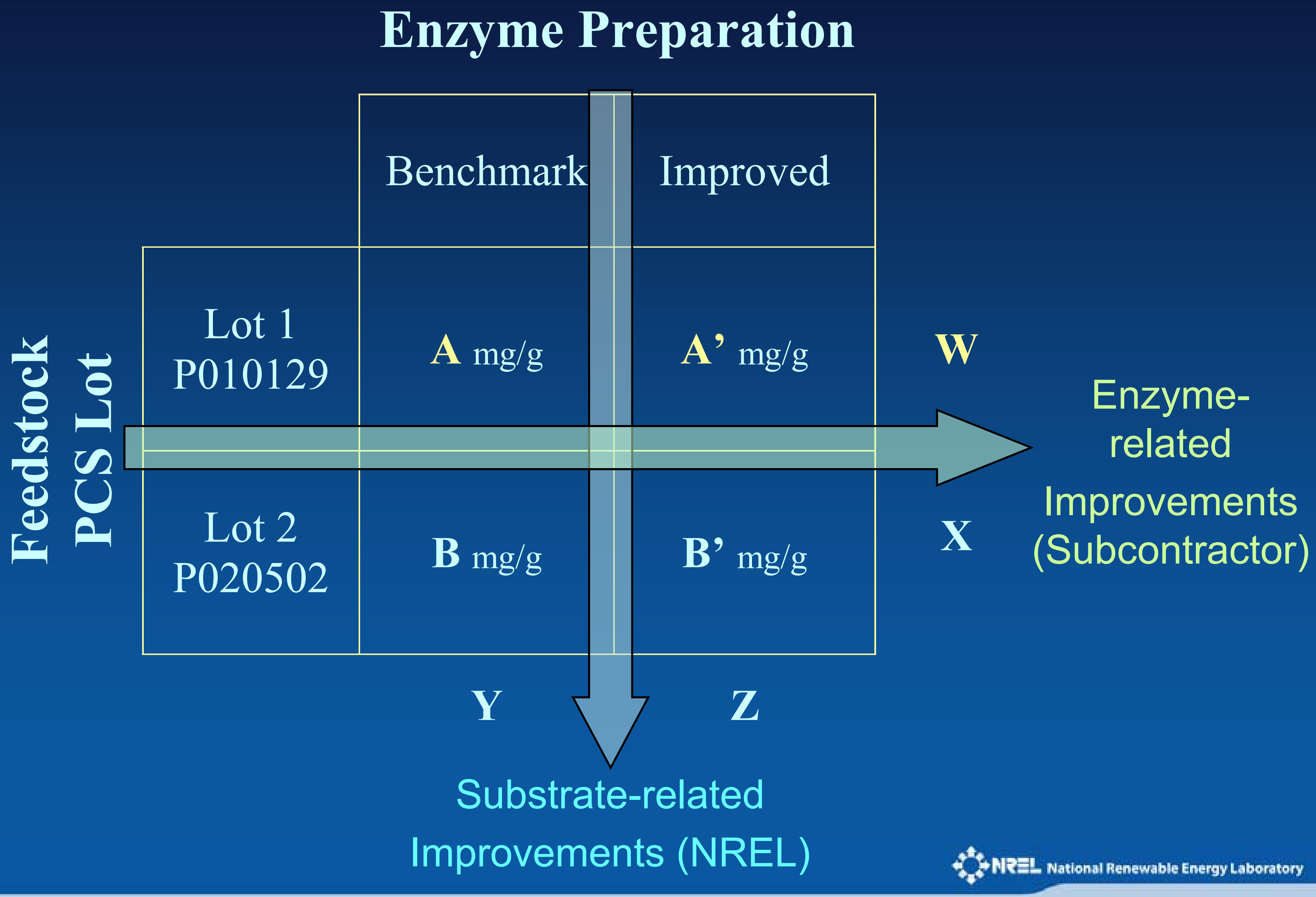
Benchmarking Performance



Measuring Improvement



Overall Improvement Matrix



Enzyme Preparation

Feedstock PCS Lot

	Benchmark	Improved	
Lot 1 P010129	**A** mg/g	**A'** mg/g	**W**
Lot 2 P020502	**B** mg/g	**B'** mg/g	**X**
	Y	**Z**	

Enzyme-related Improvements (Subcontractor)

Substrate-related Improvements (NREL)

Industry-led Cellulase Cost Reduction

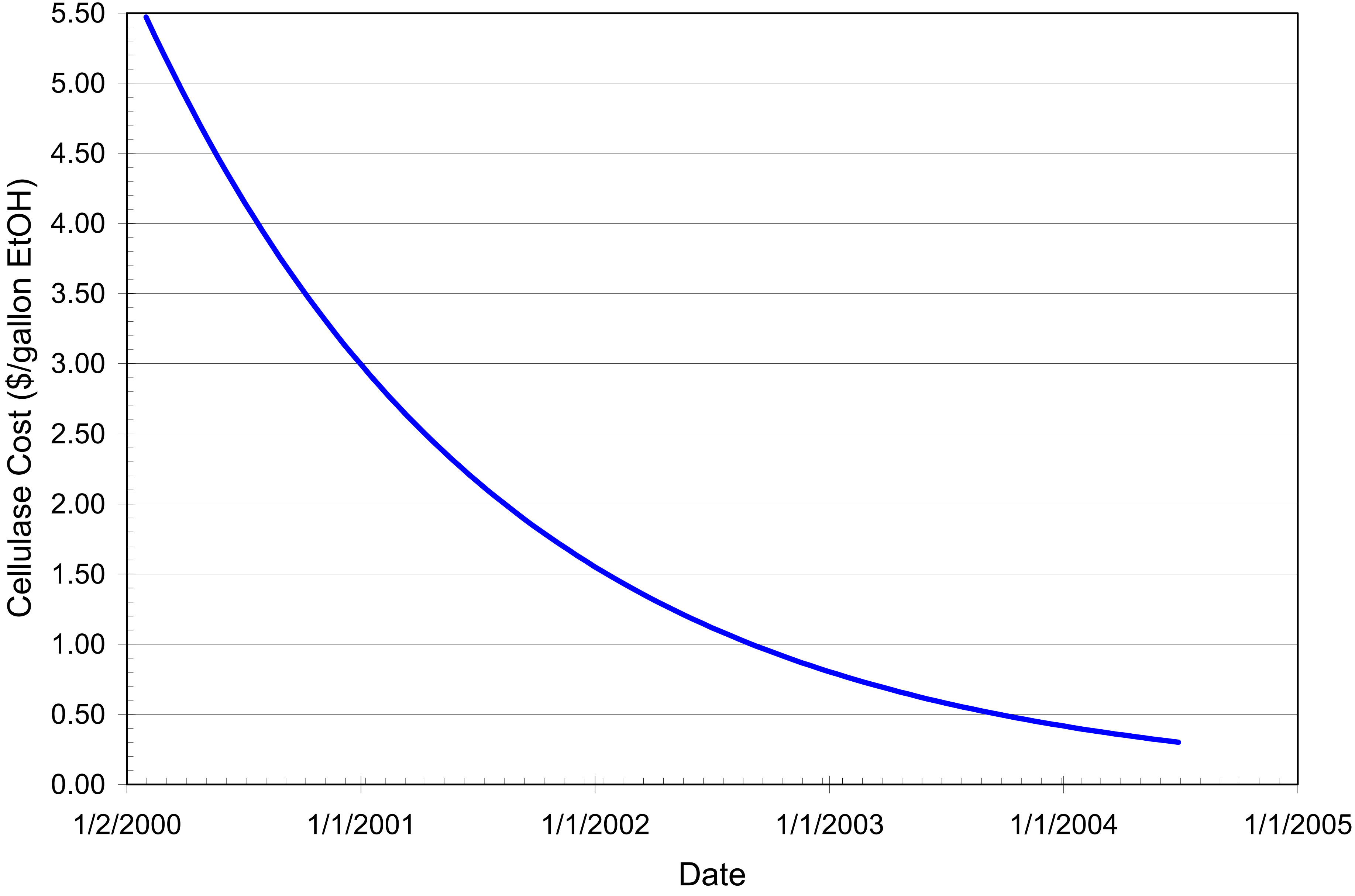
- Similar Subcontracts set up with Genencor and Novozymes to reduce cost of commodity cellulases by tenfold or greater
  - 3 year periods of performance + 1 year extensions
  - 20% cost share by industry
  - Annual performance milestones with ultimate 3 yr 10X goal relative to benchmark established at 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.genencor.com*
    - *http://www.novozymes.com*
      - Go to the companies press web site archives and search on “biomass”

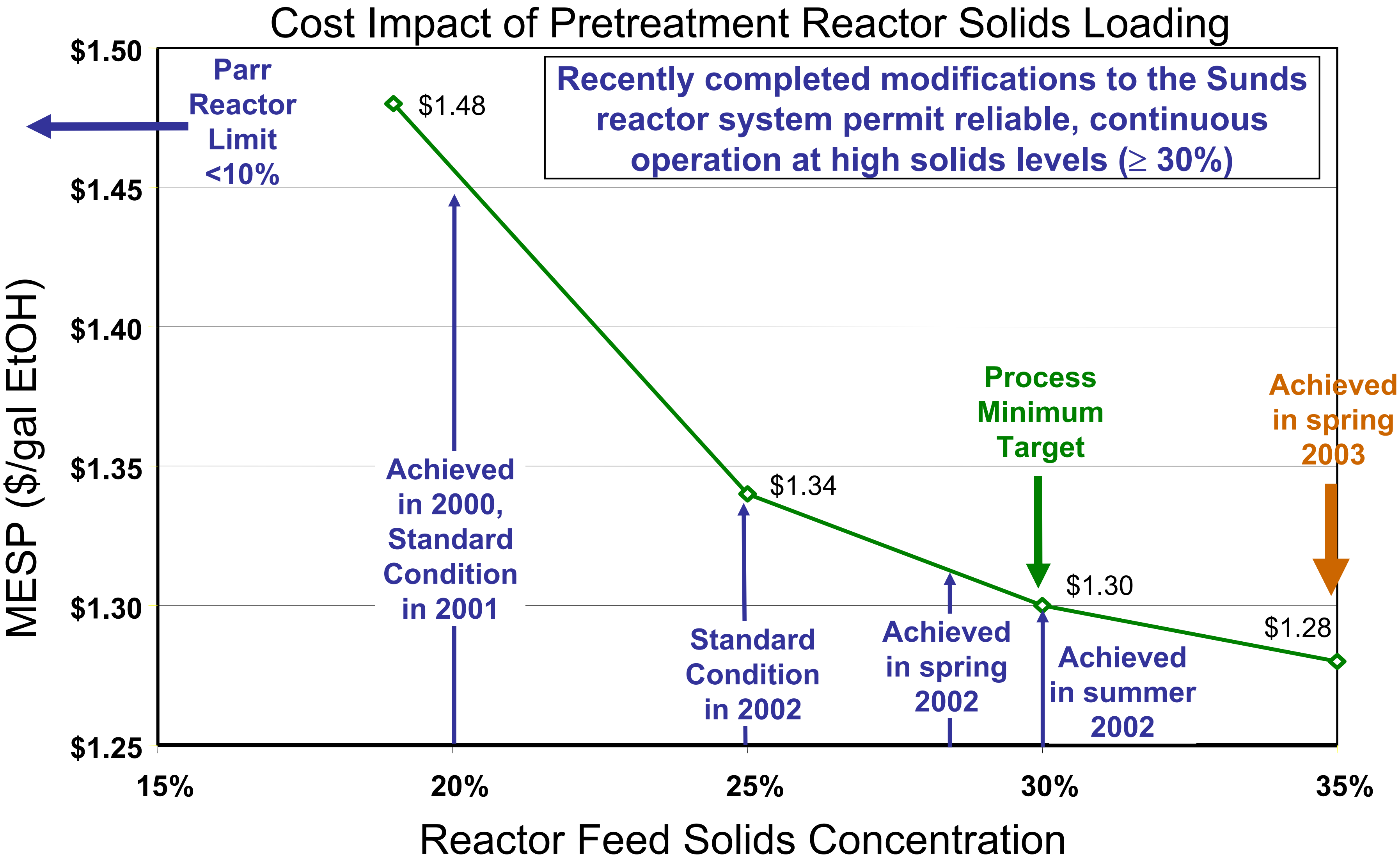
- Highlights/Summary of Reported Accomplishments
  - Both companies exceeded 3 yr 10X cost reduction goal, decreasing estimated enzyme costs from ~$5.00 to $0.30-0.40 per gal EtOH
  - Cost reduction efforts continuing
    - One year extensions finished in 11/04 (Genencor) or 1/05 (Novozymes)

Cellulase Costs Falling Rapidly

Excellent progress being made by industry through DOE subcontracts



Reducing Performance Risk: Demonstrating High-solids Processing



Recently completed modifications to the Sunds reactor system permit reliable, continuous operation at high solids levels (≥ 30%)

Reducing Deployment Risk: Showing Base-line Engineering Feasibility

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

Minimum Pretreatment Performance Targets

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

- 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

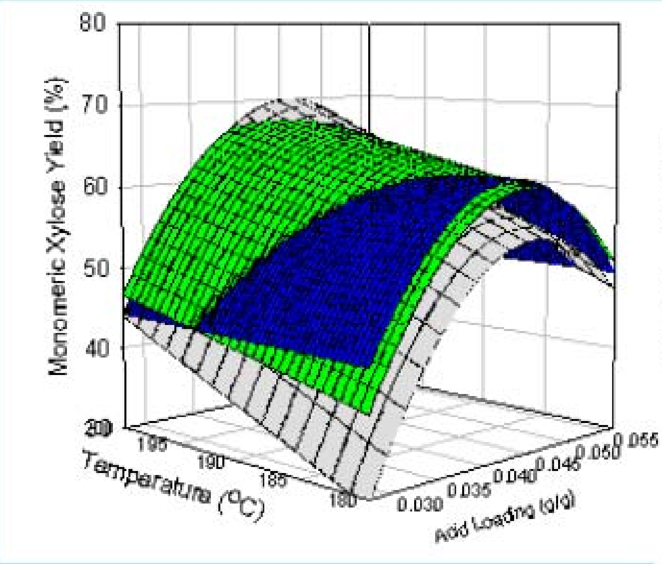
Dilute Sulfuric Acid Pretreatment of Corn Stover

Pretreatment at solids loadings from 25% to 35%

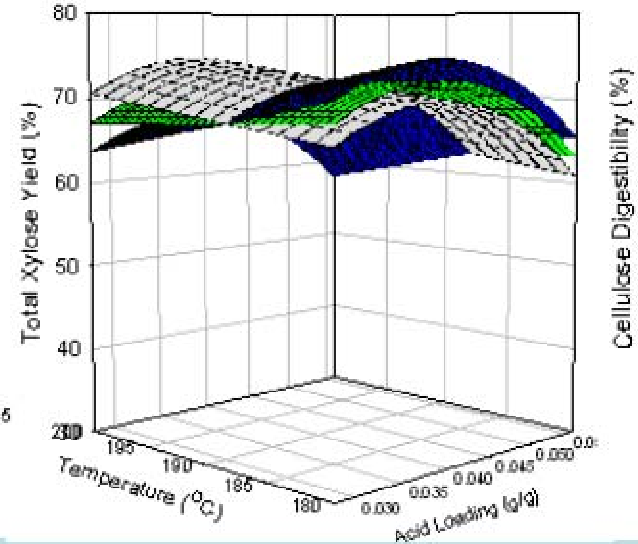
High Solids Pretreatment Performance

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

Xylan Solubilization as a Measure of Hemicellulose Extraction/Hydrolysis Efficiency

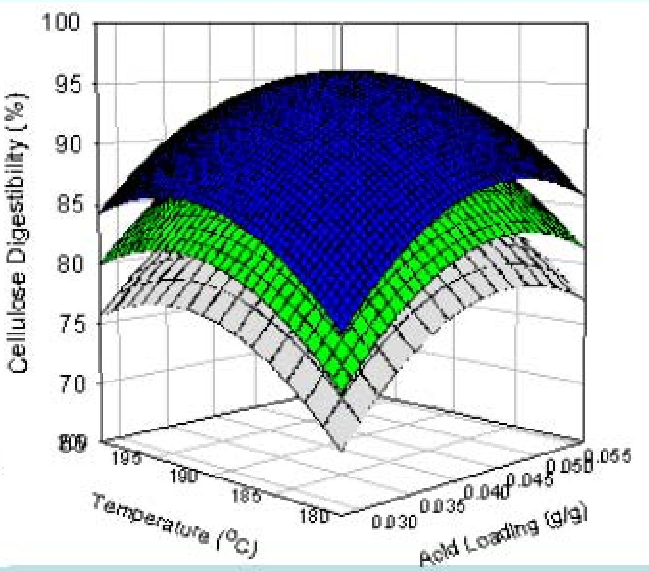


Monomeric Xylose Yield



Total Xylose Yield

Enzymatic Digestibility of Pretreated Solids



Cellulose Digestibility

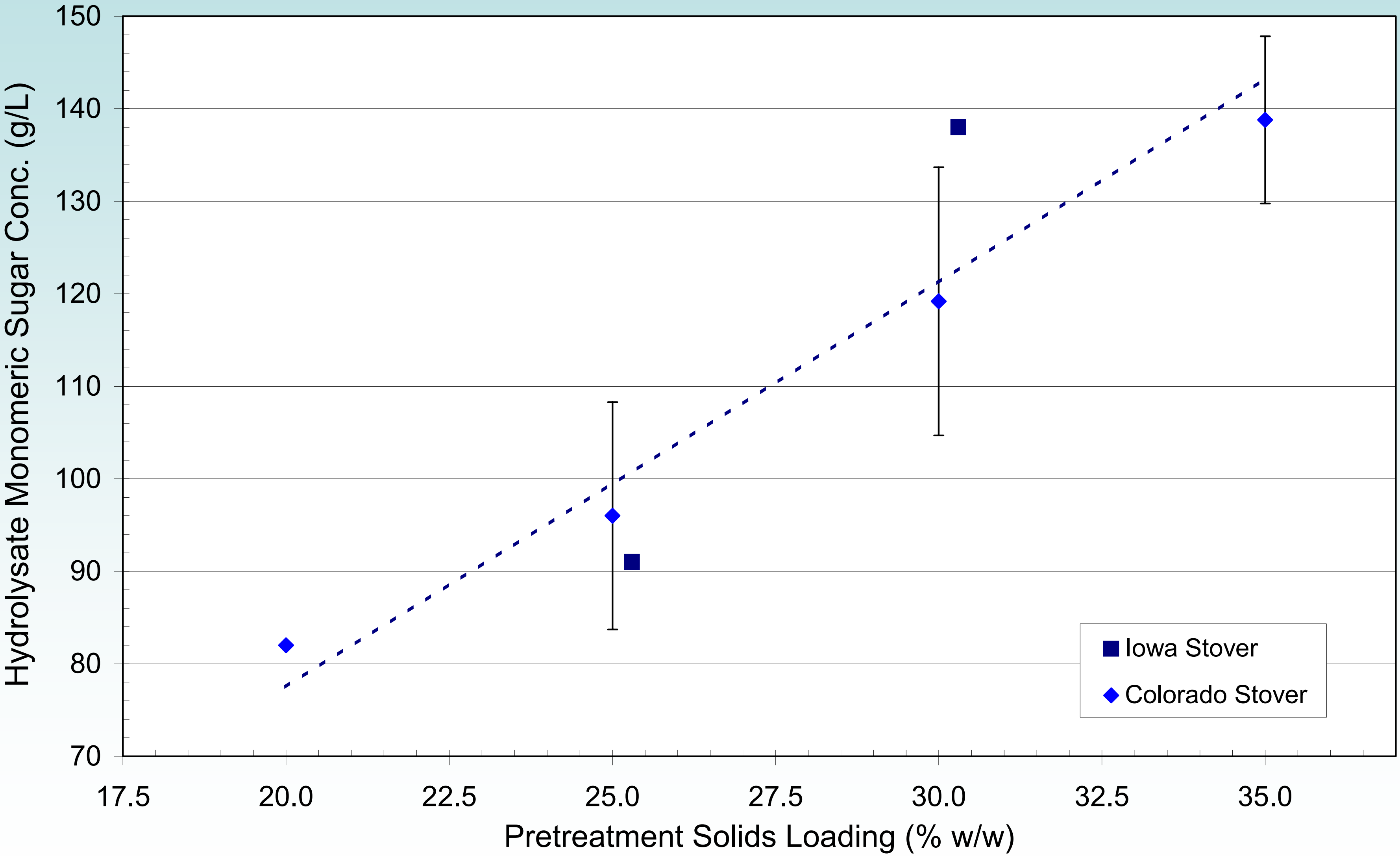
- ⬜ 25% Solids
- 🟩 30% Solids
- 🟦 35% Solids

Examples of Corn Stover Dilute-acid Hemicellulose Hydrolyzate Liquors

Component	Concentration (g/L) (20% solids)	Concentration (g/L) (30% solids)
Glucose	9.24	17.7
Xylose	**59.7**	**93.6**
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

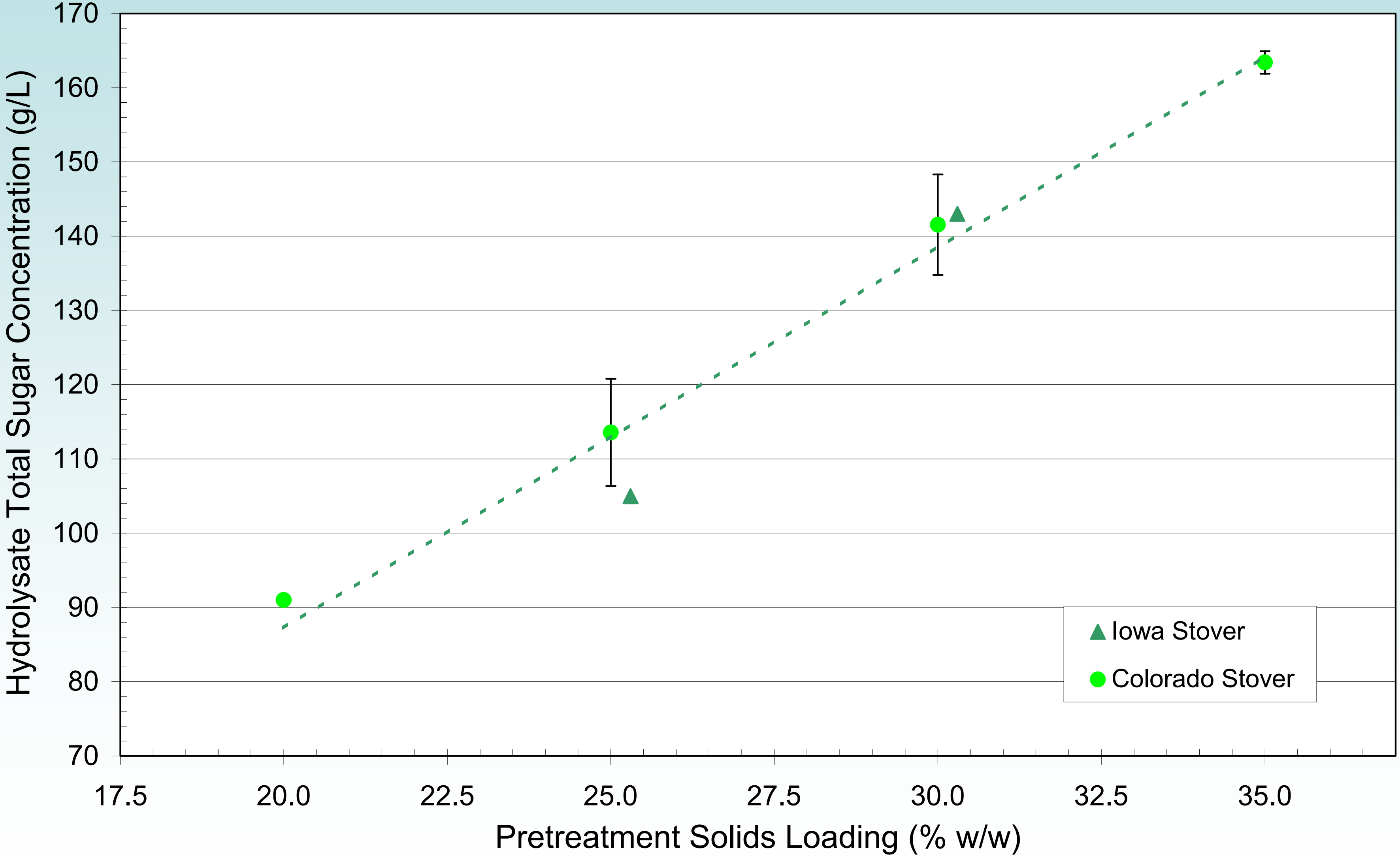
Sugar Concentration = f(Solids Loading)

Ranges in Monomeric Sugar Concentrations



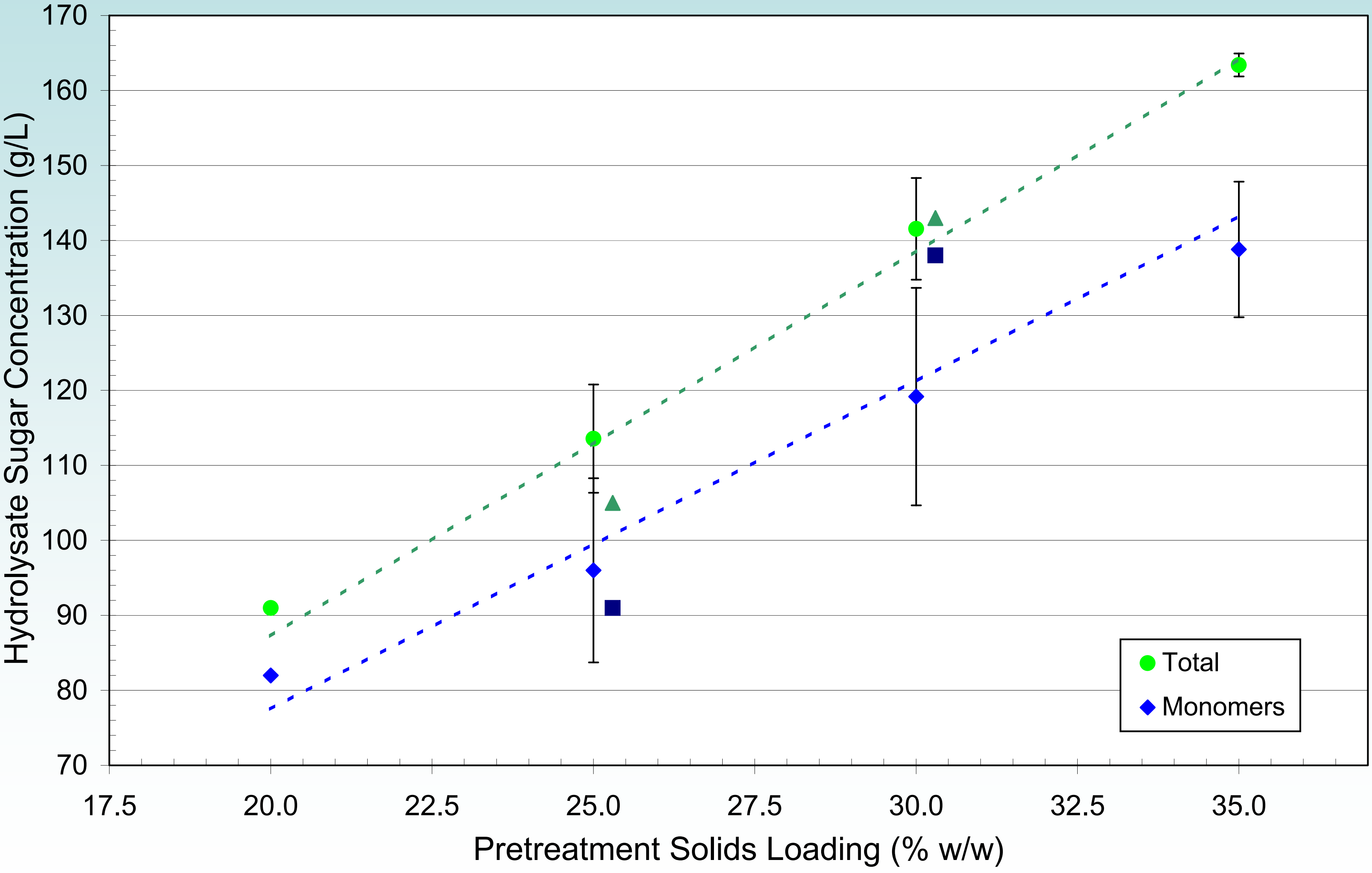
Sugar Concentration = f(Solids Loading)

Ranges in Total Sugar Concentrations



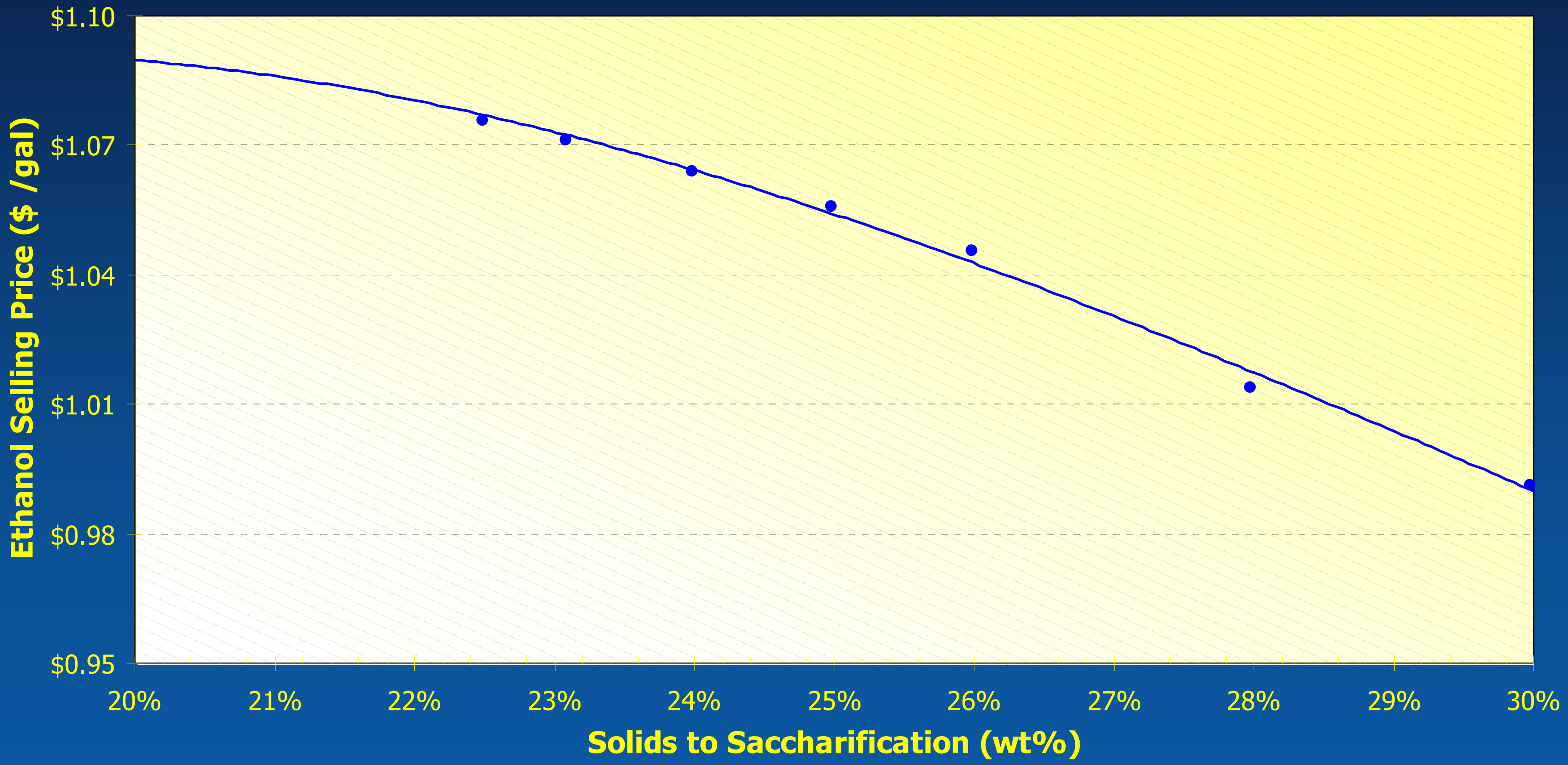
Sugar Concentration = f(Solids Loading)

Comparison of Monomeric versus Total Sugar Concentrations



Impact of Saccharification Solids Loading

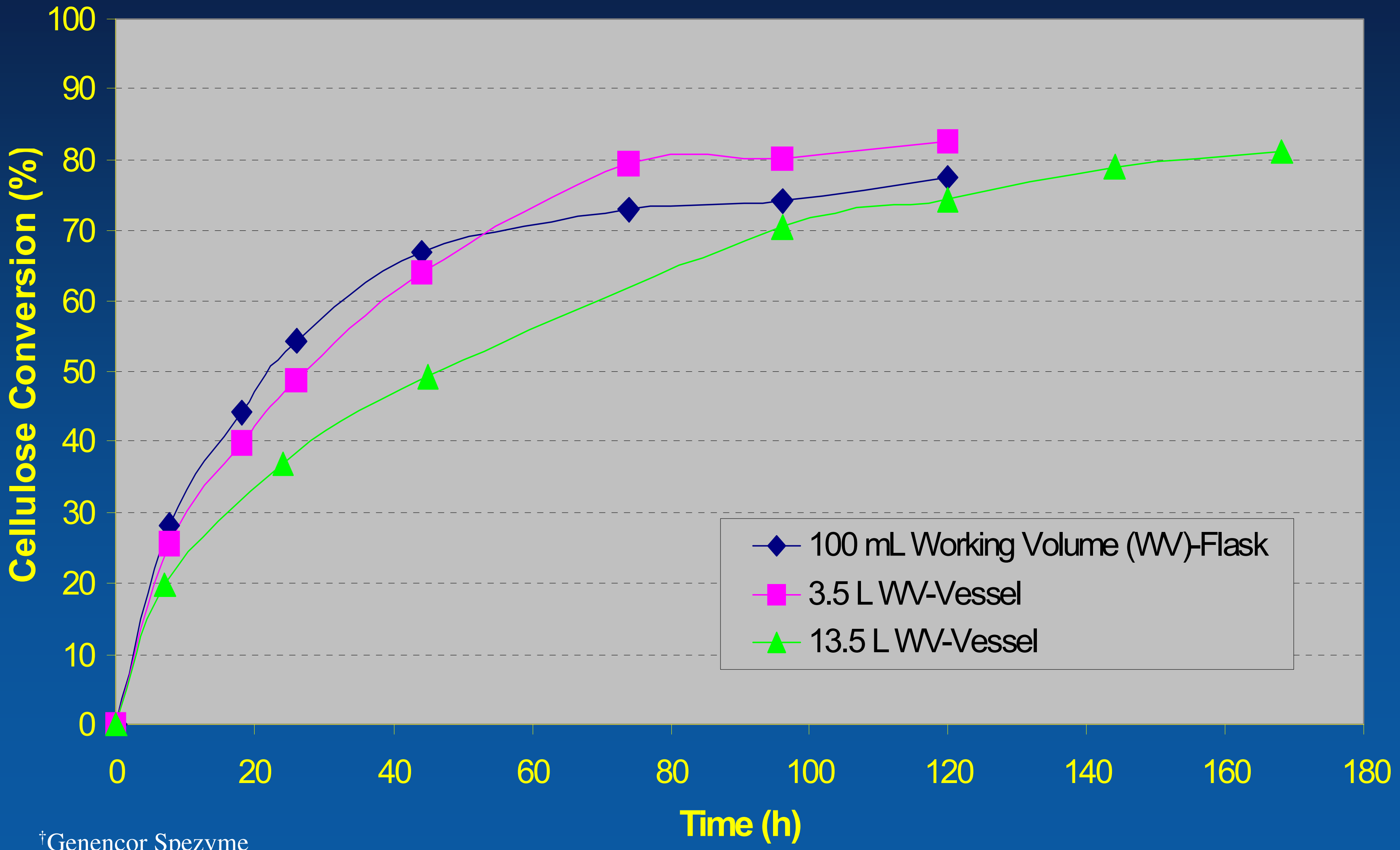
Results of Preliminary Techno-Economic Modeling



Cellulose Saccharification

Assessing Potential Scale-up Issues

Pretreated corn stover, 10% solids loading, 20 mg cellulase[†] protein/g cellulose, 45°C



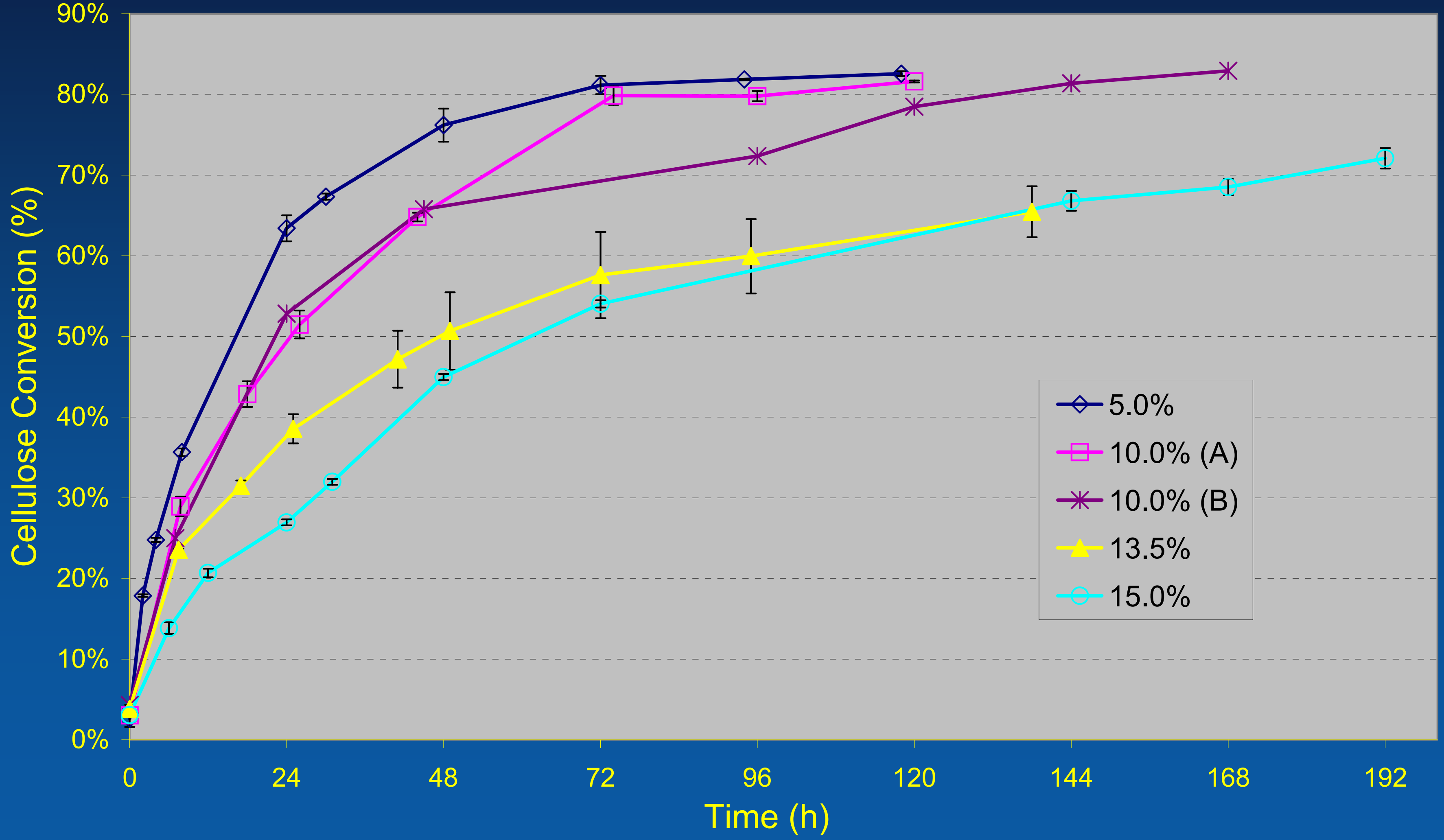
[†] Genencor Spezyme

Cellulose Saccharification

Impact of Solids Loading – Preliminary Results

Pretreated corn stover, 20 mg cellulase[†] 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



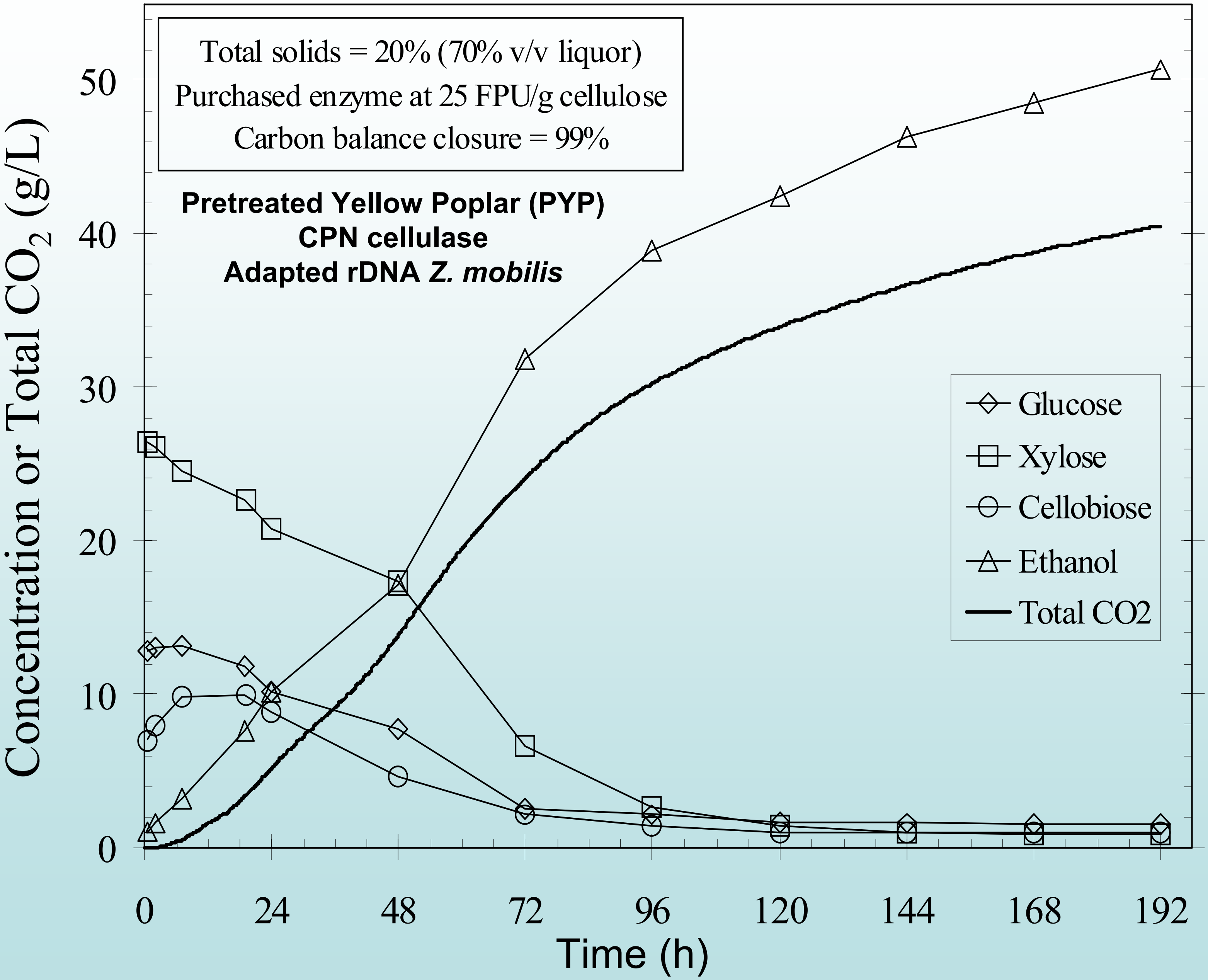
[†] Genencor Spezyme

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

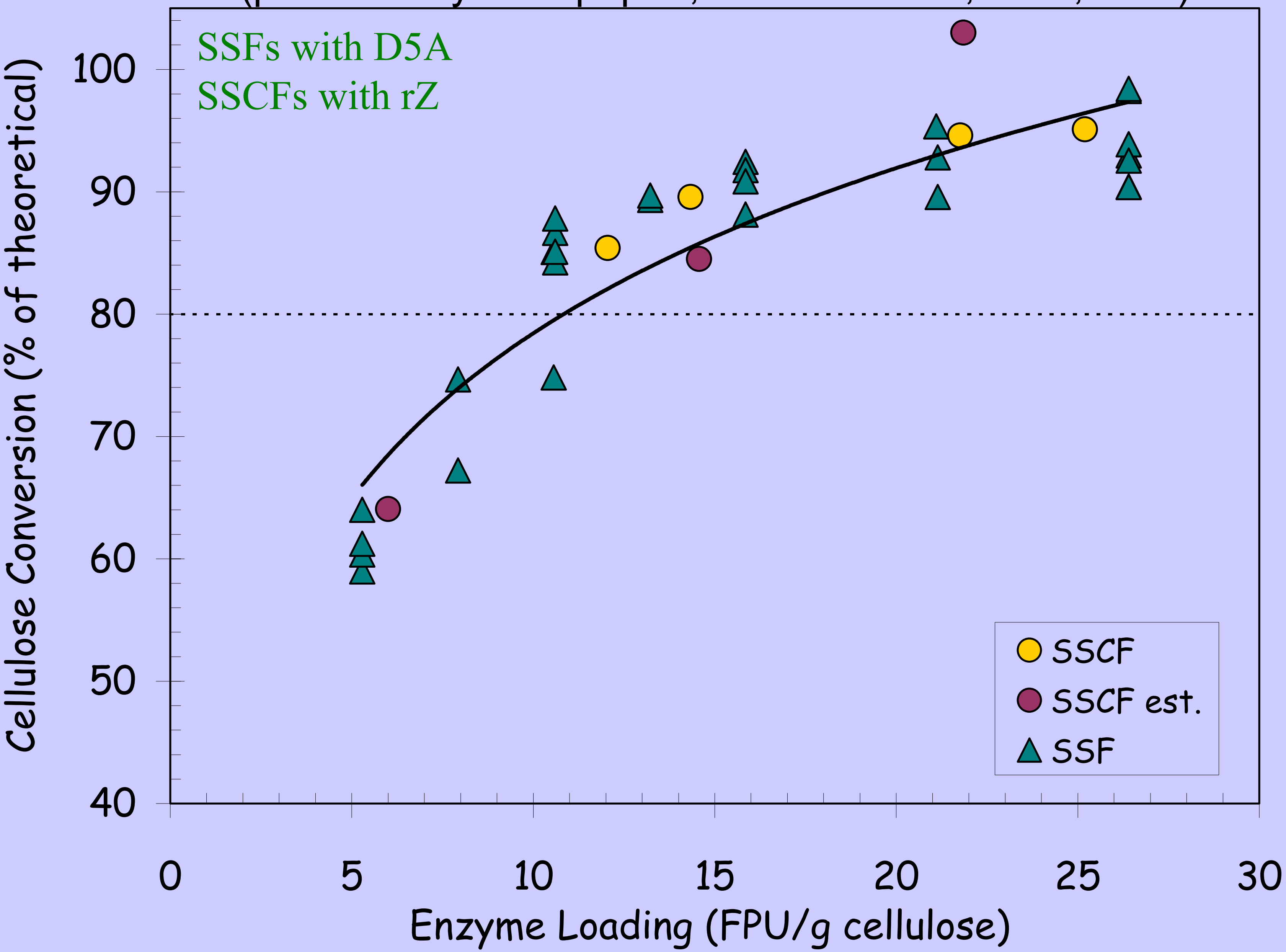


Total solids = 20% (70% v/v liquor)
Purchased enzyme at 25 FPU/g cellulose
Carbon balance closure = 99%

Pretreated Yellow Poplar (PYP)
CPN cellulase
Adapted rDNA *Z. mobilis*

Shakeflask SSF as a Predictor of Integrated SSCF

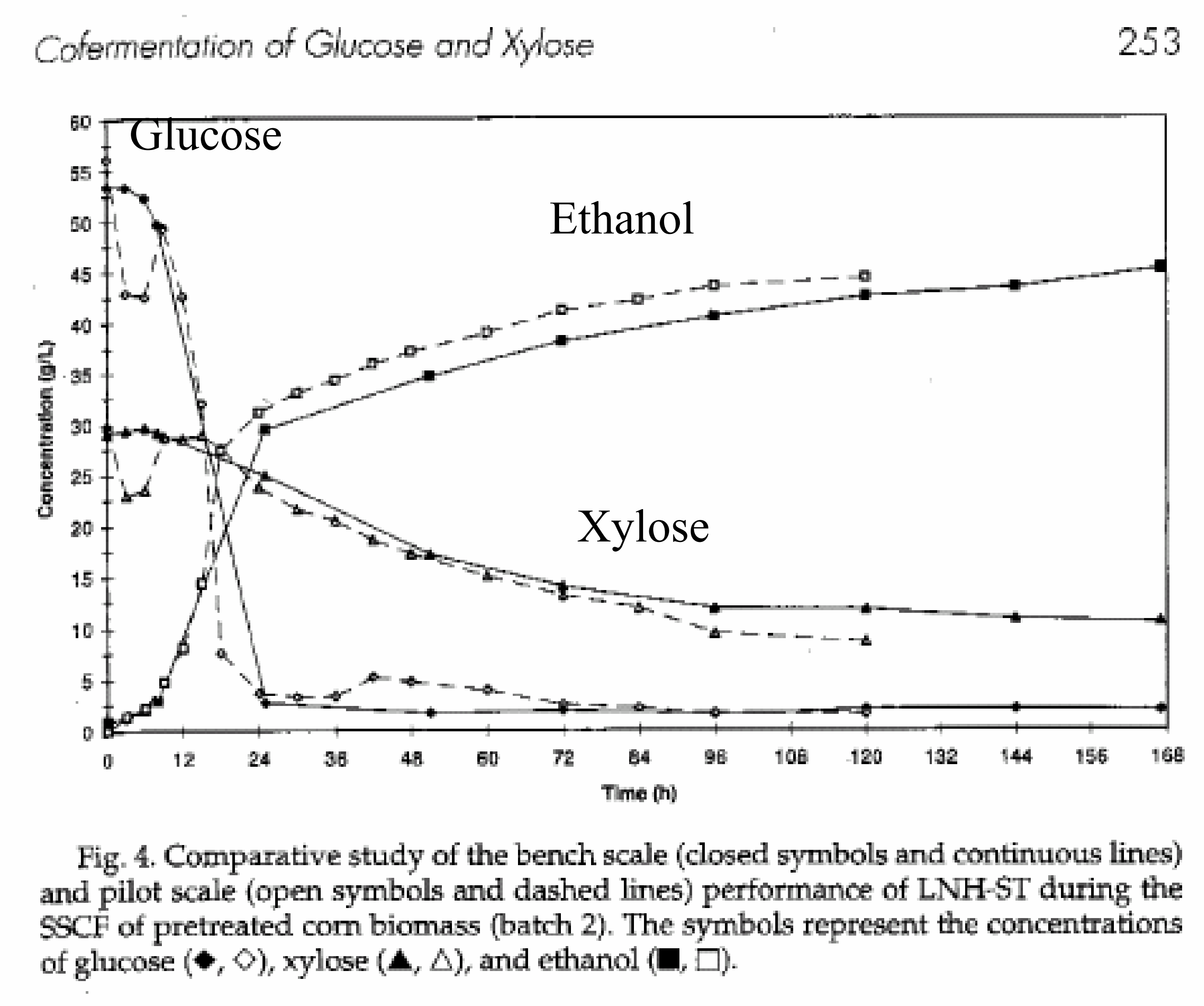
(pretreated yellow poplar, ~6% cellulose, CPN, 32ºC)



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



Cofermentation 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 (■, □).

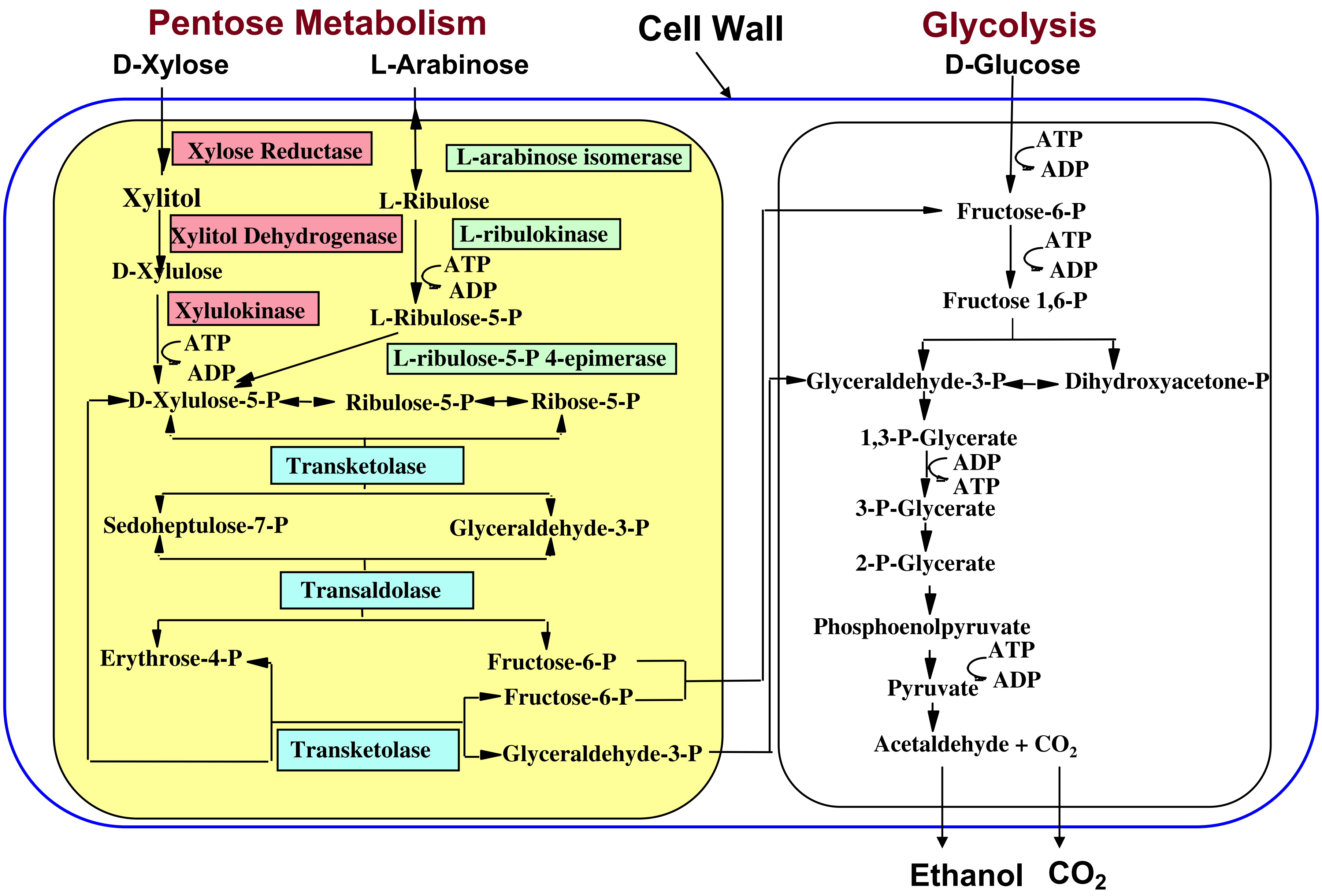
* Figure from: Toon et al.. 1997. Appl. Biochem. Biotechnol. **63-65**: 243-255.

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



Pentose Metabolism

Cell Wall

Glycolysis

D-Xylose

L-Arabinose

D-Glucose

Xylose Reductase

L-arabinose isomerase

Xylitol

L-Ribulose

Xylitol Dehydrogenase

L-ribulokinase

D-Xylulose

ATP

ADP

Xylulokinase

L-Ribulose-5-P

ATP

ADP

L-ribulose-5-P 4-epimerase

D-Xylulose-5-P

Ribulose-5-P

Ribose-5-P

Transketolase

Sedoheptulose-7-P

Glyceraldehyde-3-P

Transaldolase

Erythrose-4-P

Fructose-6-P

Fructose-6-P

Transketolase

Glyceraldehyde-3-P

ATP

ADP

Fructose-6-P

ATP

ADP

Fructose 1,6-P

Glyceraldehyde-3-P

Dihydroxyacetone-P

1,3-P-Glycerate

ADP

ATP

3-P-Glycerate

2-P-Glycerate

Phosphoenolpyruvate

ATP

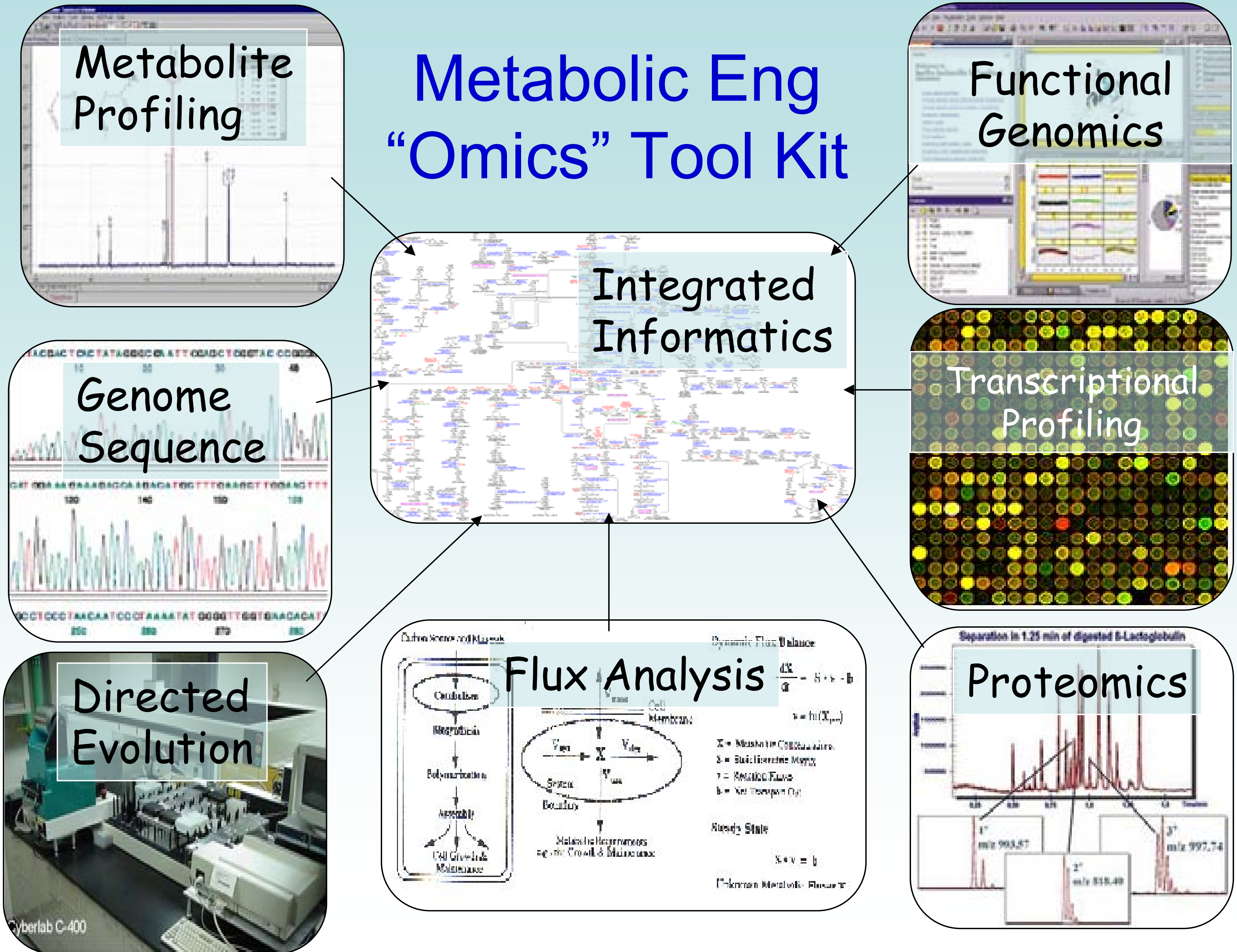
Pyruvate

ADP

Acetaldehyde + CO_2

Ethanol **CO_2**

Metabolic Eng "Omics" Tool Kit



Metabolite Profiling

Functional Genomics

Integrated Informatics

Genome Sequence

Transcriptional Profiling

Directed Evolution

Flux Analysis

Proteomics

Outline

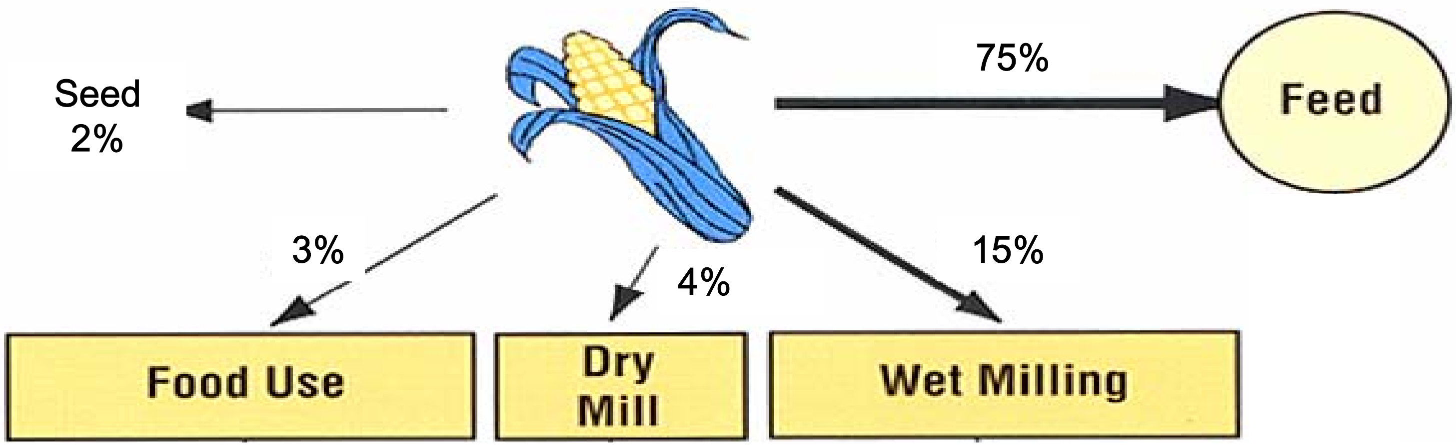
- Biomass Basics
- Overview of Conversion Options
- Details of Enzyme-based Technology
- Biorefining Now and in the Future

Todays 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



Seed
2%

75%

Feed

3%

4%

15%

Food Use

Dry Mill

Wet Milling

Directly Consumed
- Sweet corn
- Popcorn

Processed to
- Flours
- Grits
- Bran
- Tortillas
- Chips

Processed to
- Ethanol
- Feed

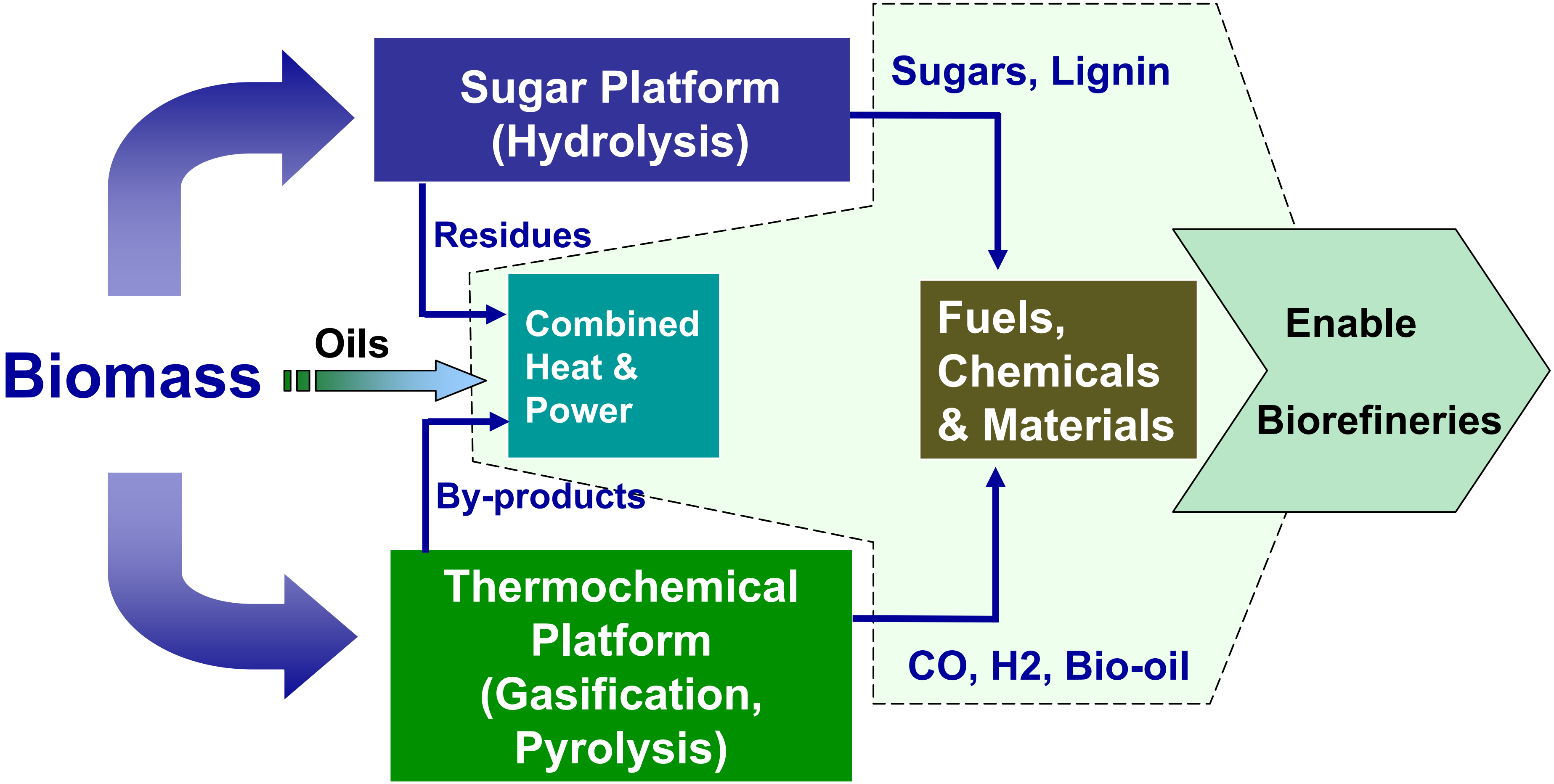
Processed to

- Oil
- Gluten
- Foods
- Starch
- Industrial Products

<u>Starch to Sugar Products</u>
- Syrups
- Ethanol
- Industrial Fermentation Products (many)

Emerging products
- polymers & chemicals

Biomass Conversion Technology "*Platforms*"



Biomass

Oils

Sugar Platform (Hydrolysis)

Residues

Sugars, Lignin

Combined Heat & Power

By-products

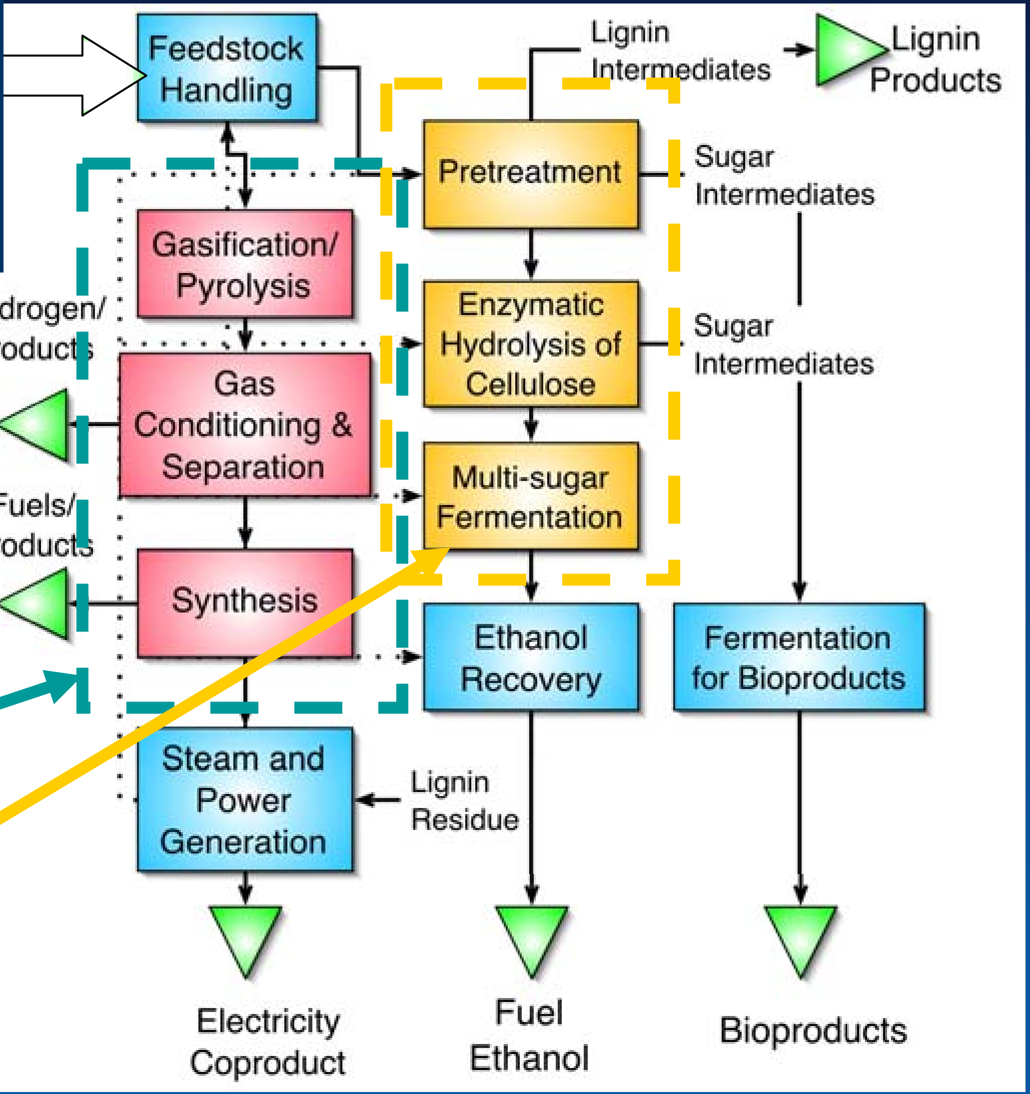
Thermochemical Platform (Gasification, Pyrolysis)

CO, H2, Bio-oil

Fuels, Chemicals & Materials

Enable Biorefineries

Cellulosic Biorefinery Vision


Feedstock Handling
Lignin Intermediates
Lignin Products
Pretreatment
Sugar Intermediates
Gasification/ Pyrolysis
Hydrogen/ Products
Enzymatic Hydrolysis of Cellulose
Sugar Intermediates
Gas Conditioning & Separation
Multi-sugar Fermentation
Fuels/ Products
Synthesis
Ethanol Recovery
Fermentation for Bioproducts
Steam and Power Generation
Lignin Residue
Electricity Coproduct
Fuel Ethanol
Bioproducts

An integrated biorefinery will make use of:

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

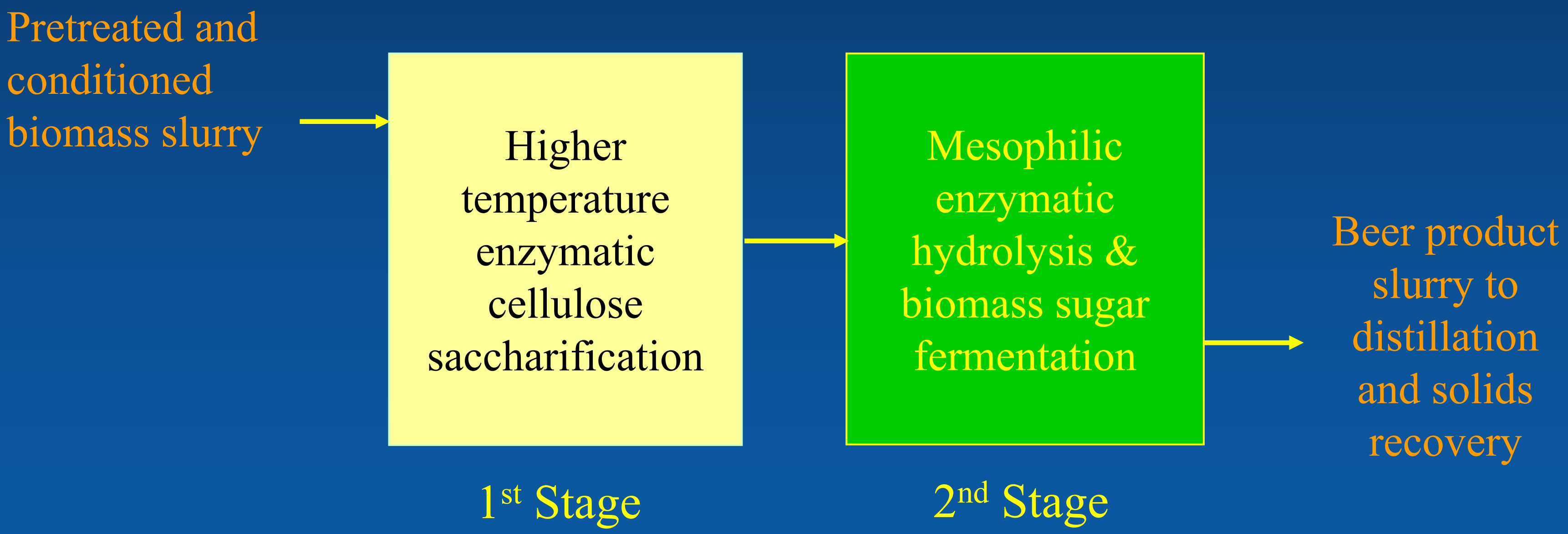
Challenges to Deploying Future Lignocellulosic Biorefineries

- 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
  - 1st stage: Operate at enzymes’ T_{opt} to exploit thermostability and produce an intermediate sugar stream (consistent with “sugar platform” concept)
  - 2nd stage: Inoculate, run in SSF/SSCF mode to achieve high cellulose conversion yield



Pretreated and conditioned biomass slurry

Higher temperature enzymatic cellulose saccharification

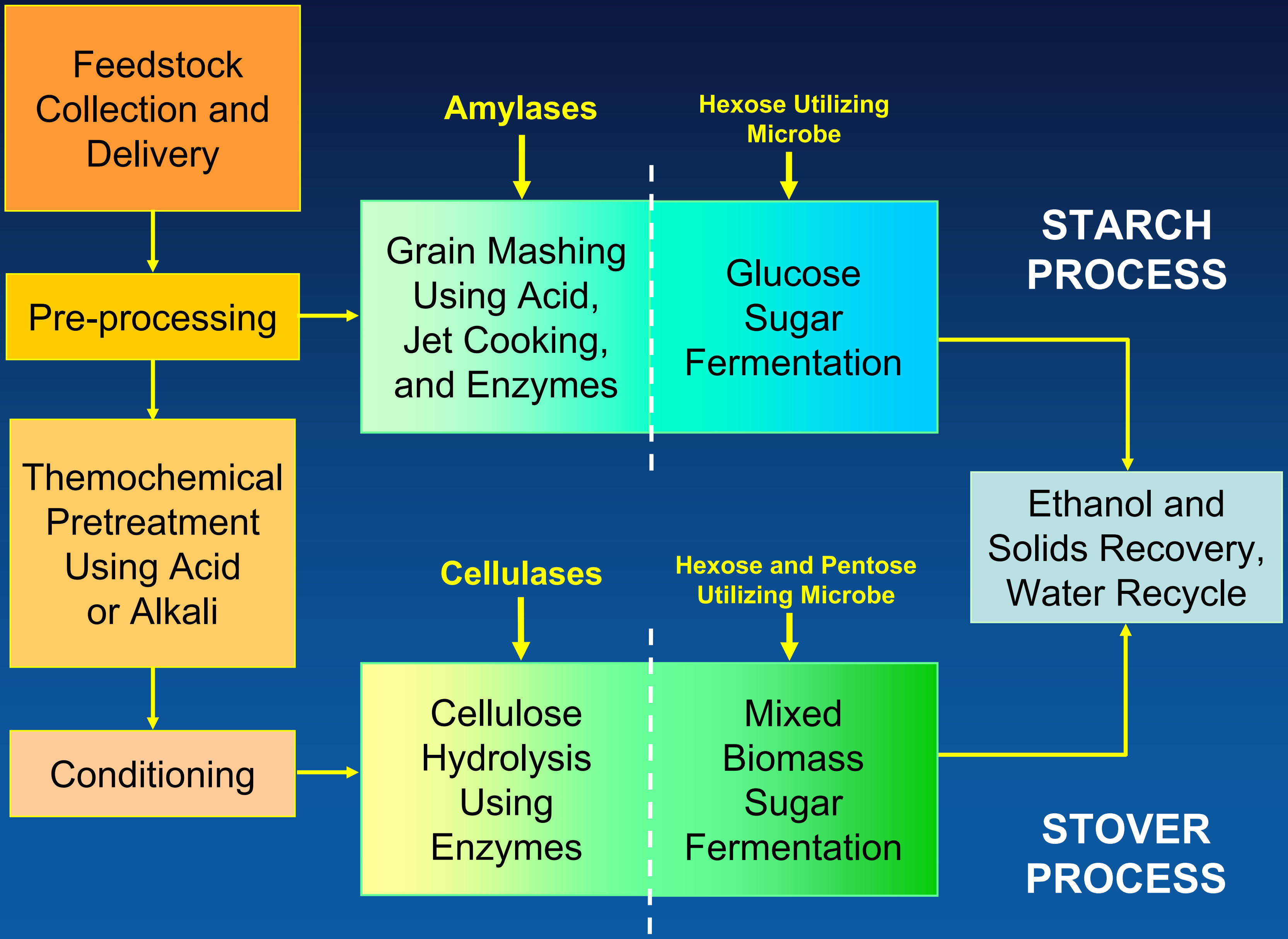
Mesophilic enzymatic hydrolysis & biomass sugar fermentation

Beer product slurry to distillation and solids recovery

1st Stage

2nd Stage

Conversion Process Steps



Feedstock Collection and Delivery

Pre-processing

Themochemical Pretreatment Using Acid or Alkali

Conditioning

Amylases

Hexose Utilizing Microbe

Grain Mashing Using Acid, Jet Cooking, and Enzymes

Glucose Sugar Fermentation

STARCH PROCESS

Cellulases

Hexose and Pentose Utilizing Microbe

Cellulose Hydrolysis Using Enzymes

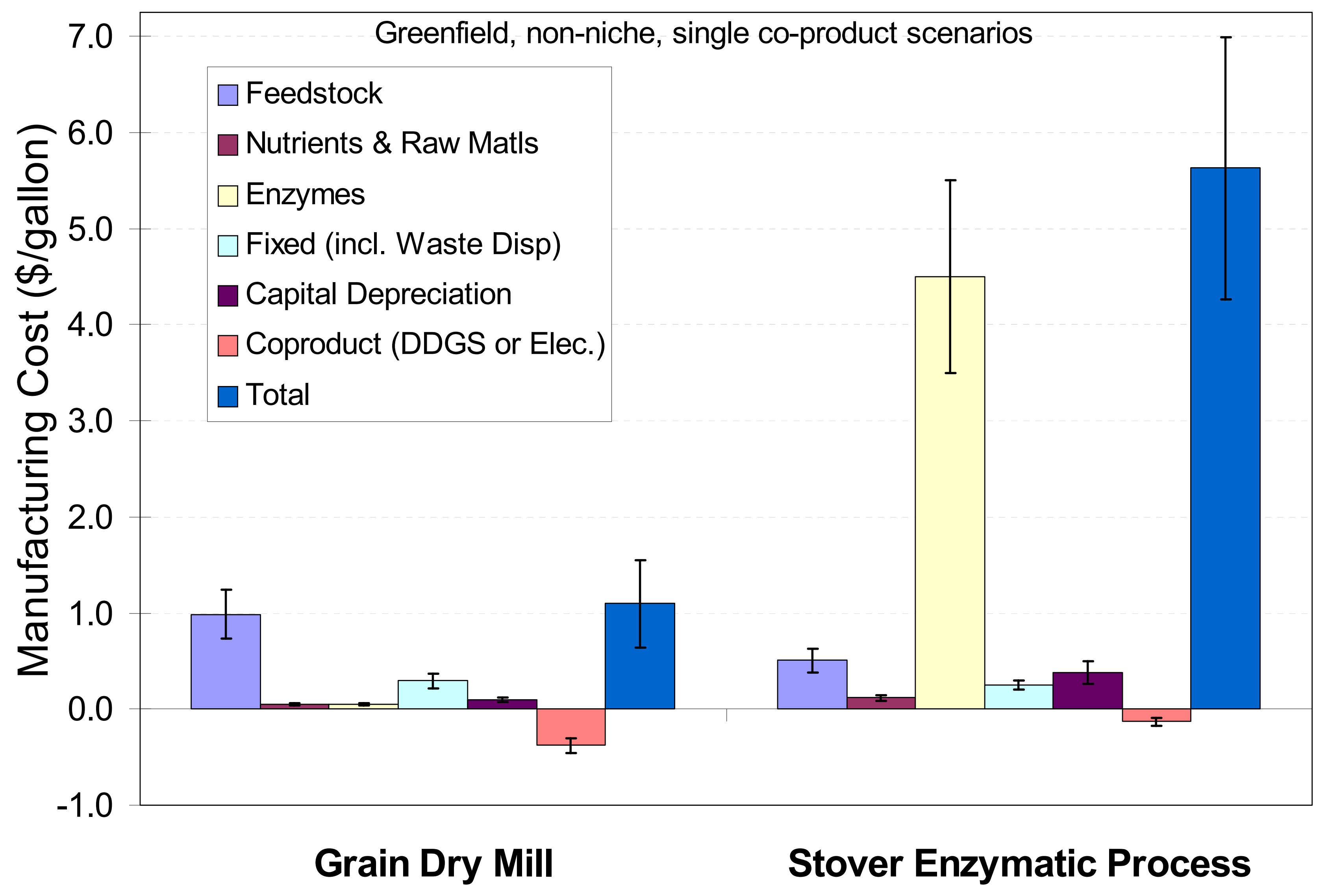
Mixed Biomass Sugar Fermentation

STOVER PROCESS

Ethanol and Solids Recovery, Water Recycle

Comparative Economics

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



Key Findings

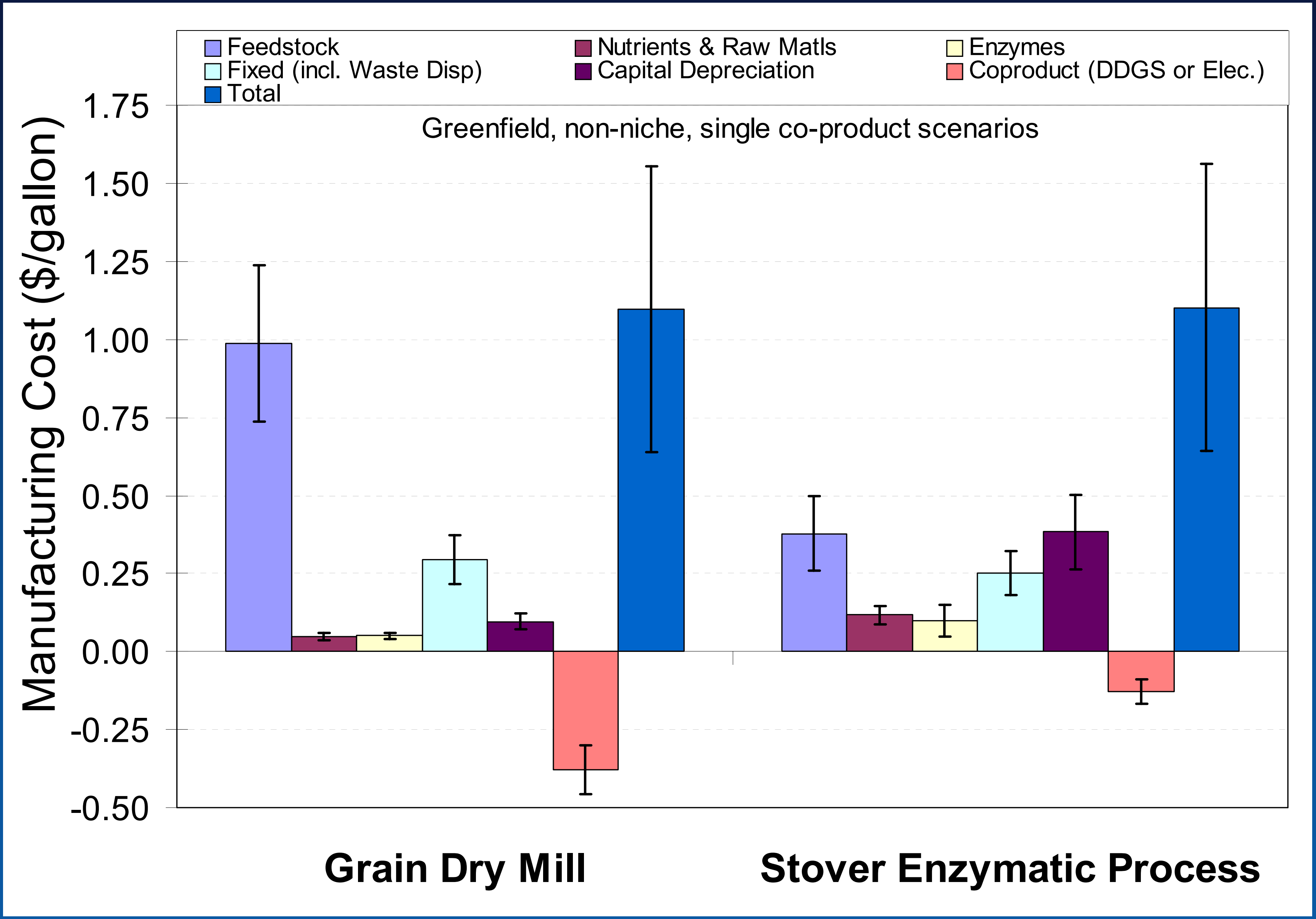
- Costs driven by
  - Feedstock (grain or stover)
  - Enzymes (stover)
  - Utilities prices (gas and electricity; grain)
  - Capital equipment (stover)

➢ *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

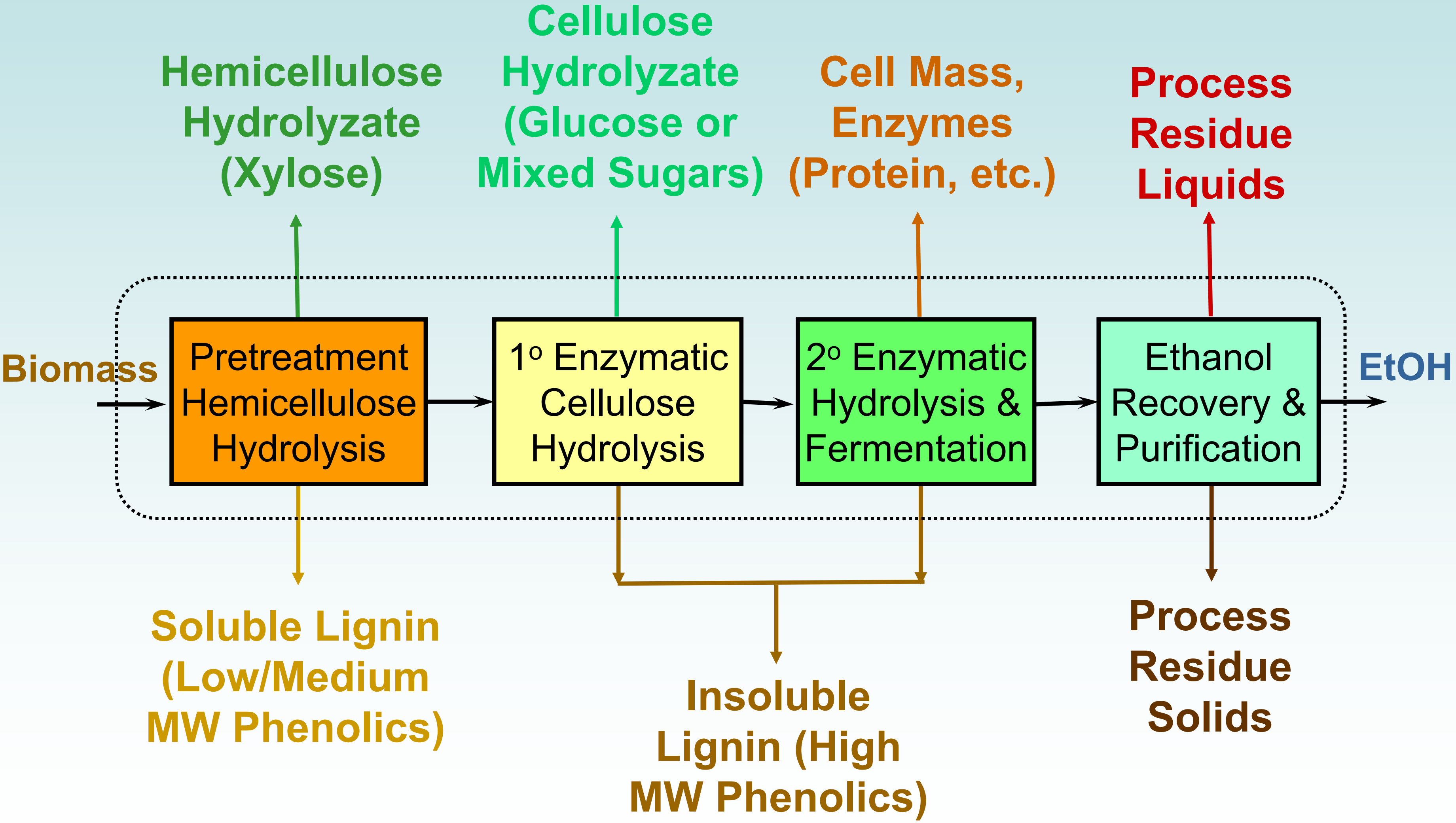
- Lower operating cost
  - Operating cost less enzymes potentially 20-40% lower processing stover
  - Diversifying feedstock options provide hedge against rising grain prices

- 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 “crade to grave” Life Cycle Analysis completed

Potential for Novel Coproducts from Enzymatic Sugar Platform Process



Hemicellulose Hydrolyzate (Xylose)

Cellulose Hydrolyzate (Glucose or Mixed Sugars)

Cell Mass, Enzymes (Protein, etc.)

Process Residue Liquids

Biomass

Pretreatment Hemicellulose Hydrolysis

1^o Enzymatic Cellulose Hydrolysis

2^o Enzymatic Hydrolysis & Fermentation

Ethanol Recovery & Purification

EtOH

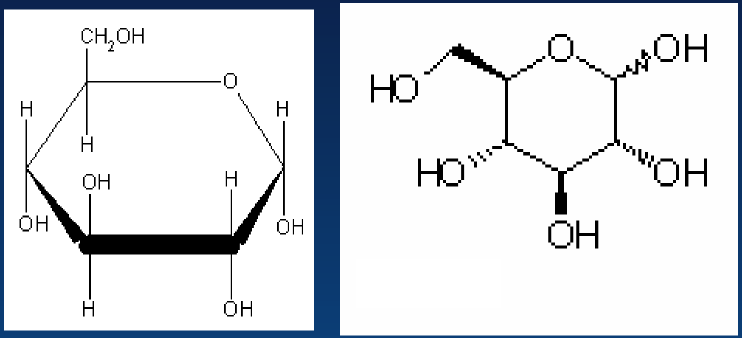
Soluble Lignin (Low/Medium MW Phenolics)

Insoluble Lignin (High MW Phenolics)

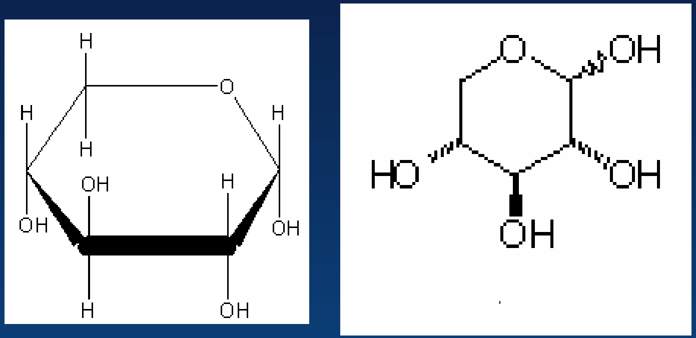
Process Residue Solids

Potential Opportunities for D-Xylose

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



α-D-Glucose

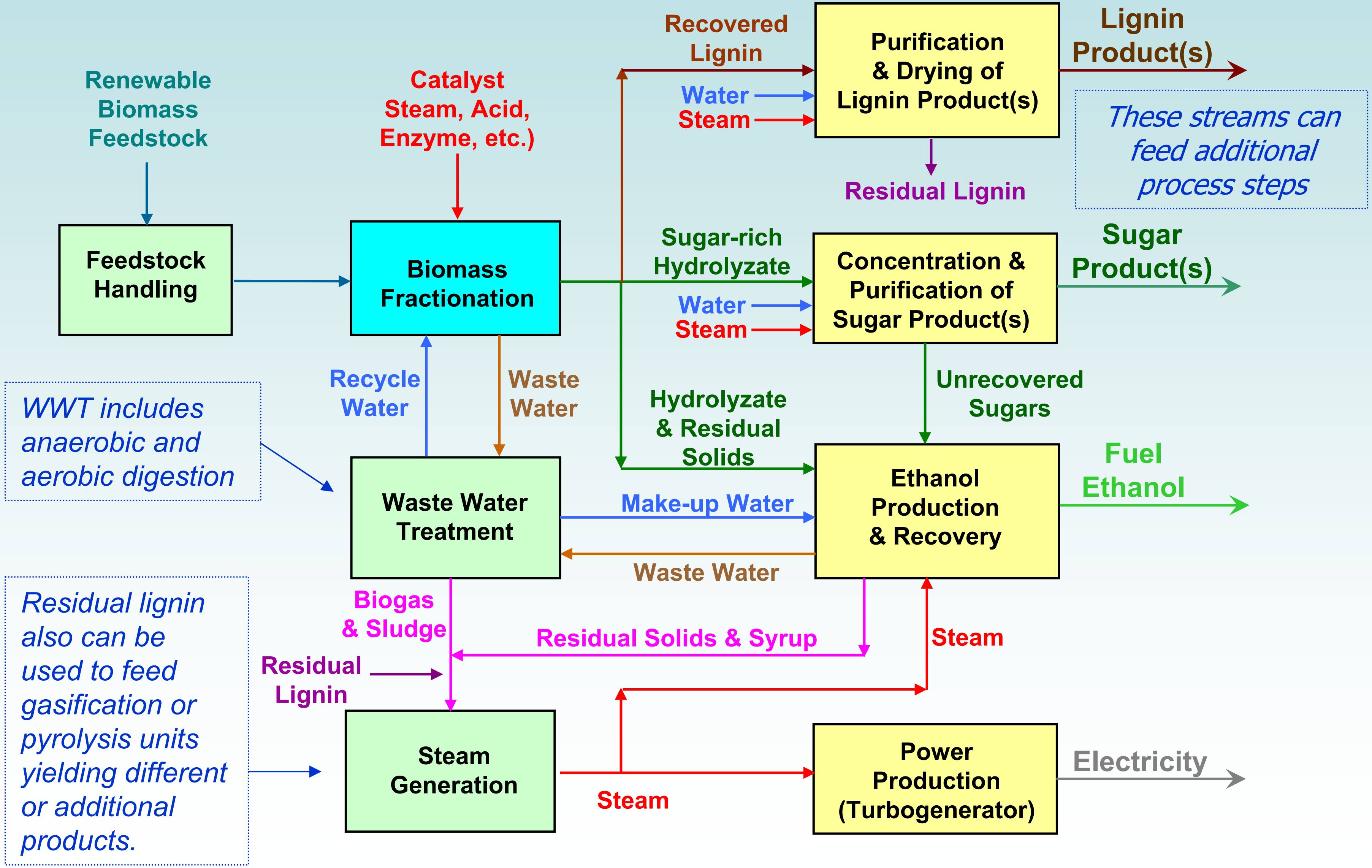


α-D-Xylose

- 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., ↓ μ_{max}, ↓ OUR_{max}

Multiproduct Lignocellulose Biorefinery

Sugar (and Lignin) Platform Example



Renewable Biomass Feedstock

Feedstock Handling

Catalyst Steam, Acid, Enzyme, etc.)

Biomass Fractionation

Recovered Lignin

Water

Steam

Purification & Drying of Lignin Product(s)

Lignin Product(s)

Residual Lignin

These streams can feed additional process steps

Sugar-rich Hydrolyzate

Water

Steam

Concentration & Purification of Sugar Product(s)

Sugar Product(s)

Unrecovered Sugars

Recycle Water

Waste Water

WWT includes anaerobic and aerobic digestion

Hydrolyzate & Residual Solids

Ethanol Production & Recovery

Fuel Ethanol

Waste Water Treatment

Make-up Water

Waste Water

Biogas & Sludge

Residual Solids & Syrup

Steam

Residual Lignin

Residual lignin also can be used to feed gasification or pyrolysis units yielding different or additional products.

Steam Generation

Steam

Power Production (Turbogenerator)

Electricity

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

• Biomass chemistry and physical properties
• Fractionation
• Catalysis
  • Chemical
  • Biological (enzymes and microorganisms)
• Genetic and protein engineering
	• Process integration
	• Material and energy balances
	• Solids handling and feeding
	• Reactor design
	• Catalyst production
	• Reaction kinetics
	• Separation technology
	• Materials of construction
	• Control systems and automation
		• Decrease financial risk (in the context of energy price fluctuations)
		• Process knowledge at large scale
		• Lower capital and operating costs
		• Reduce environmental risk (minimize waste)
		• **Integrate systems for fuels, chemicals, materials, and power for optimum product slate**

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^2 Process Demonstration Unit
  - 6,000 ft^2 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^2 Integrated Process Development Unit (PDU)

6,000 ft^2 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 capabililities 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 (± 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
    - ✓ 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

Acknowledgments

- Data from NREL’s Sugar Platform R&D
  - Sugar Platform Integration team (Dan Schell et al.)
  - Enzyme Subcontract Liaison (Jim McMillan et a.)
- Comparative economics from NREL-USDA joint study
  - USDOE/NREL: Kelly Ibsen, Robert Wallace
  - USDA ARS: Andrew McAloon, Frank Taylor, Winnie Yee
- Funding
  - USDOE’s EERE’s Office of the Biomass Program