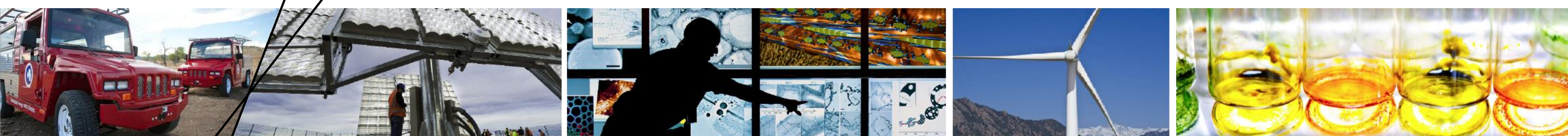
2013 DOE Bioenergy Technologies Office (BETO) Project Peer Review

Biochemical Processing Integration



May 20, 2013
Biochemical Conversion Area

Daniel Schell
NREL

This presentation does not contain any proprietary, confidential or otherwise restricted information

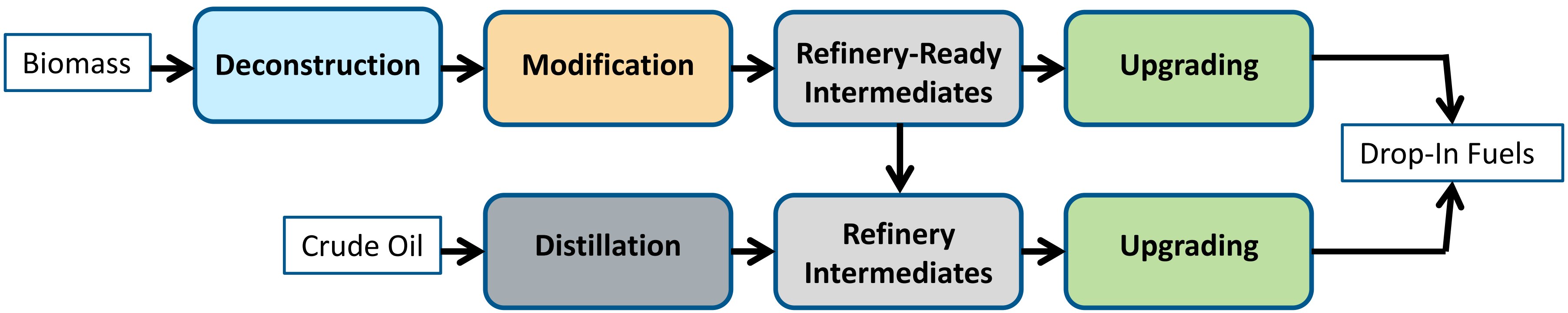
High-Level Project Goal

Produce integrated process performance data that when used in an economic model produces a cost estimate that meets the biofuel production cost targets in the MYPP.

Supports the mission of BETO and the Biochemical Conversion Technology Area to independently demonstrate integrated process performance and transfer this knowledge to industry, further facilitating BETO's mission to deploy cost-effective biofuels production technology.

Transitioning from Ethanol to Hydrocarbons

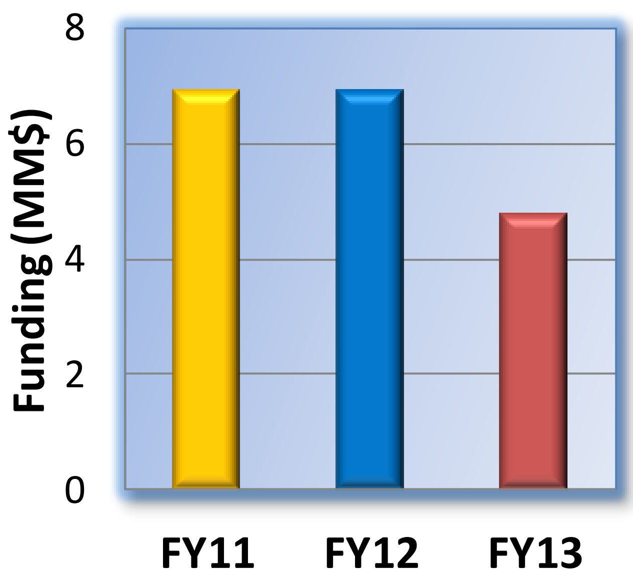
For the last two years, the primary focus of this project was achieving performance results that meets the cellulosic ethanol cost target ($2.15/gal) defined in the predecessor MYPP.



The new strategic goal defined in BETO’s current MYPP is to “develop commercially viable technologies for converting biomass feedstocks into energy dense, fungible liquid transportation fuels, as well as bioproducts or chemical intermediates and biopower.”

Overview

- Project start date: FY01
- Project end date (EtOH): FY12
- Project end date (HC): FY17

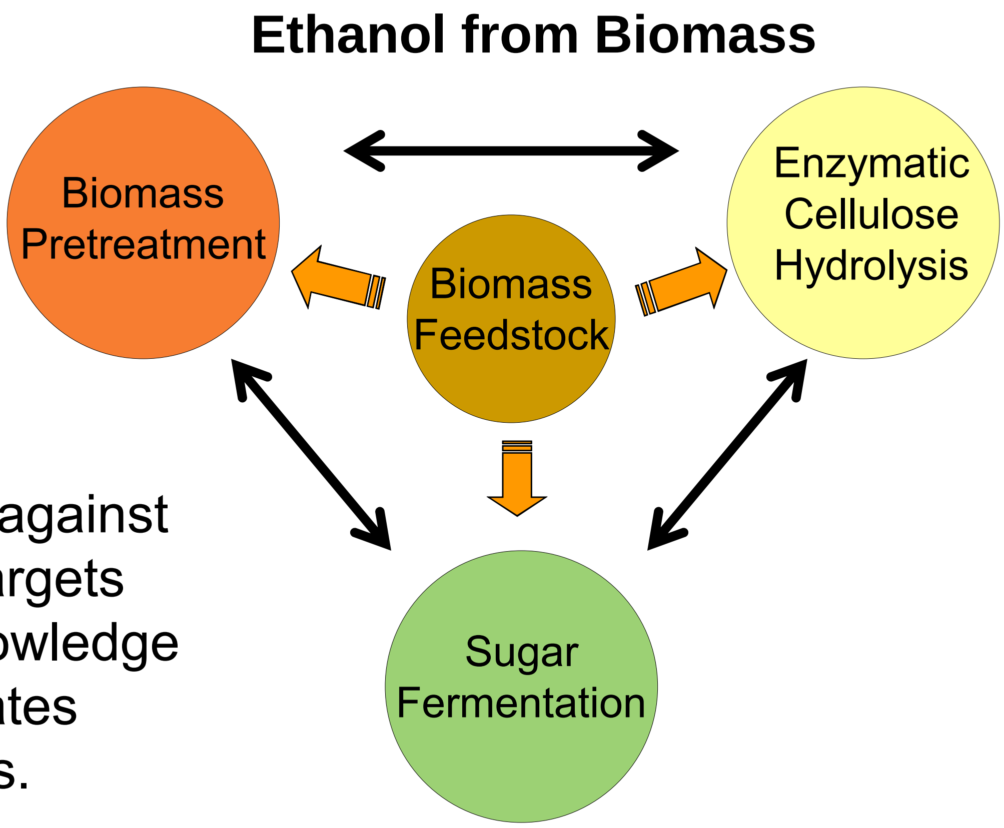


Average annual funding: $4.4MM

- **Barrier**
  - Biochemical conversion process integration
- **MYPP targets**
  - 2012, demonstrate integrated pilot-scale ethanol production at $2.15/gal
  - 2017, validate integrated production of a biologically-derived hydrocarbon (HC) fuel against interim cost target (TBD)
  - 2022, validate integrated pilot-scale production of a HC fuel at $3.00 gge
- **Subcontracts**
  - Colorado State University, membrane studies
  - Hazen Research, S/L separation work
  - University of Louisville, mixing studies
  - Harris Group/Brown & Caldwell, waste water treatment analysis and design
- **Other collaborations**
  - MAST Center, membrane fundamentals
  - ORNL, microbial fuel cells/waste water

Project Overview

Biochemical Processing Integration (BPI) was established in FY2001 to perform integrated process research at bench- and pilot-scale using corn stover as near-term and readily-available feedstock.

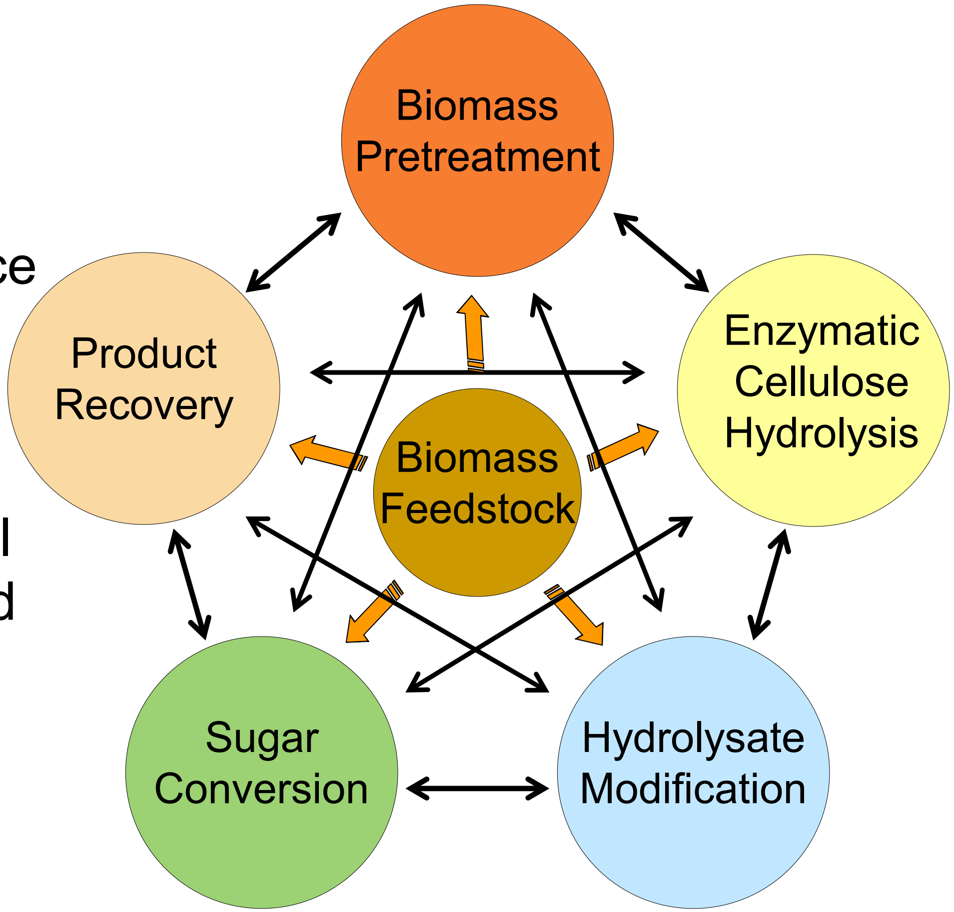


We evaluate integrated process performance and gauge progress against the current year's yield and cost targets and inform BETO of progress. Knowledge gained from our efforts also facilitates current and future validation efforts.

Specific Project Objectives

- Generate integrated performance data
  - Evaluate and optimize integrated performance at the bench scale
  - Translate results to pilot scale operations and produce performance results for economic analysis
- Investigate other process performance issues and challenges beyond the major unit operations, e.g., waste water treatment, recycle water
- Develop new and improved analytical methods and deploy to academia and industry as needed
- Supply feedstocks, pretreated materials, and process residues to industry and academia for their research efforts

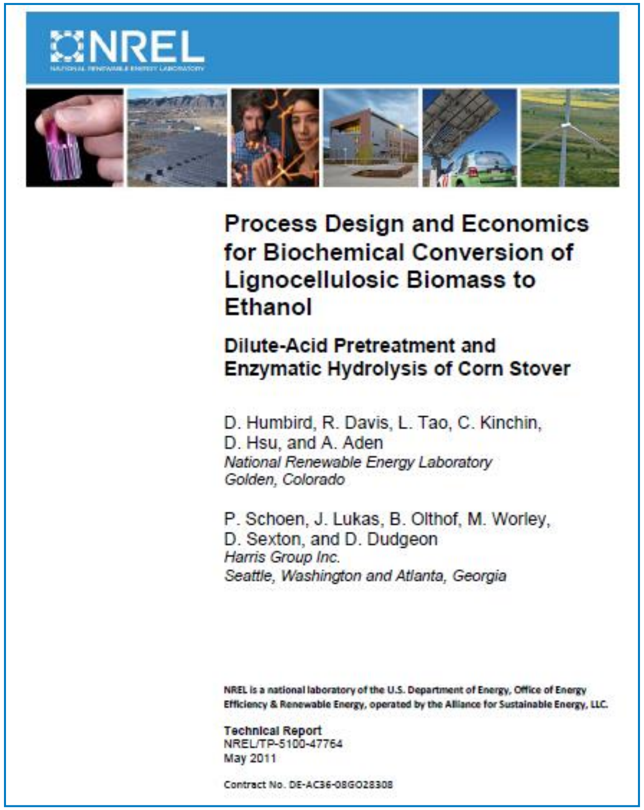
Hydrocarbon Fuel from Biomass



Approach

Multi-year effort to benchmark and understand/improve integrated process performance and generate cost information using accumulated BPI research results, results from other Biochemical Conversion area work, and the latest advances from industry and academia.

- High-level objectives and timelines defined in the MYPP
- Activities (work breakdown structure) and intermediate objectives (milestones) developed in multi-year plans (Gantt chart) that are updated yearly
- Detailed yearly plans developed and defined in Annual Operating Plans with specific quantifiable milestones


NREL

Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol

Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover

D. Humbird, R. Davis, L. Tao, C. Kinchin, D. Hsu, and A. Aden
National Renewable Energy Laboratory
Golden, Colorado

P. Schoen, J. Lukas, B. Olthof, M. Worley, D. Sexton, and D. Dudgeon
Harris Group Inc.
Seattle, Washington and Atlanta, Georgia

NREL is a national laboratory of the U.S. Department of Energy, Office of Energy Efficiency & Renewable Energy, operated by the Alliance for Sustainable Energy, LLC.

Technical Report
NREL/TP-5100-47764
May 2011

Contract No. DE-AC36-08GO28308

Project Organization (Subtask Structure: FY11-FY12)

BPI
Dan Schell

Analytical Development
Ed Wolfrum

- New analytical methods and tools
- Rapid analysis methods and models

Arabinose Utilization
Jeff Linger

- Arabinose utilizing *Zymomonas mobilis* strain (risk reduction strategy implemented in 2010)

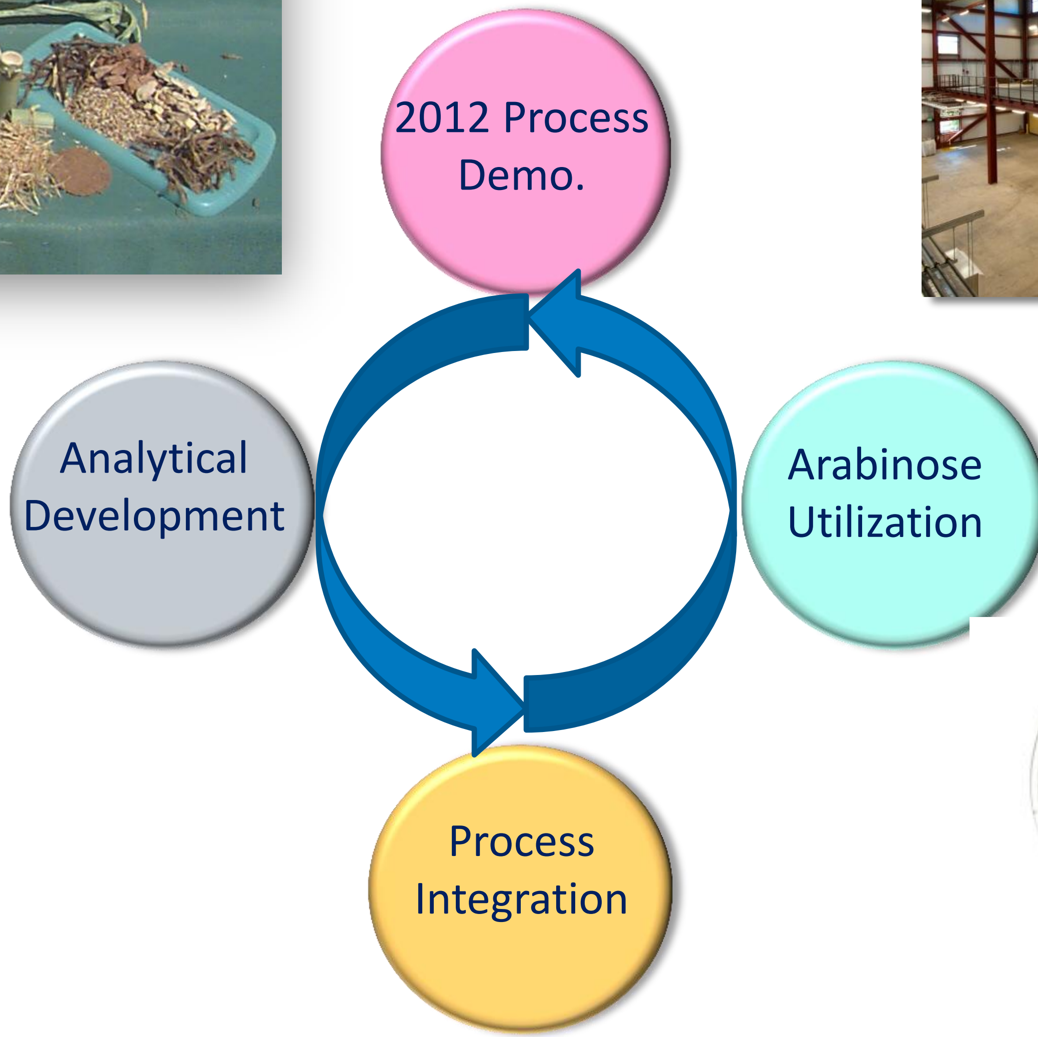
Process Integration
Nancy Dowe

- Biocatalyst evaluations
- Integrated bench-scale process evaluations

2012 Process Demonstration
Dan Schell

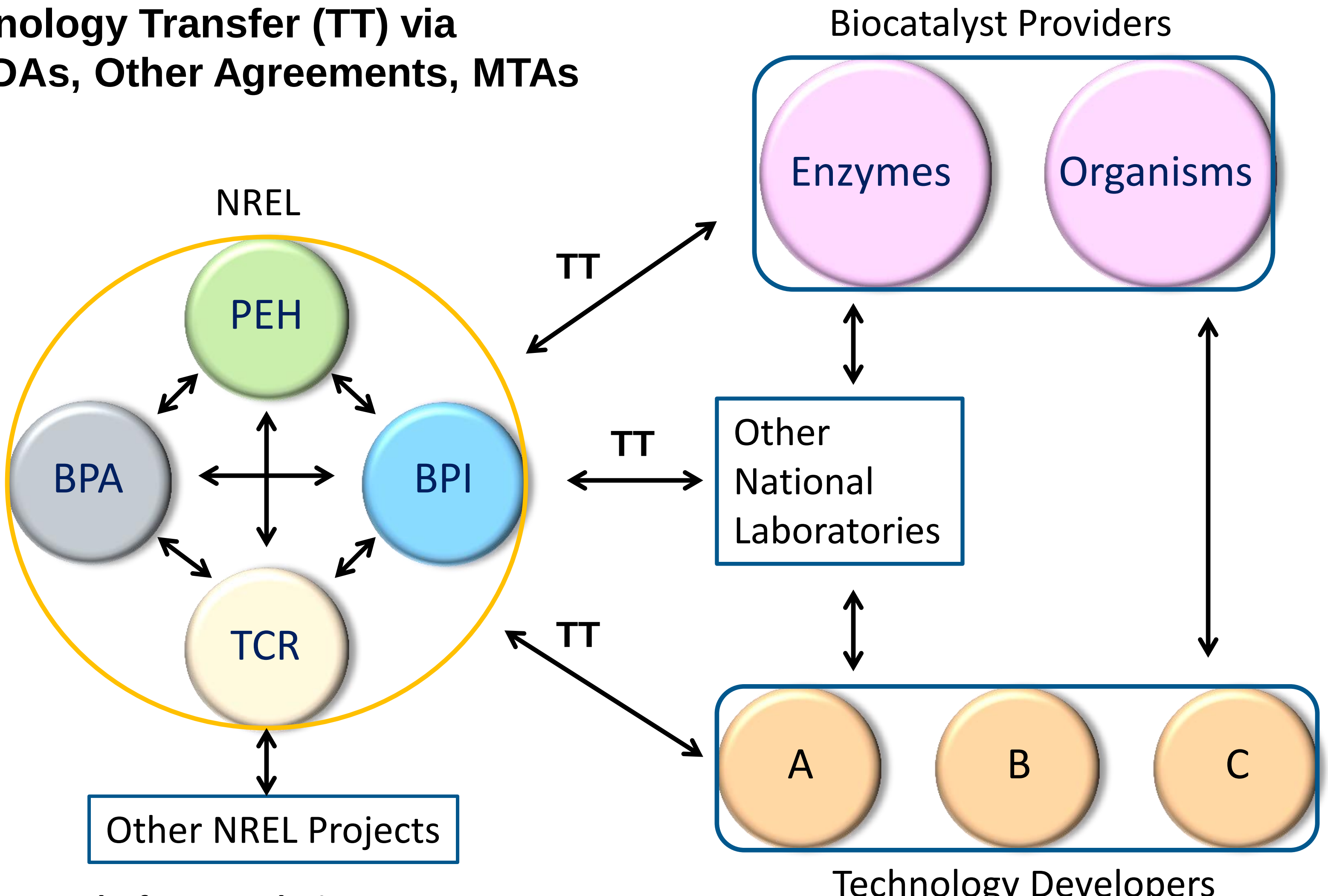
- Integrated pilot-scale runs
- Impact of other unit operations (e.g., WWT)
- Pilot plant maintenance/modifications

Project Interactions

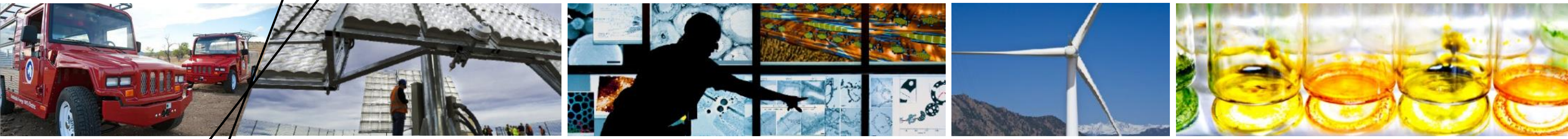


Information/Technology Flow

Technology Transfer (TT) via CRADAs, Other Agreements, MTAs



BPA-Biomass Platform Analysis
TCR-Targeted Conversion Research
PEH-Pretreatment and Enzymatic Hydrolysis



Technical Accomplishments

Analytical Development

Supplemental information on technical accomplishments provided in the back of the presentation.

Laboratory Analytical Procedures (LAPs)

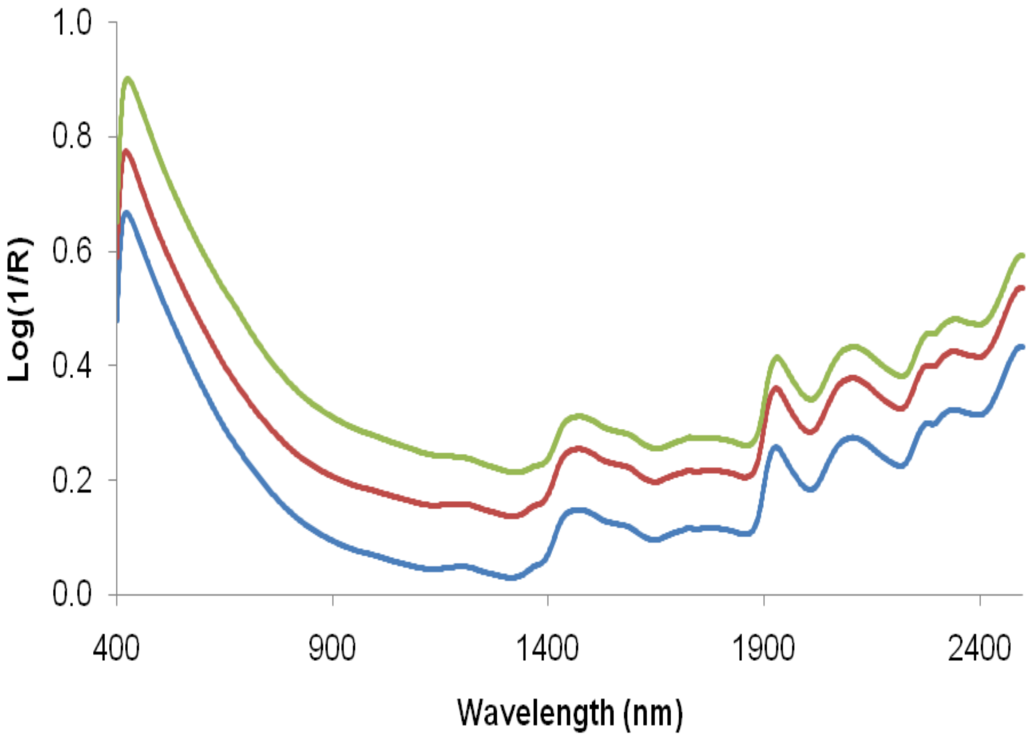
- Continued work to develop and improve biomass analysis methods (LAPs) being used by industry and academic researchers
- Methods available at: http://www.nrel.gov/biomass/analytical_procedures.html
- Over 19,000 unique visits to the NREL LAP web site from November 2011 thru February 2013

NIST Reference Materials

New book chapter discusses use and integration of the biomass analysis LAPs : "Methods for Biomass Compositional Analysis", in “Catalysis for the Conversion of Biomass and Its Derivatives”, A. Sluiter, J. Sluiter, E. Wolfrum, Max Planck Research Library for the History and Development of Knowledge, Proceedings 2.” Berlin: Edition Open Access (2013), ISBN 978-3-8442-4282-9.

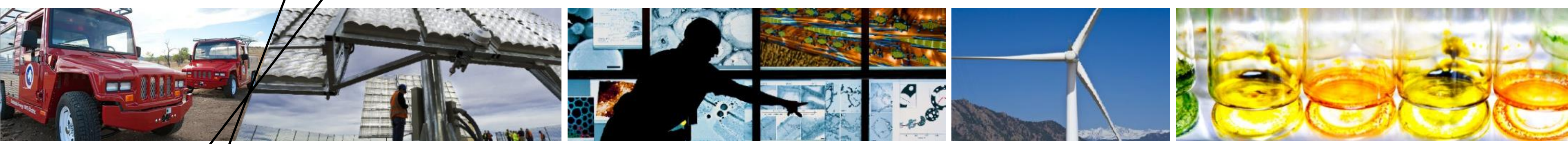
Rapid Biomass Compositional Analysis

- Used Multivariate Analysis methods to correlate wet chemistry data with NIR spectroscopy data
- These methods provide high-throughput biomass analysis with accuracies comparable to classical techniques



Models implemented on multiple spectrometers for:

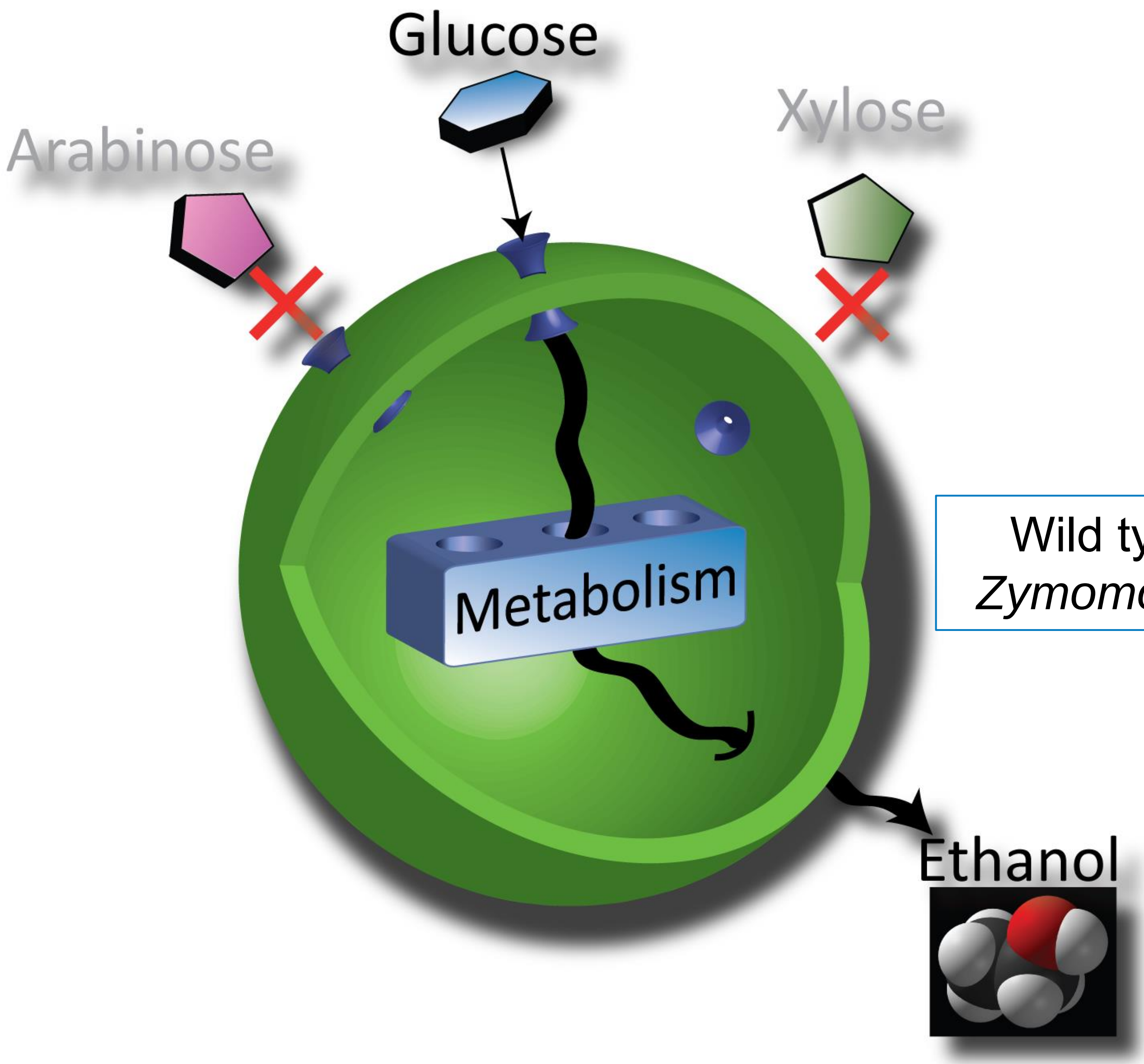
- Corn stover
- Sorghum
- Miscanthus (preliminary)
- Pretreated Corn Stover (PCS) solids (washed, dried, and milled)
- PCS hydrolysate liquor
- PCS whole slurry
- Mixed feedstocks (including switchgrass, sorghum, corn stover, miscanthus, rice straw
- ***Received DOE permission to copyright & license NIR data***
- ***First licensing agreements executed in 2012, more are under negotiation***



Technical Accomplishments

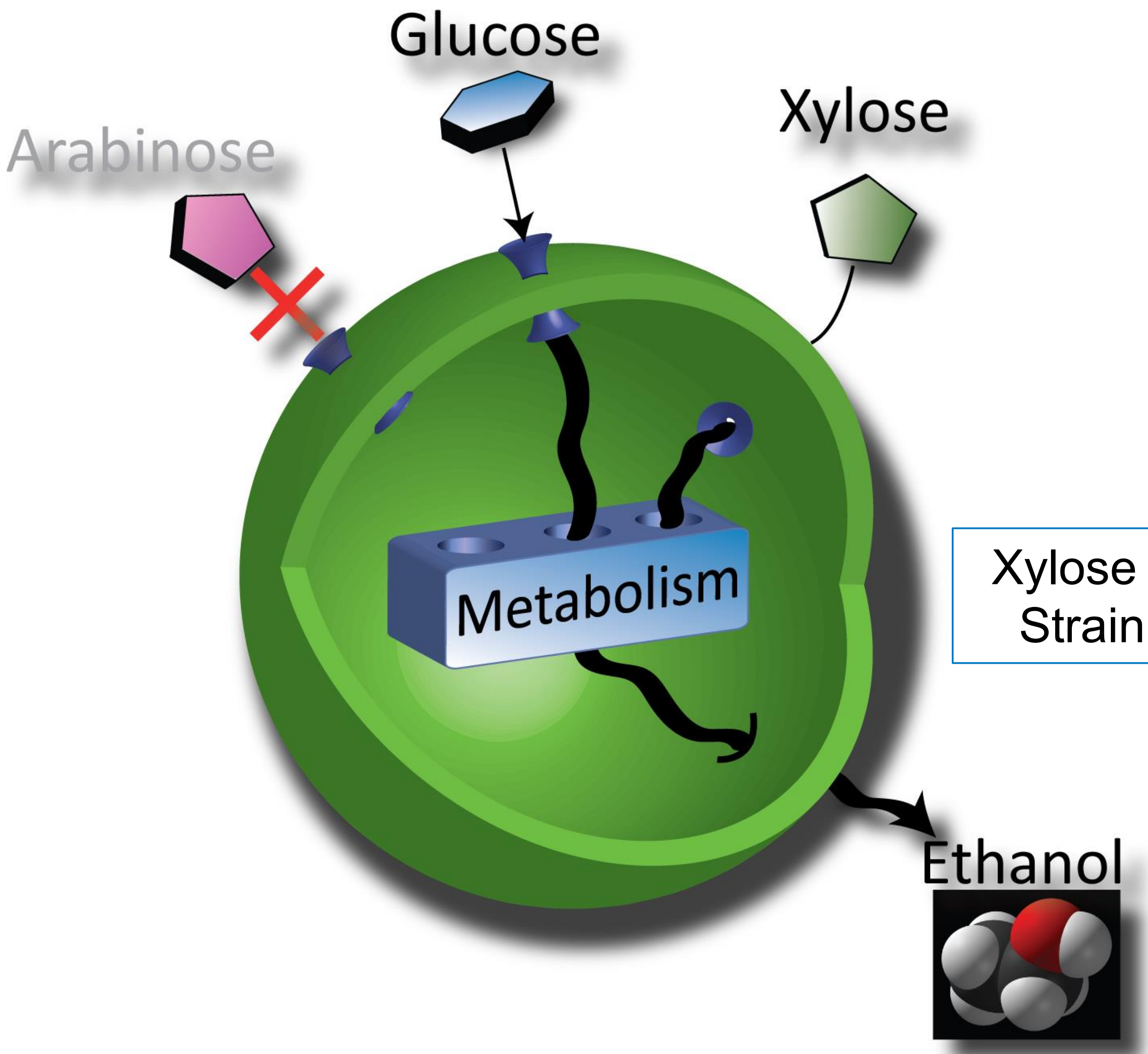
New Arabinose Utilizing Z. mobilis Strain

Engineering New *Zymomonas* Strains



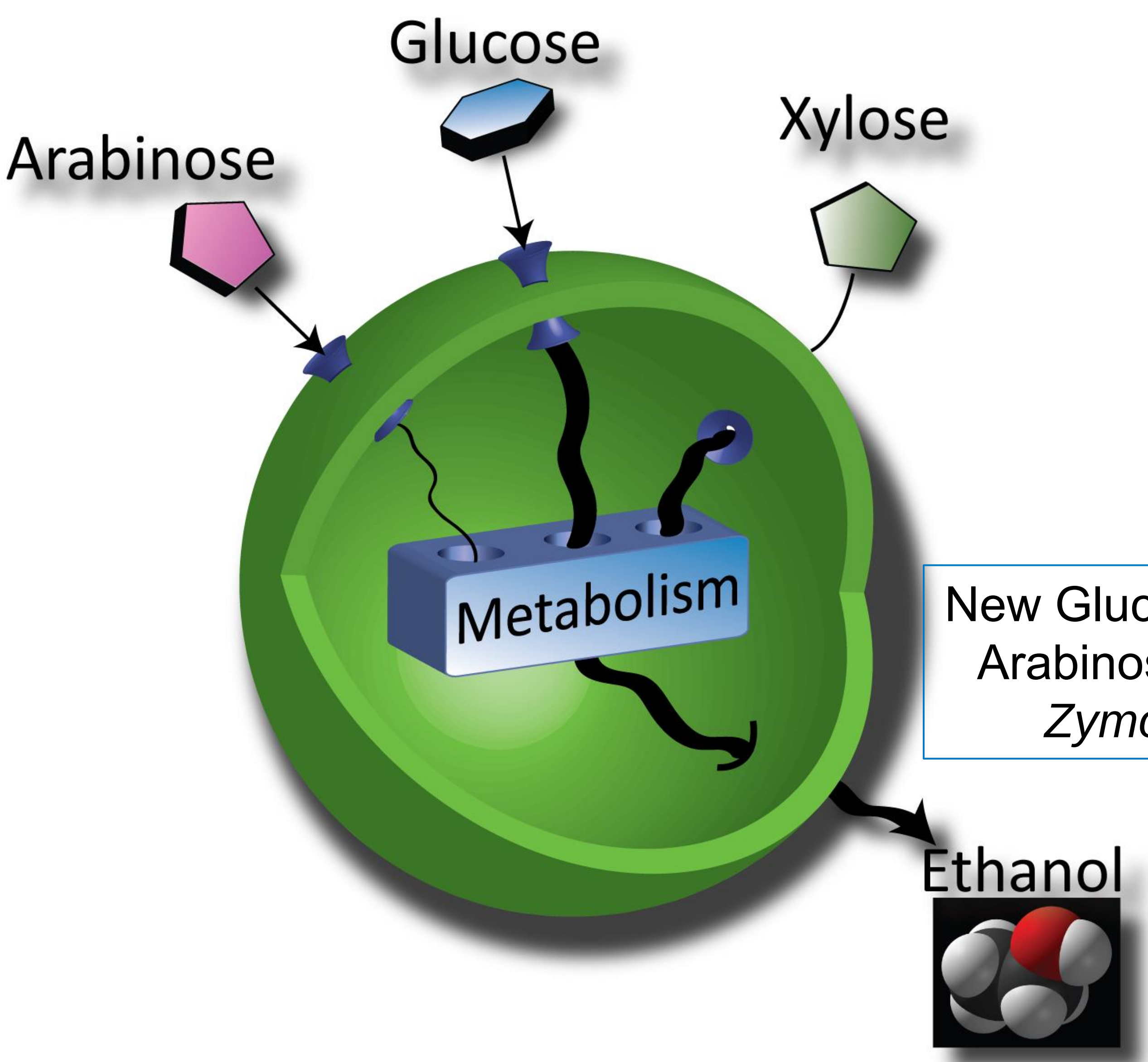
Wild type *Zymomonas*

Engineering New *Zymomonas* Strains



Xylose Utilizing Strain (8b)

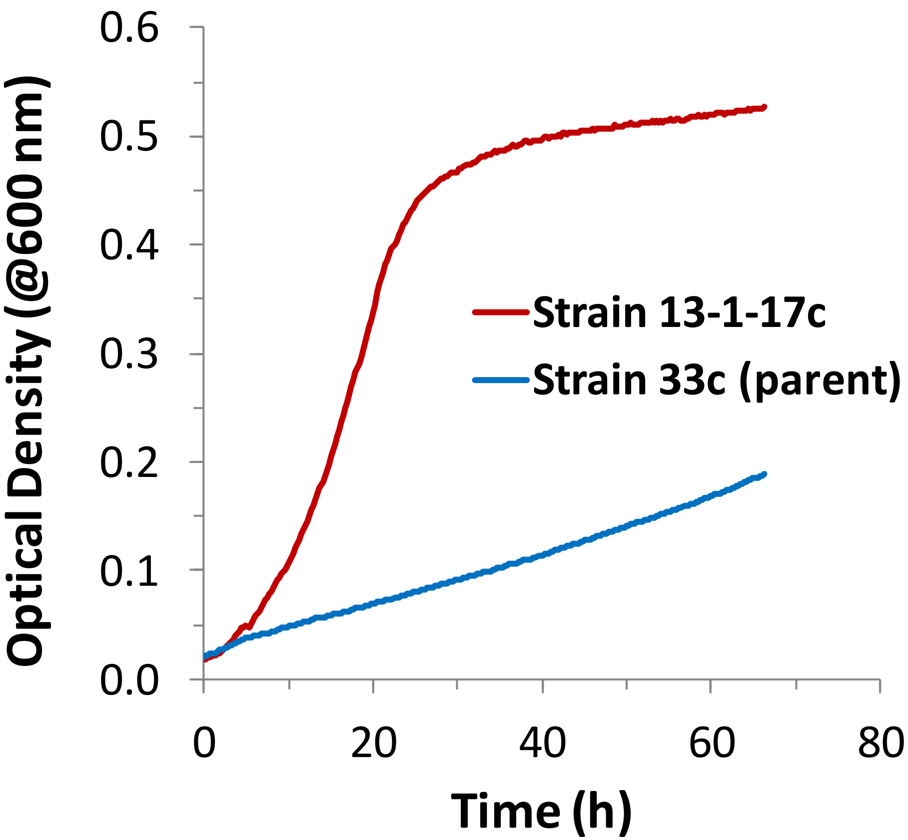
Engineering New *Zymomonas* Strains



New Glucose-Xylose-Arabinose Utilizing *Zymomonas*

Improved Arabinose Utilizing Strain 13-1-17

Growth of two arabinose-utilizing *Z. mobilis* strains on pure arabinose

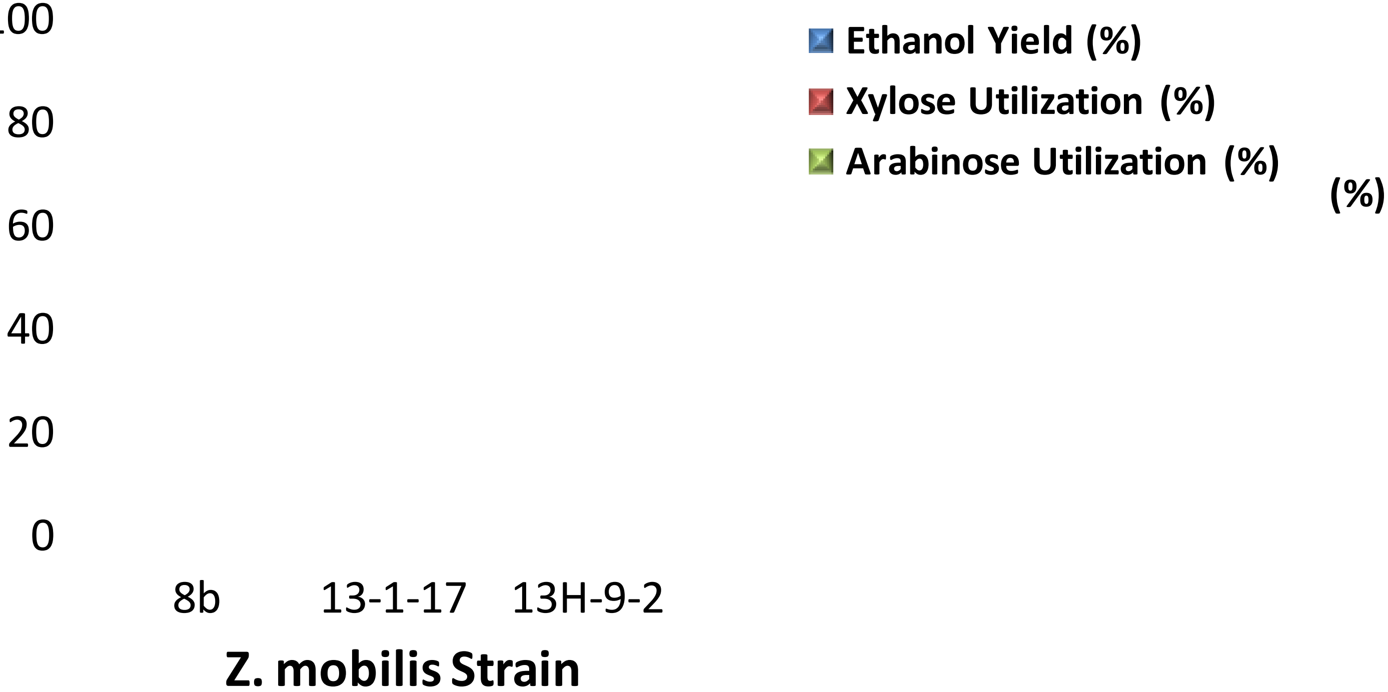


a single genomic
that was responsible for
growth rates/ethanol
ı on arabinose

ɔtection is being pursued
:ations are in preparation

Performance of Several Engineered Strains

Strain performance evaluated in 20% (w/w) total solids pretreated corn stover slurry



ıot tolerant of
· hydrolysates

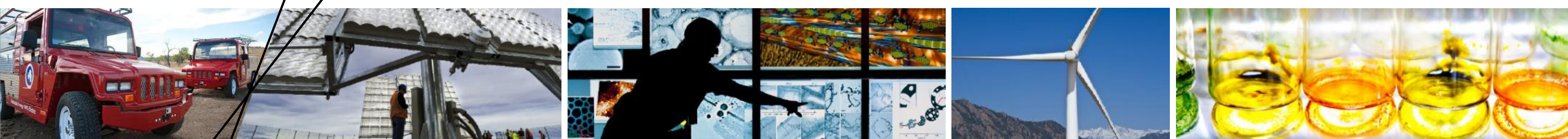
ıically-integrated
by UV or chemical
n 13-1-17c

ains able to use
ɑs well as 8b and
nose to ethanol

- No further work is occurring; strain can be deployed

All microorganisms completely consume glucose, so glucose utilization is not shown.

NATIONAL RENEWABLE ENERGY LABORATORY

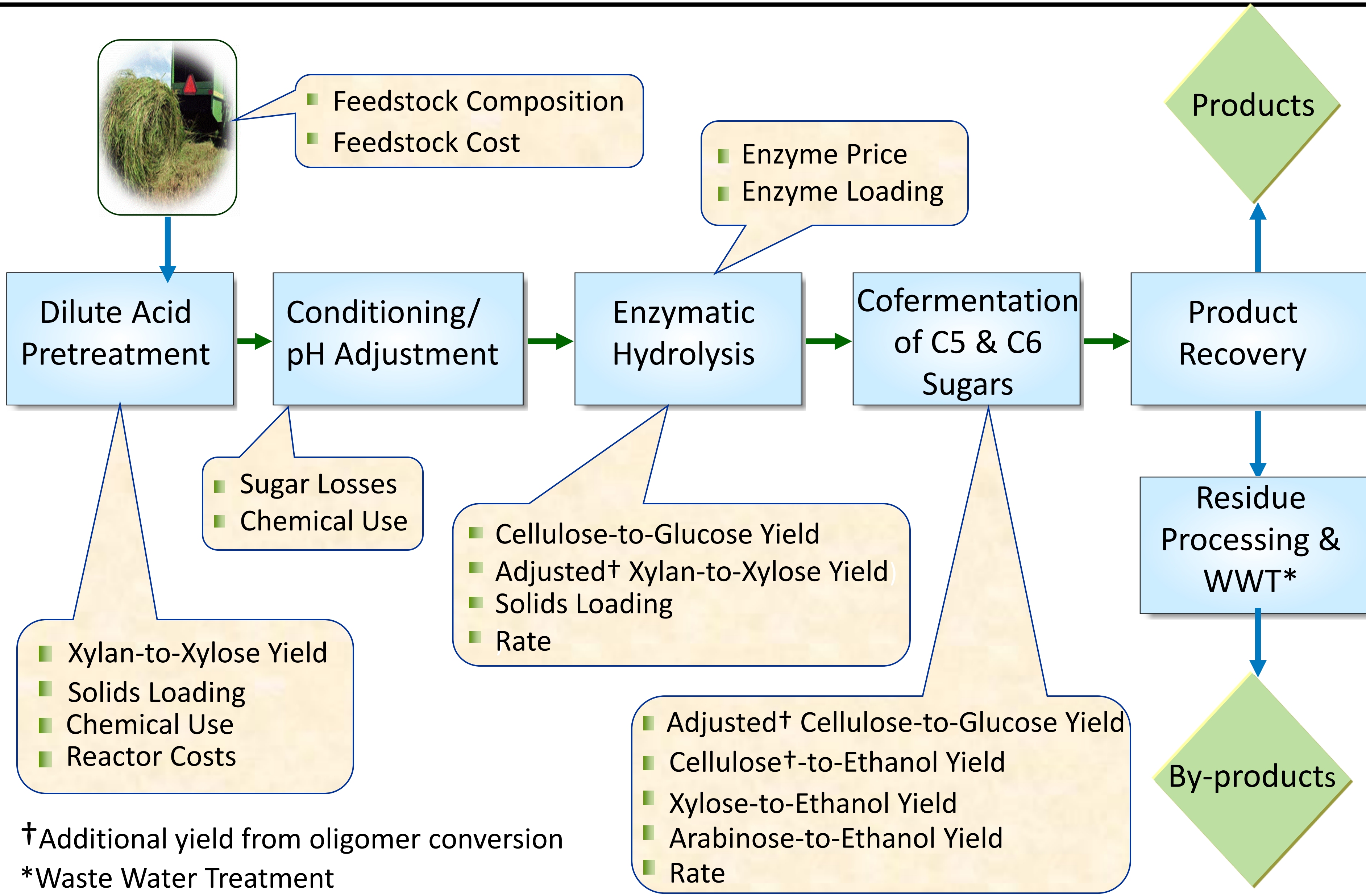


Technical Accomplishments

Bench-Scale Performance Results

2005 to 2012

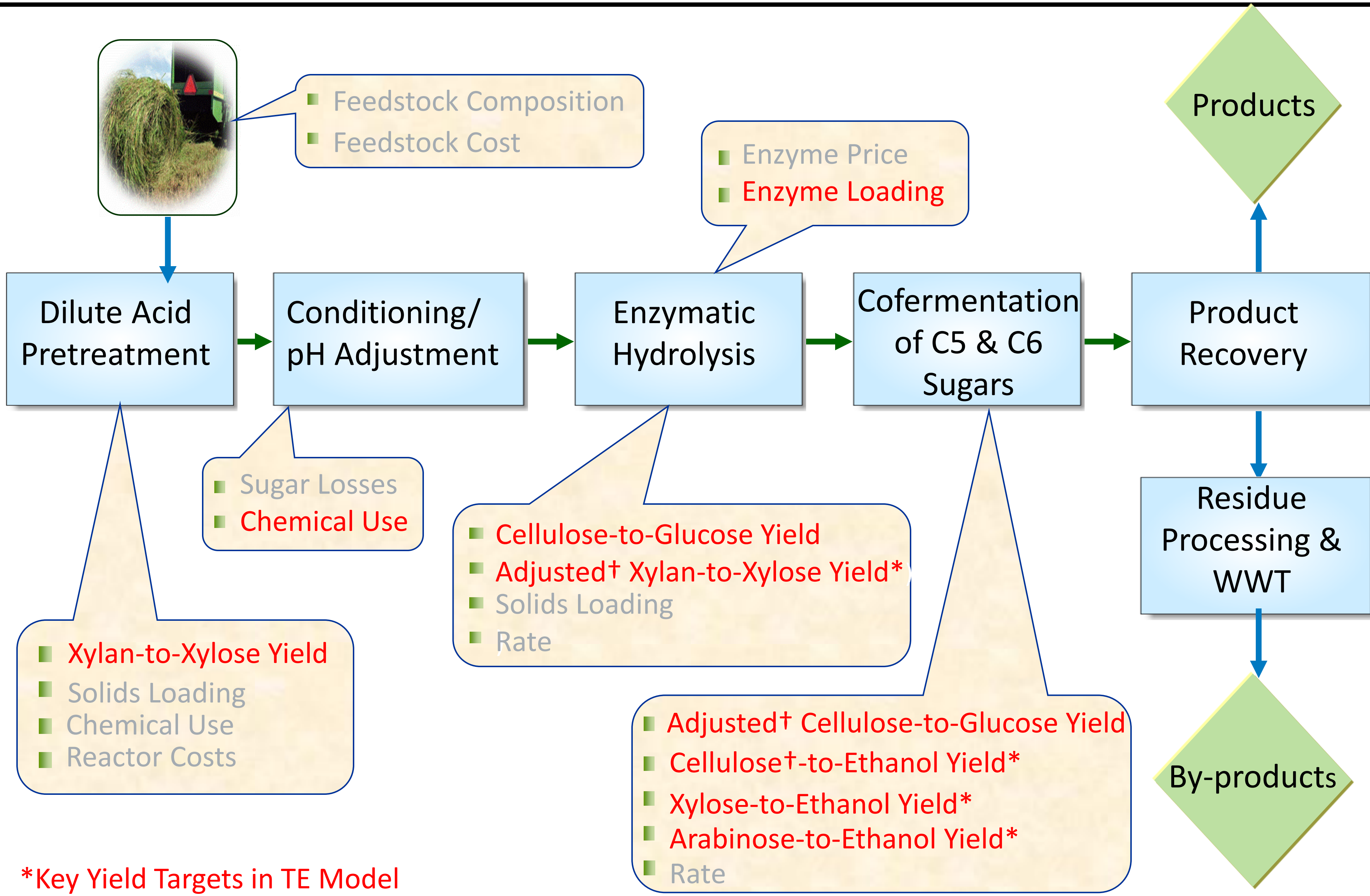
Biochemical Process — Key Process Variables



†Additional yield from oligomer conversion

*Waste Water Treatment

Biochemical Process — Most Impactful Variables



*Key Yield Targets in TE Model

Bench-Scale Methodology

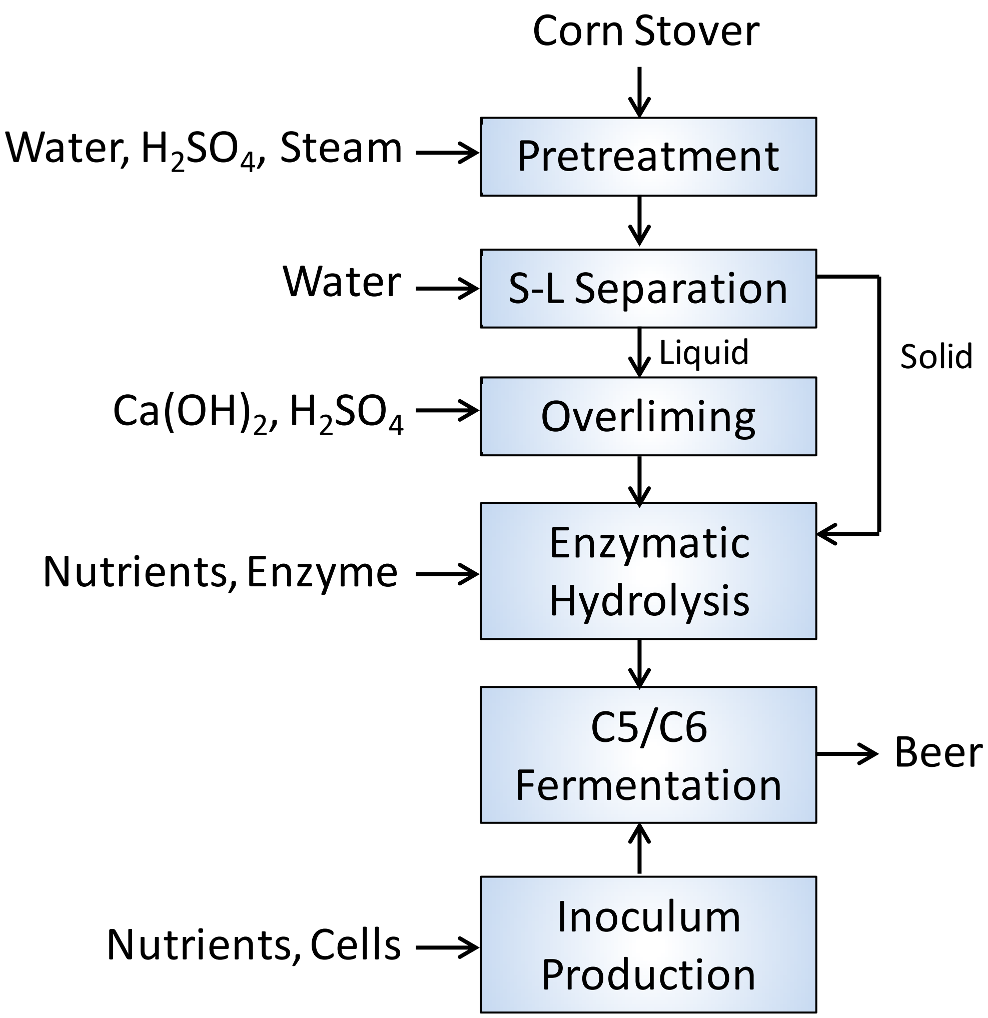
- Pretreated corn stover
  - Produced in pilot scale reactors, operating conditions varied over time
- Enzymatic hydrolysis
  - 20% (w/w) solids loading
  - 40 mg protein*/g cellulose enzyme loading
  - Enzymes change over time
- Fermentation
  - Two cofermenting *Zymomonas mobilis* strains tested
  - Media: 5 g/L yeast extract, 1 g/L KH_2PO_4 or 0.25% Corn Steep Liquor
  - Initial cell density ~ 0.5 g/L (dry basis, cell paste or 10% v/v transfer)

*Protein measured by BCA assay

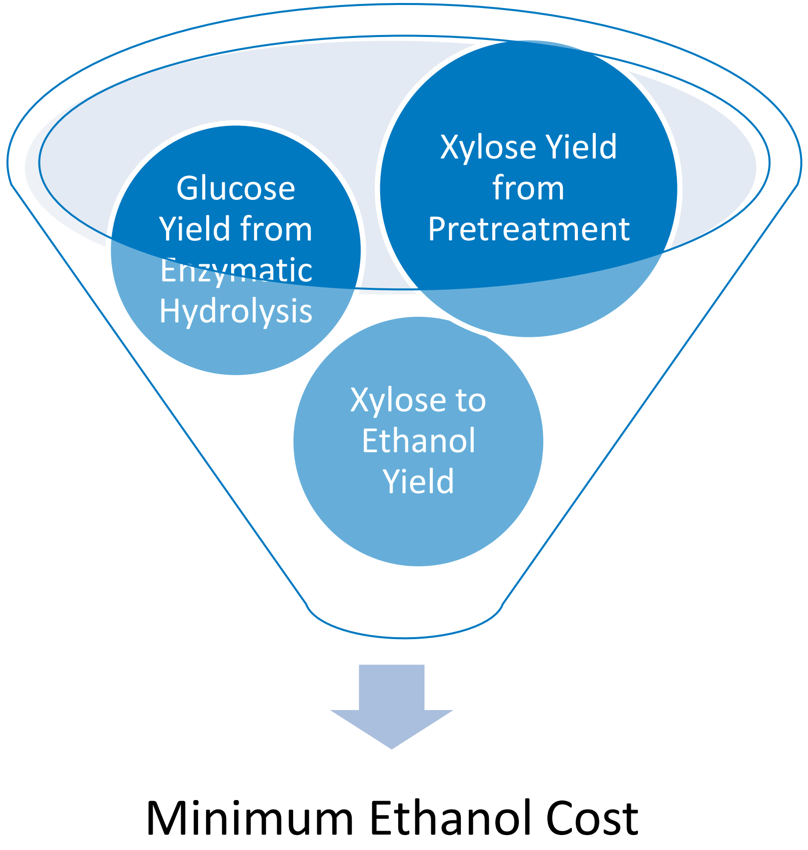
Bench-Top Shaking Incubator

500-mL Fermentors

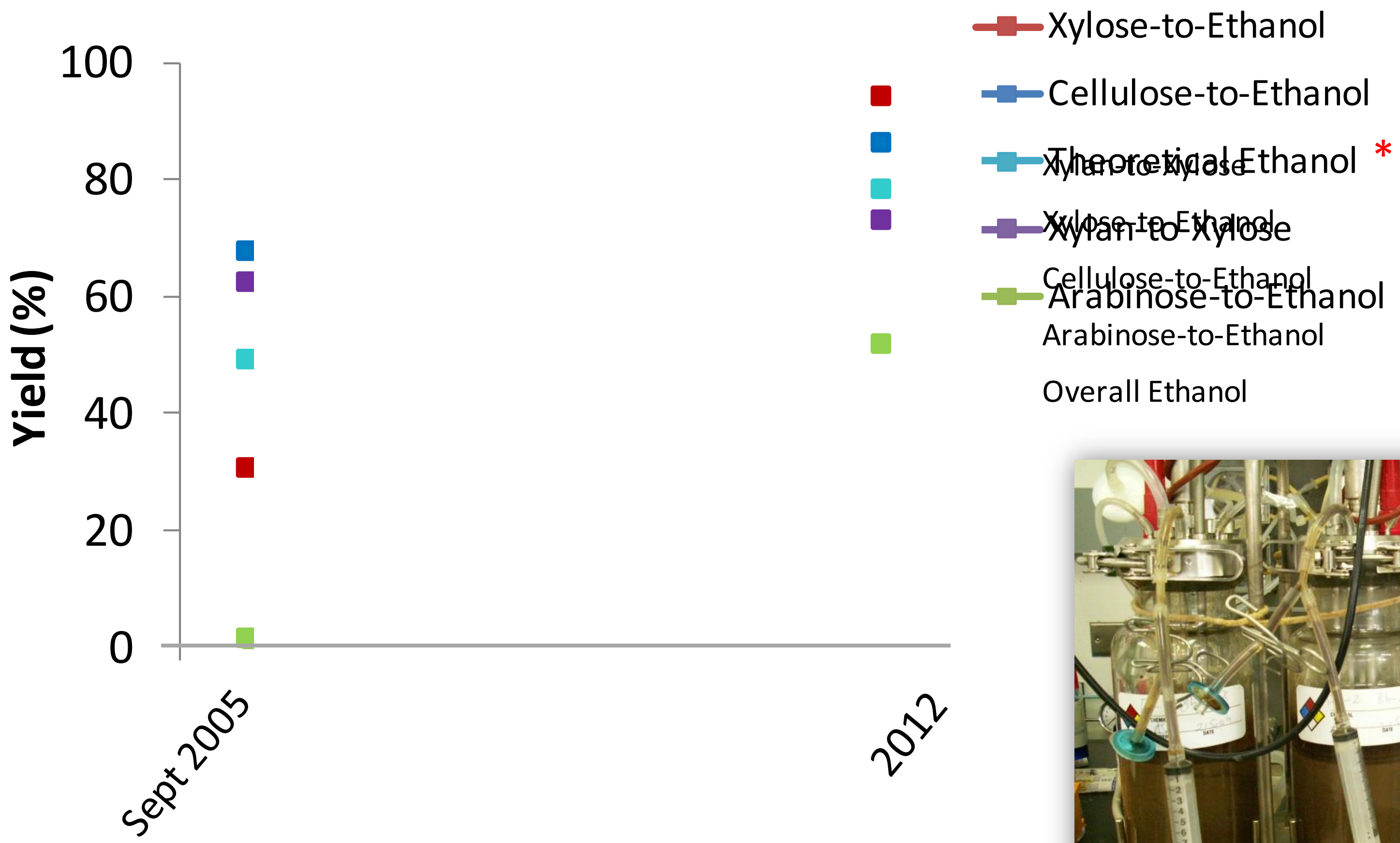
2005 Process Configuration



Minimizing Ethanol Cost ≠ Maximizing Yields for Each Unit Operation



2005

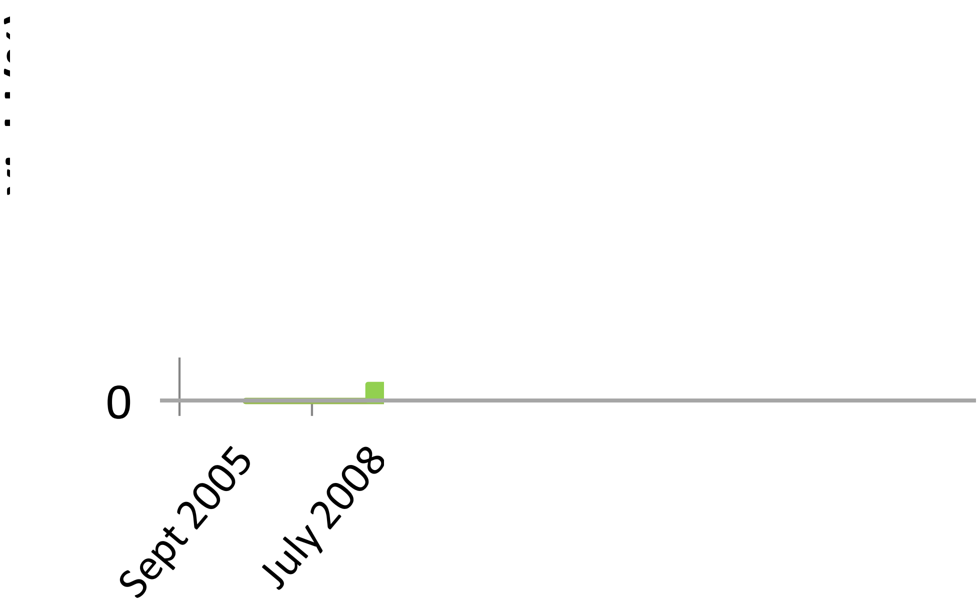


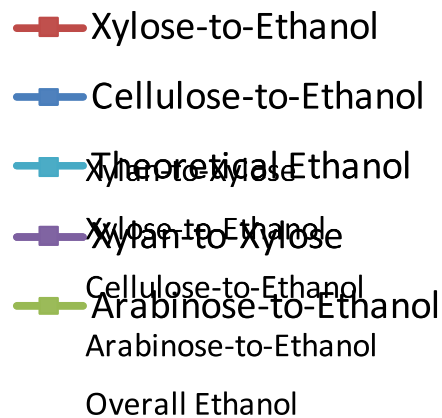
Enzyme: Spezyme CP (Genencor)

Microbe: *Zymomonas mobilis* 8b

* Percent of theoretical ethanol yield from initial sugars present at start of fermentation

2008



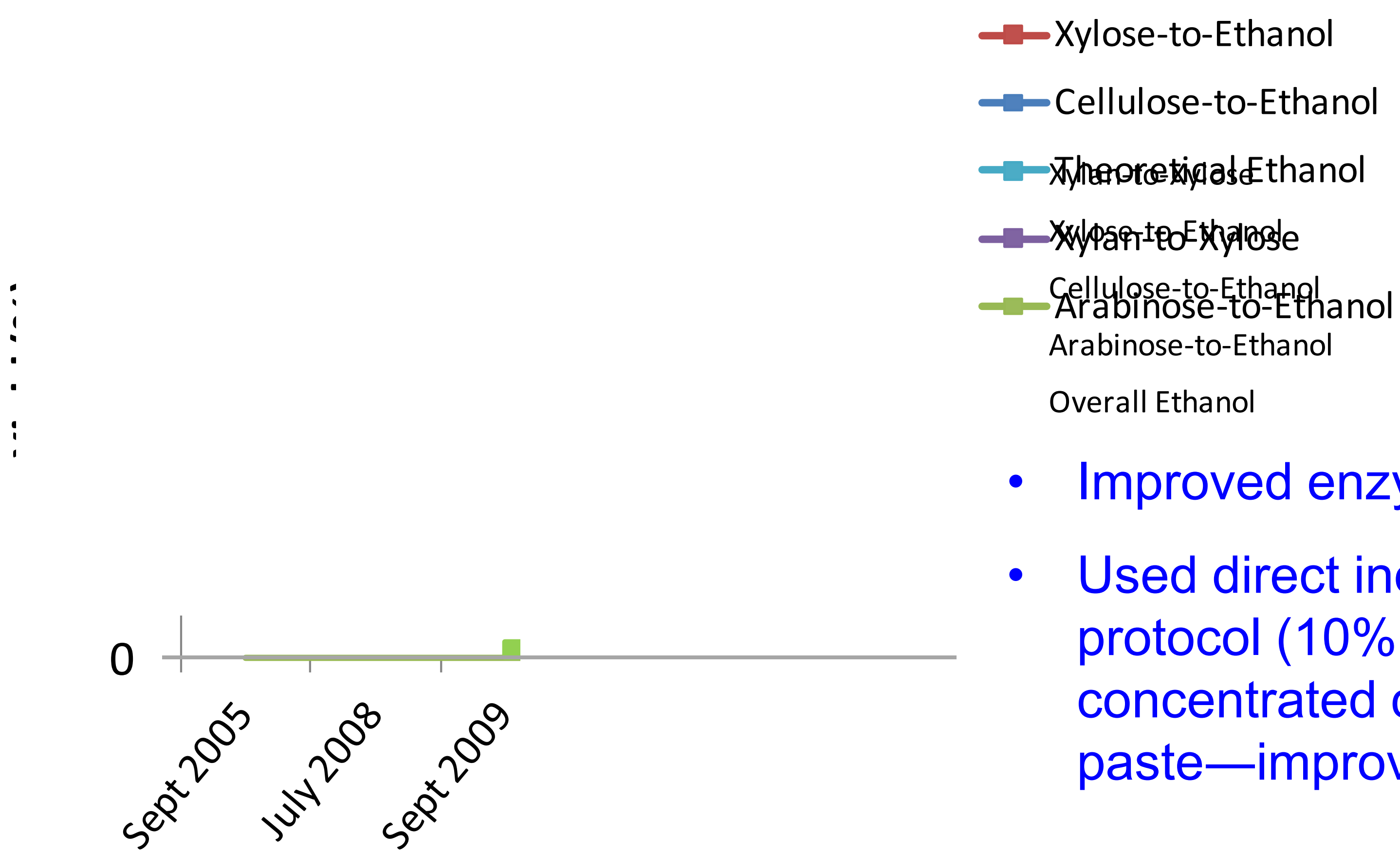


- Eliminated overliming process [$Ca(OH)_2$]—caused sugar losses
- Added whole slurry NH_4OH conditioning—significantly reduced sugar losses (no S/L separation step required)

Enzyme: GC-220 (Genencor)

Microbe: *Zymomonas mobilis* 8b

2009

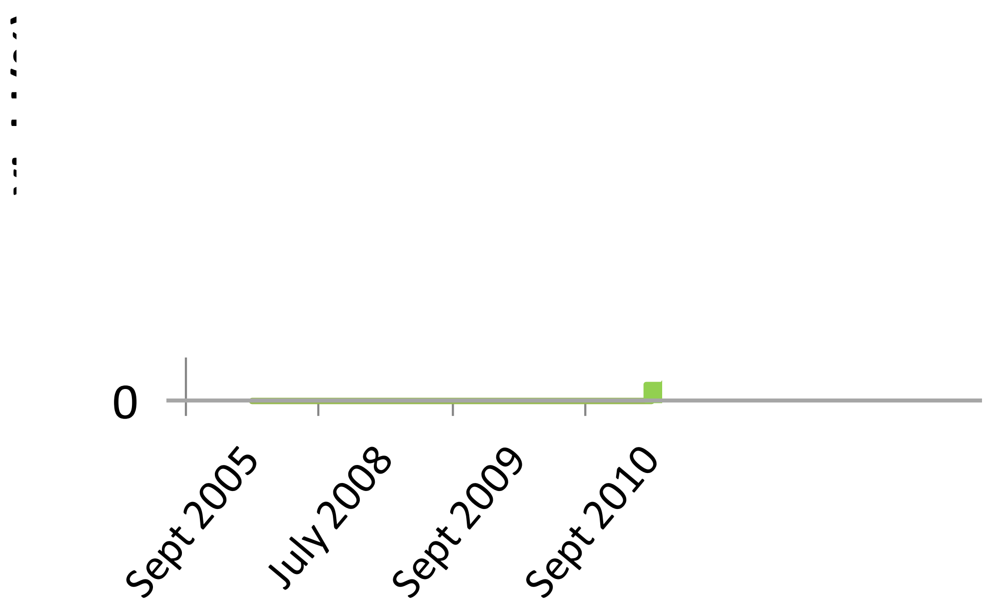


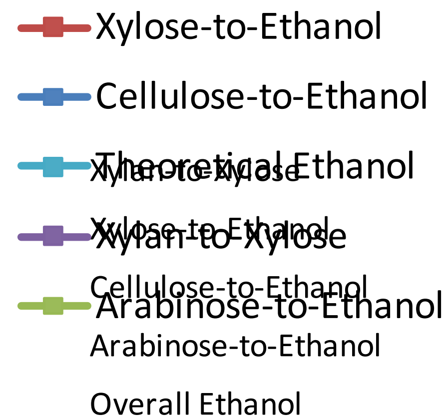
- Improved enzyme
- Used direct inoculum transfer protocol (10% v/v) instead of concentrated cell paste—improved cell viability

Enzyme: Pre-CTec 1 (Novozymes)

Microbe: *Zymomonas mobilis* 8b

2010



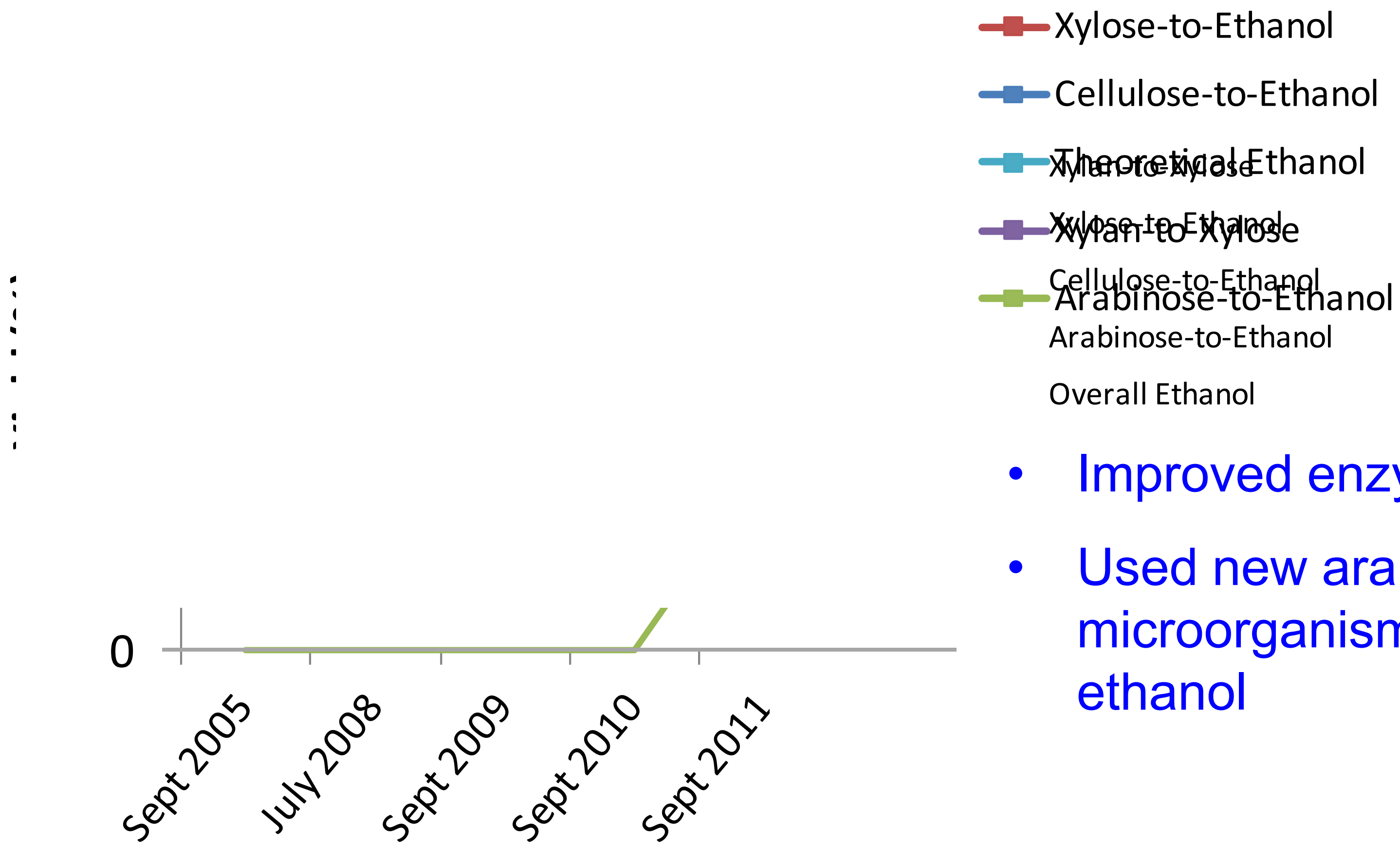


- Lowered pretreatment acid loading—lower severity pretreatment reduced cellulose digestibility
- Produced more monomeric xylose—xylo-oligomer conversion to monomers by a secondary acid cook step

Enzyme: CTec 1 (Novozymes)

Microbe: *Zymomonas mobilis* 8b

2011



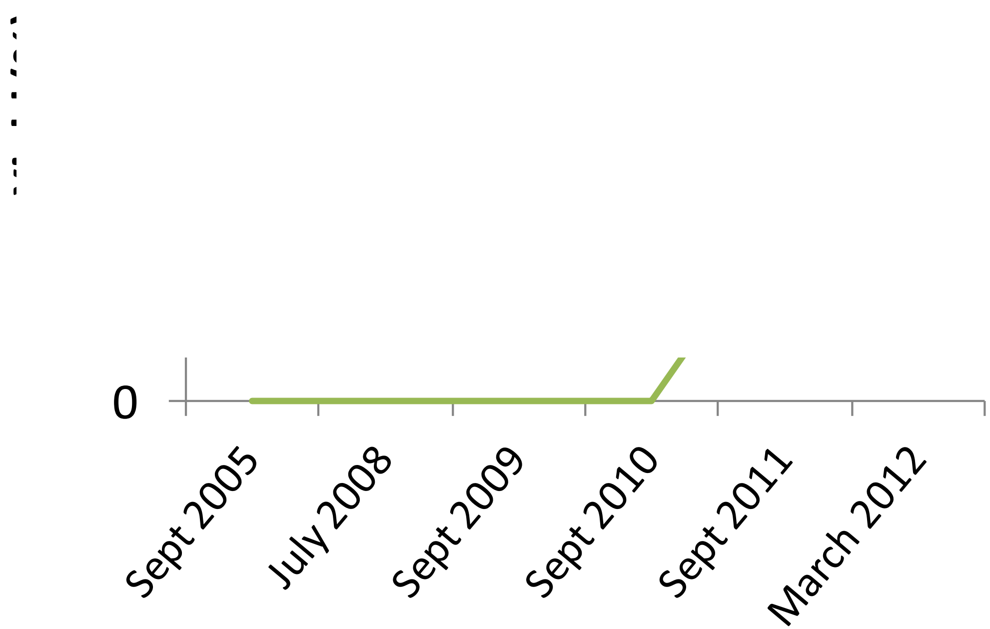
- Improved enzyme
- Used new arabinose-fermenting microorganism—produced more ethanol

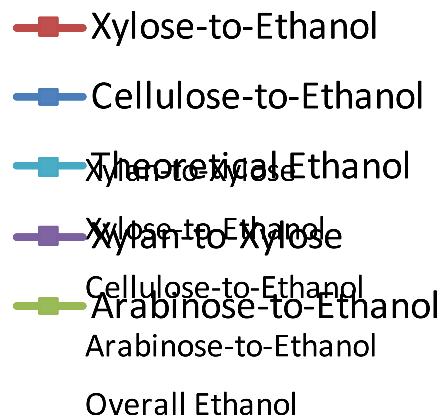
Enzyme: CTec 2 (Novozymes)

Microbe: *Zymomonas mobilis* A7*

*DuPont strain

2012



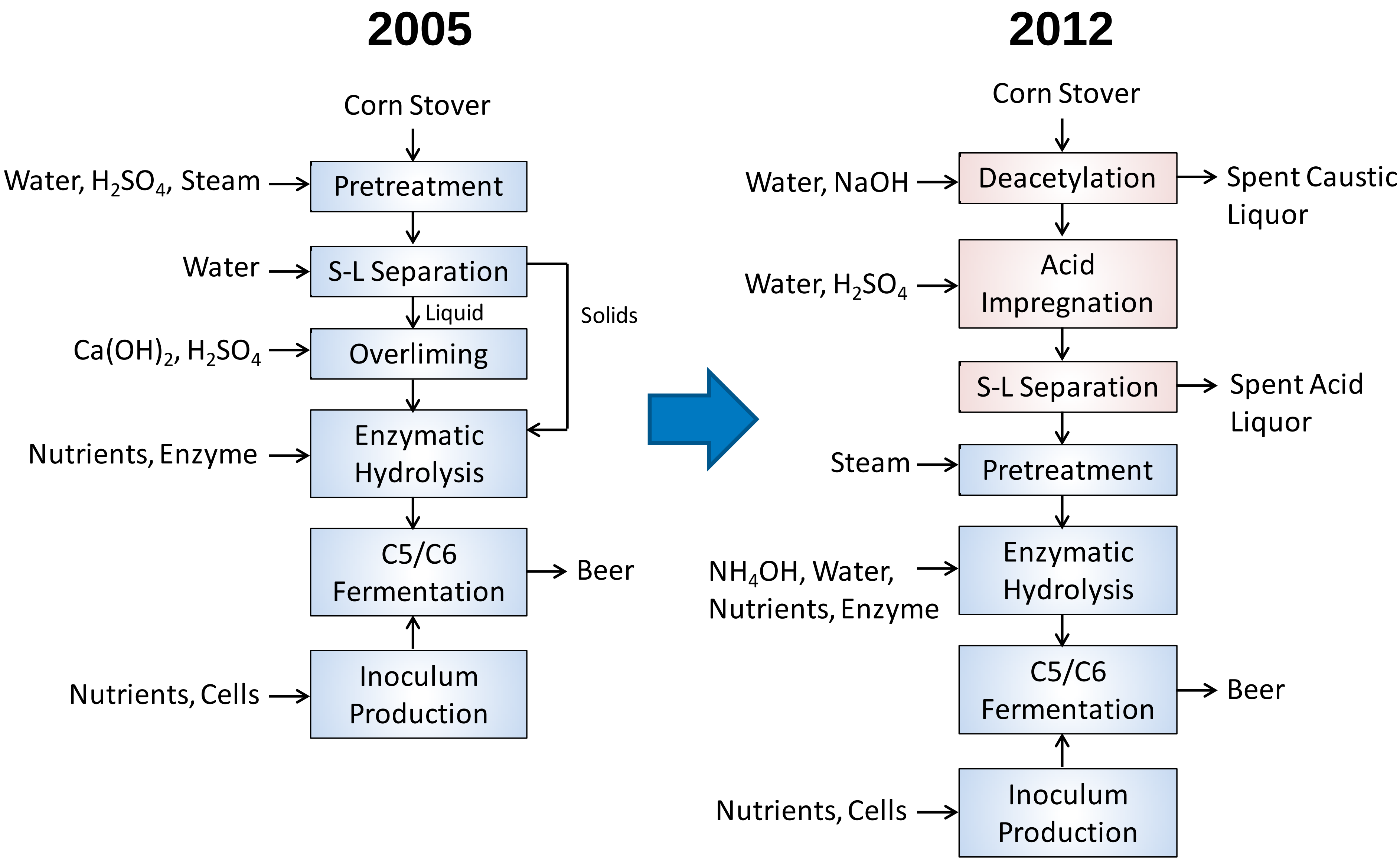


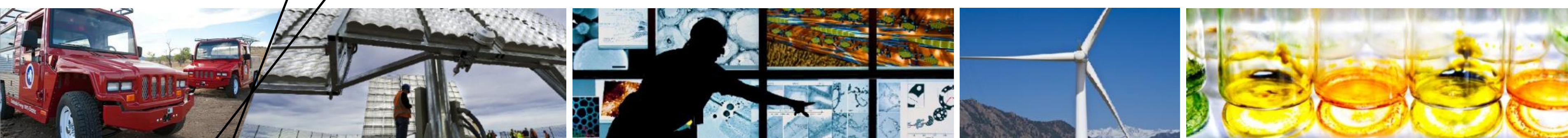
Enzyme: CTec 2 (Novozymes)

Microbe: *Zymomonas mobilis* A7

- Added feedstock deacetylation step—reduced concentration of inhibitory compounds (acetic acid, lignin derived phenolics)
- Eliminated xylo-oligomer conversion step—not needed because of good conversion of xylo-oligomers to monomers during enzymatic hydrolysis

Process Configuration Changes





Technical Accomplishments

Pilot Plant Operations: Equipment and Methodology

Pilot-Scale Equipment: Feed Preparation

Corn Stover
↓
Deacetylation
↓
Acid Impregnation
↓
S-L Separation
↓
Pretreatment
↓
Enzymatic Hydrolysis
↓
C5/C6 Fermentation
↑
Inoculum Production

1900-L Horizontal Paddle Mixer

Screw Press

Pilot-Scale Equipment: Pretreatment

Corn Stover
↓
Deacetylation
↓
Acid Impregnation
↓
S-L Separation
↓
Pretreatment
↓
Enzymatic Hydrolysis
↓
C5/C6 Fermentation
↑
Inoculum Production

40 dry kg/h Vertical Pretreatment Reactor (VtR)

40 dry kg/h Horizontal Pretreatment Reactor (HzR)

Pilot-Scale Equipment: Enzymatic Hydrolysis

Corn Stover
↓
Deacetylation
↓
Acid Impregnation
↓
S-L Separation
↓
Pretreatment
↓
Enzymatic Hydrolysis
↓
C5/C6 Fermentation
↑
Inoculum Production

4000-L Horizontal Paddle Mixer

Pilot-Scale Equipment: Fermentation

Corn Stover

↓

Deacetylation

↓

Acid Impregnation

↓

S-L Separation

↓

Pretreatment

↓

Enzymatic Hydrolysis

↓

C5/C6 Fermentation

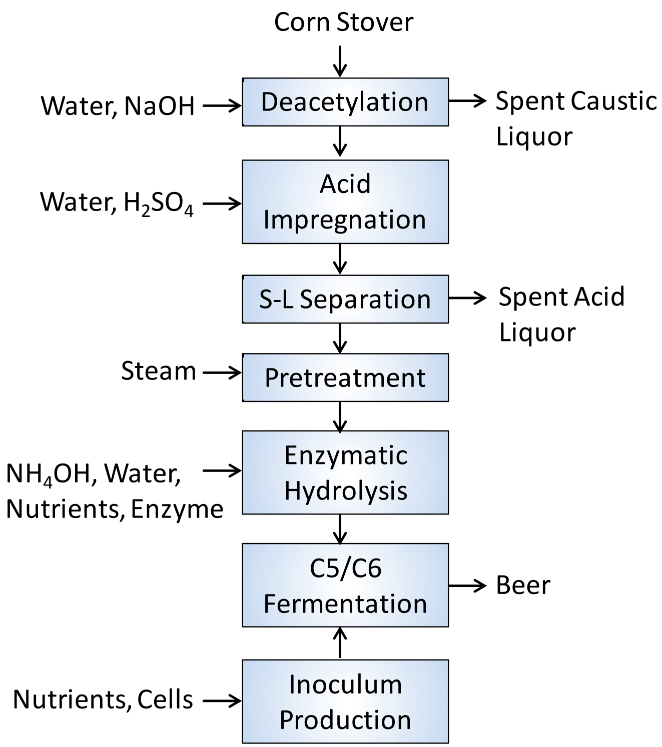
↑

Inoculum Production

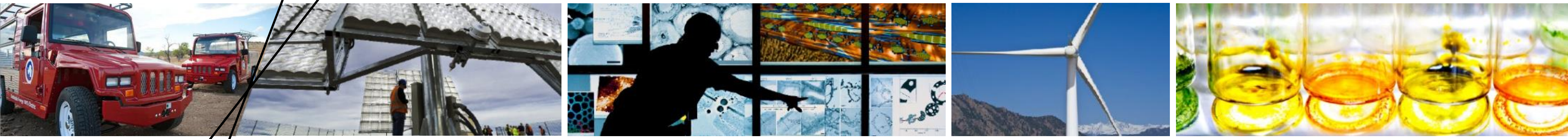
160-L Bioreactor

1500-L Bioreactor

Pilot Plant Runs



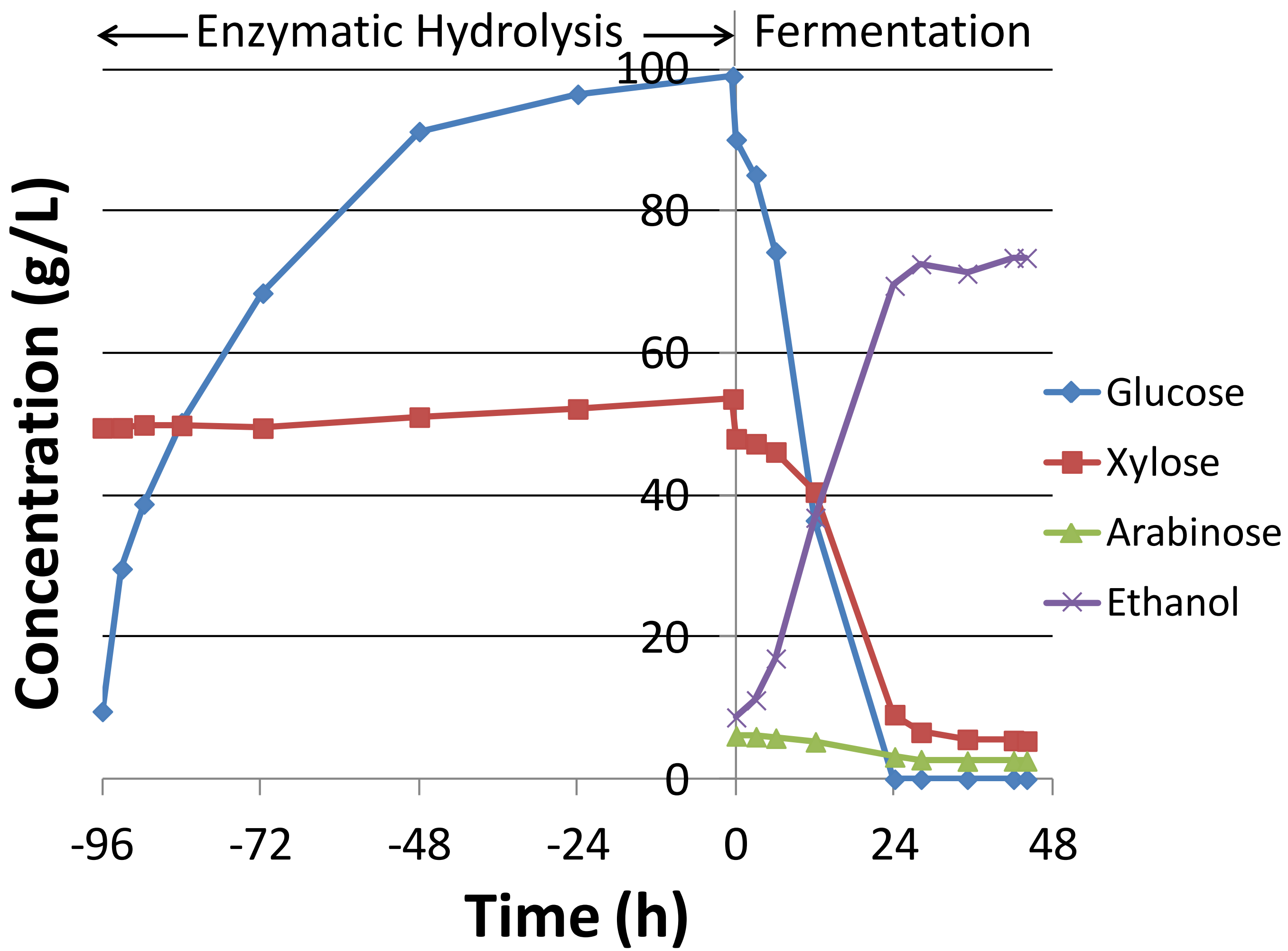
- **Six pilot-scale runs completed (five using deacetylated stover, one without) with accompanying parallel bench-scale runs**
- **Operating conditions**
  - **Pretreatment**: HzR-160°C, 10 minutes, ~0.35% (w/w) acid at reaction conditions; VtR-190°C, 1 min, ~0.35% (w/w) acid
  - **Enzymatic hydrolysis**: 20% total solids loading, 50°C, NH_4OH used to control pH at 4.8-5.2, 18-33 mg protein/g cellulose (CTec 1/2)
  - **Fermentation**: *Zymomonas mobilis* A7, 33°C, pH 5.8 controlled with NH_4OH, 10% (v/v) inoculum (~0.5 g/L initial cell density, dry basis)
- **Analytical measurements**
  - Total/insoluble solids concentration
  - Monomeric/oligomeric sugars and product concentrations by HPLC
  - Composition of solids



Technical Accomplishments

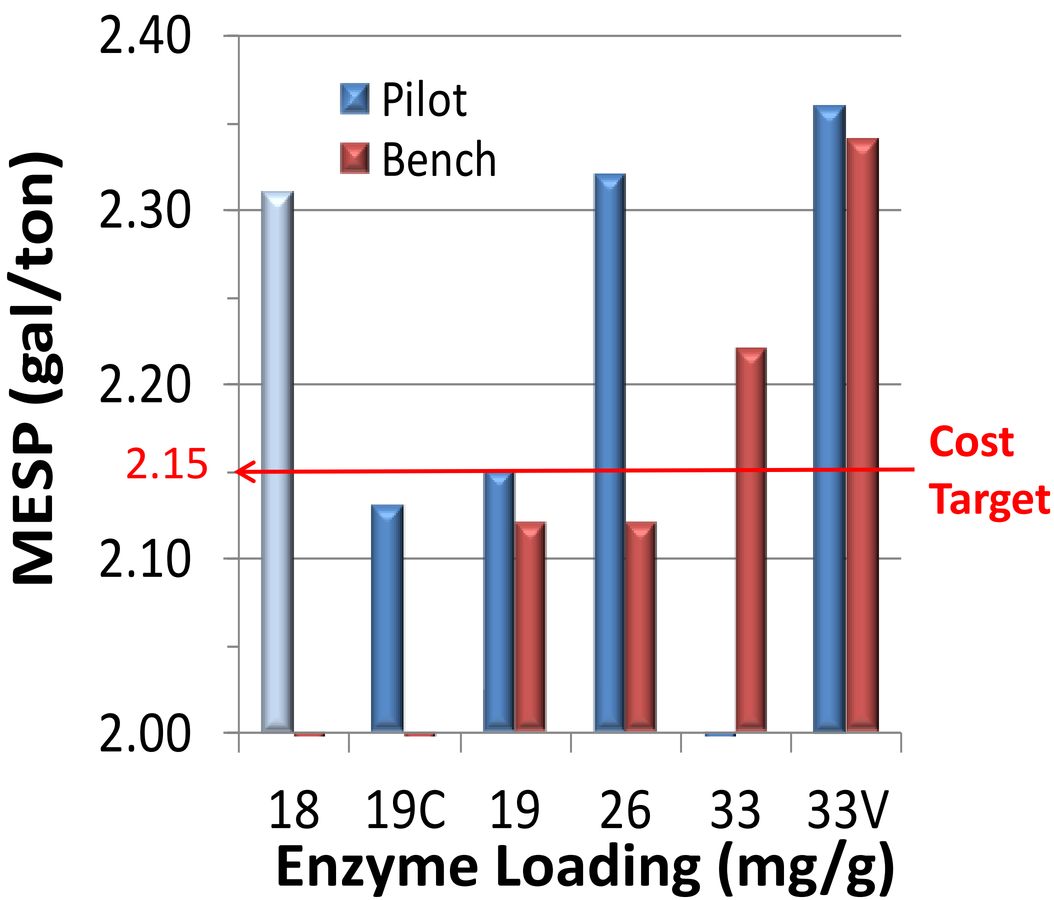
Key Pilot Plant Performance Results: Summer 2012

Representative Component Concentration Profiles



For run performed with 19 mg enzyme/g cellulose

Cost Summary-All Six Runs



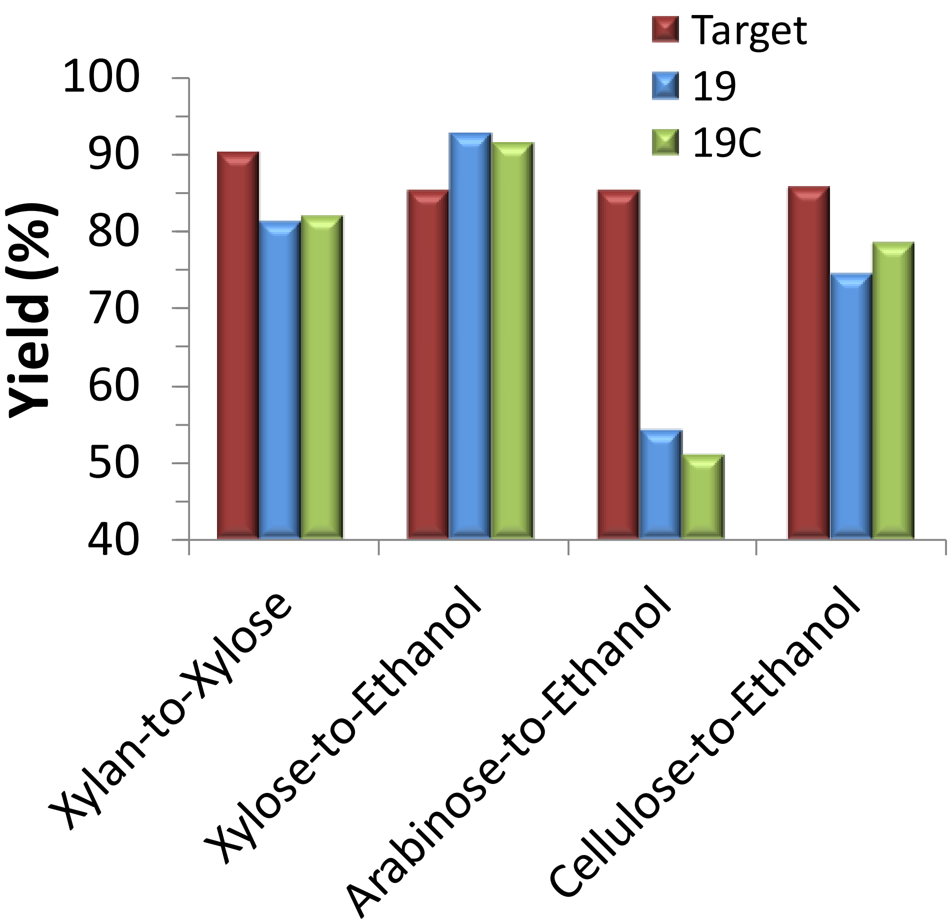
All runs used HzR, CTec 2, and deacetylated stover except as follows:

- 18 used untreated stover
- 19C used CTec 1
- 33V used VtR

- Bench-scale results generated on pretreated material produced during pilot-scale campaign
- Results for run at 33 mg/g not reported due to severe contamination
- MESP for 26 mg/g pilot-scale run were high because of slight contamination

Performance Summary

2012 cost target achieved by a combination of yield improvements and cost reductions:



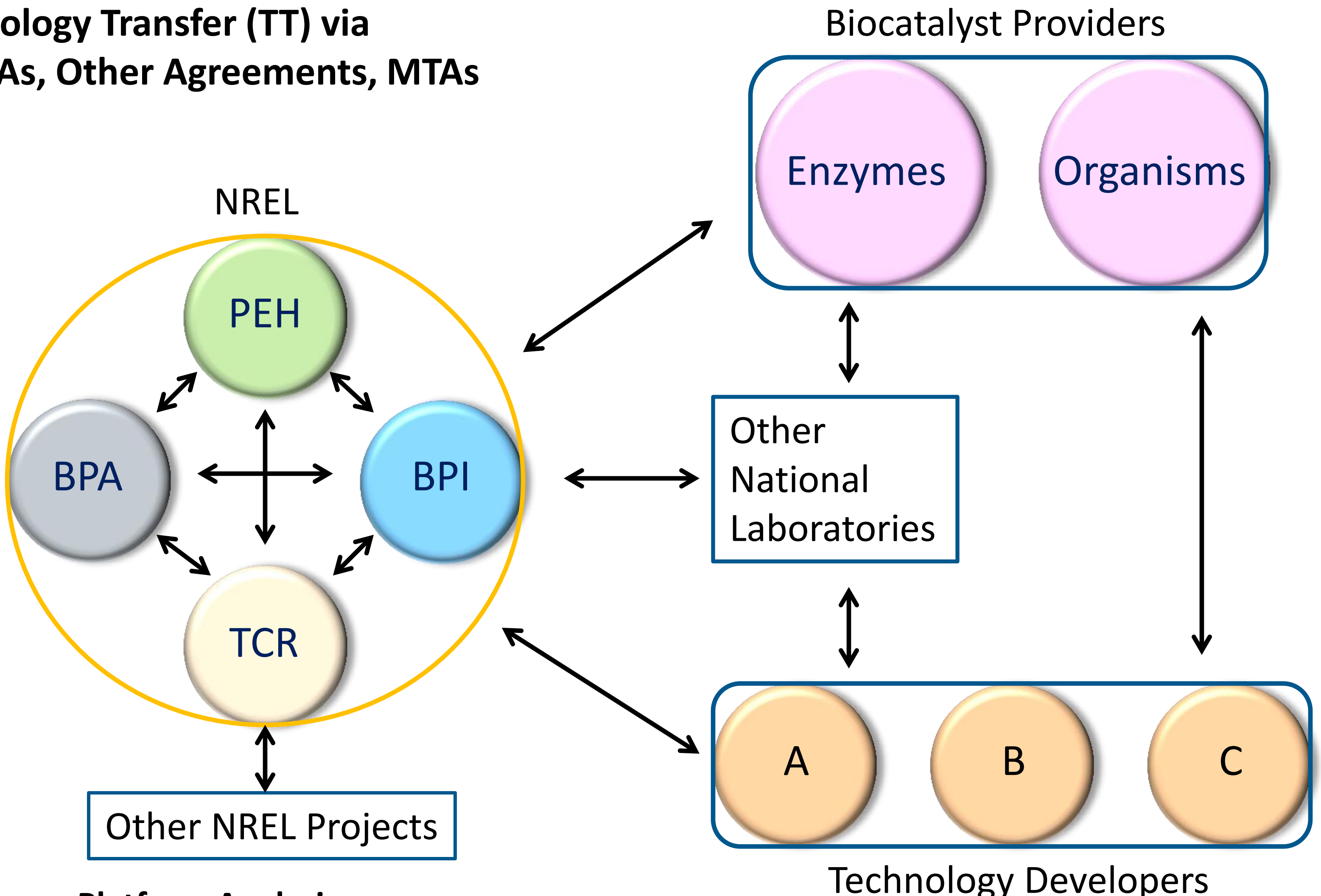
:s in enzyme

on of major
ars at high yields

sage reduced
l WWT costs

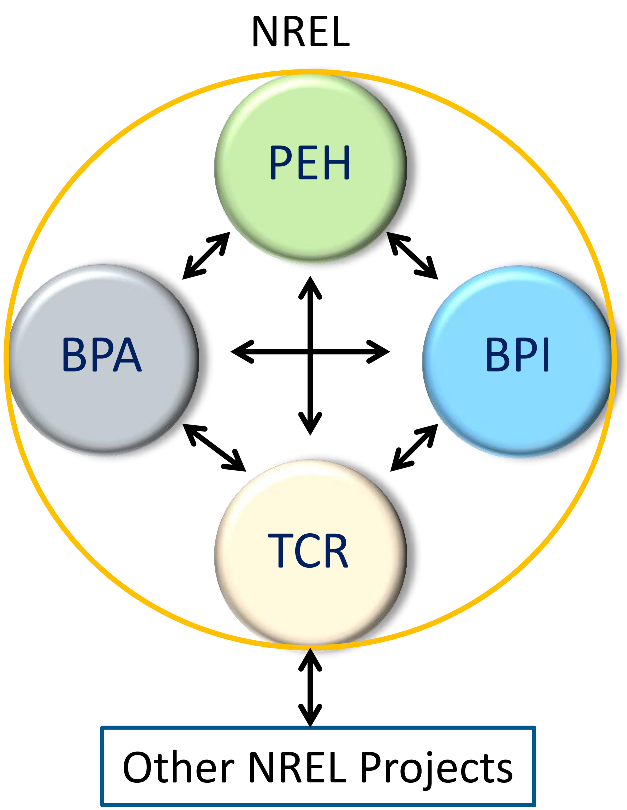
Information/Technology Flow

Technology Transfer (TT) via
CRADAs, Other Agreements, MTAs



BPA-Biomass Platform Analysis
TCR-Targeted Conversion Research
PEH-Pretreatment and Enzymatic Hydrolysis

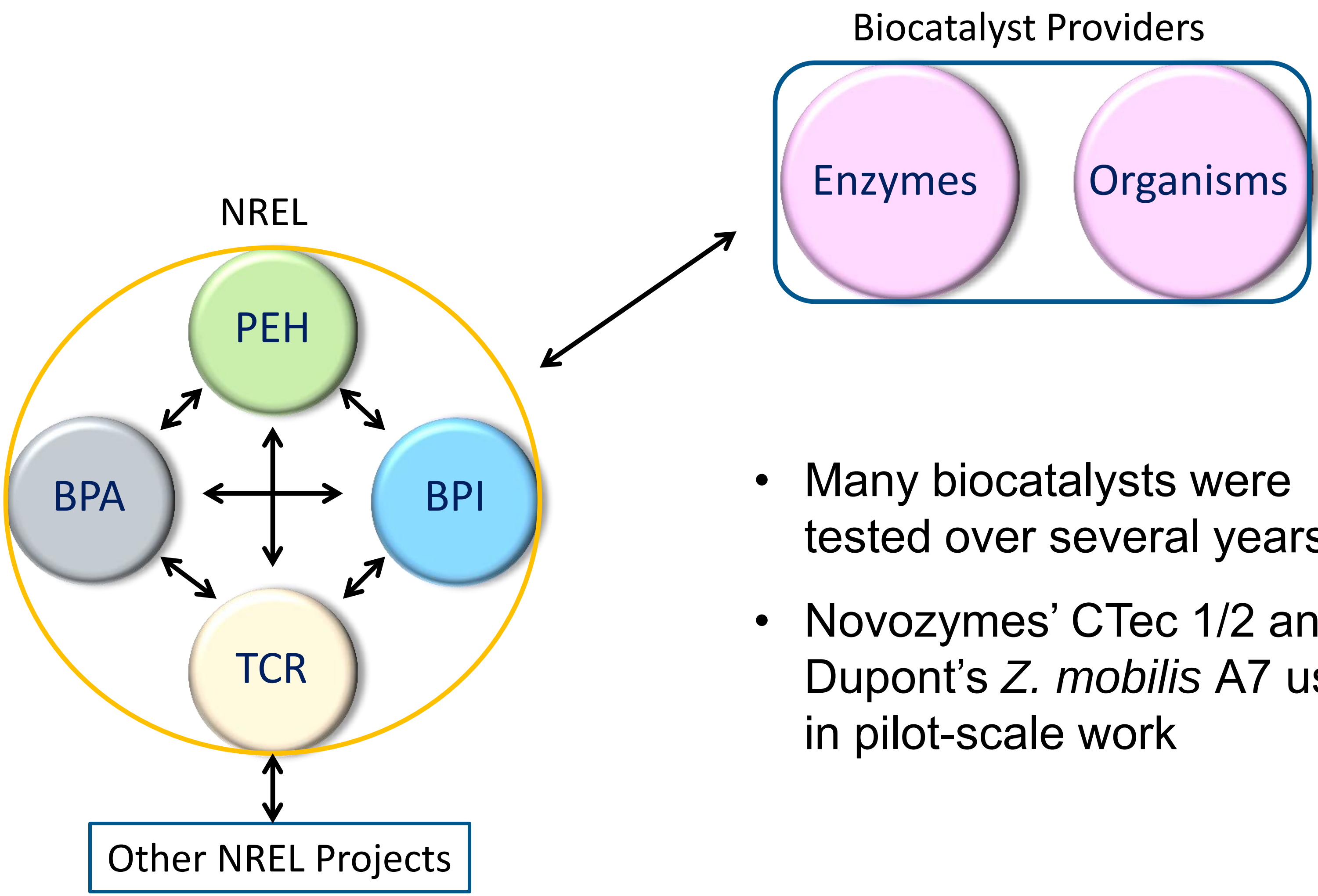
Information/Technology Flow



- Feedstock deacetylation developed by TCR and PEH utilized in pilot-scale work by BPI
- Waste water treatment design and cost update initiated and funded by BPI and performed by subcontractor and BPA using waste water sample produced in the pilot plant

BPA-Biomass Platform Analysis
TCR-Targeted Conversion Research
PEH-Pretreatment and Enzymatic Hydrolysis

Information/Technology Flow

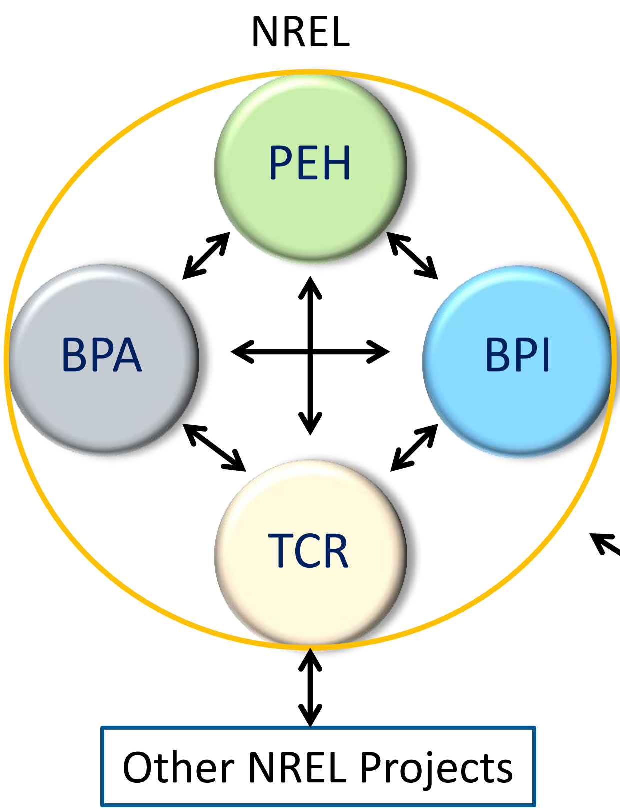


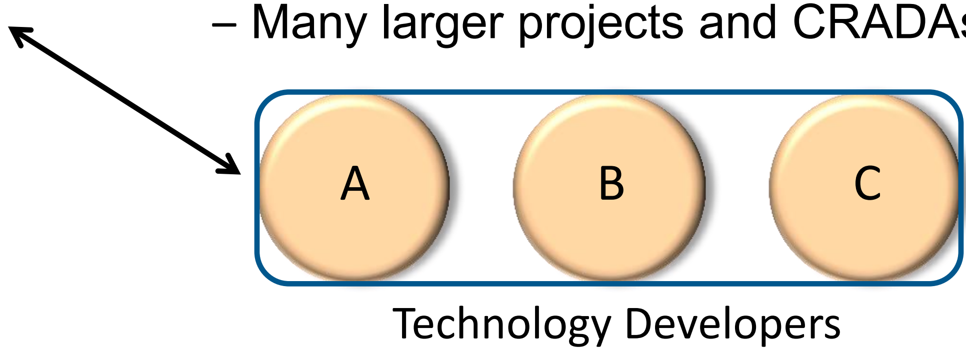
- Many biocatalysts were tested over several years
- Novozymes' CTec 1/2 and Dupont's *Z. mobilis* A7 used in pilot-scale work

BPA-Biomass Platform Analysis
TCR-Targeted Conversion Research
PEH-Pretreatment and Enzymatic Hydrolysis

Information/Technology Flow

- Distribution of new and improved analysis methods
- Knowledge and experience gained from executing DOE-funded work is utilized in our work with industry
- Many projects performed over the last two years (CRADAs)
  - Smaller efforts to test biocatalysts or provide process materials
  - Many larger projects and CRADAs





BPA-Biomass Platform Analysis
TCR-Targeted Conversion Research
PEH-Pretreatment and Enzymatic Hydrolysis

Success Factors

- Demonstrate integrated performance in bench- and pilot-scale equipment meeting 2017 and 2022 cost targets
- Advance understanding of environmental and associated regulatory/legal issues and concerns, e.g., liquid, solid and gaseous emissions, and their impacts on process economics that can only be well understood from integrated process operation
- Gather and disseminate information that enhances the ability to assess technical, market and business readiness
  – Disseminate technical information via conferences and publications
  – Develop, maintain and distribute standard analytical procedures for biomass compositional analysis
  – Use insights gained from pilot-scale operation to further understand the challenges and opportunities for process improvement

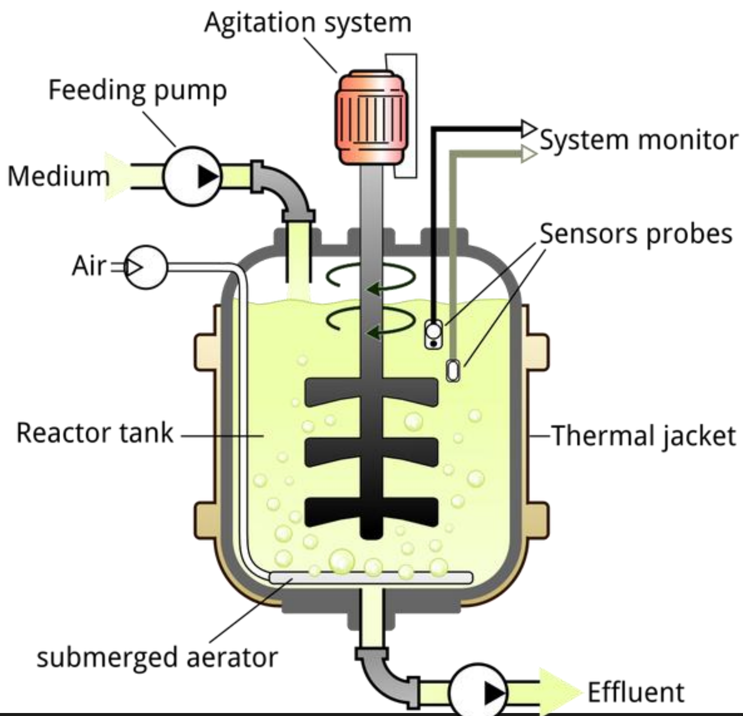
Challenges

- Improving both chemical- and enzymatic-based processes for biomass deconstruction
- Developing robust catalyst (chemical or biological) to effectively convert all biomass-derived sugars to hydrocarbons
- Finding new uses/opportunities for lignin
- Demonstrating integrated performance meeting yield/cost targets
- Demonstrating sustained continuous integrated operation for long periods of time (weeks/months)
- Reducing capital cost to increase financing opportunities

Current/Future Work

FY13 is a transition year

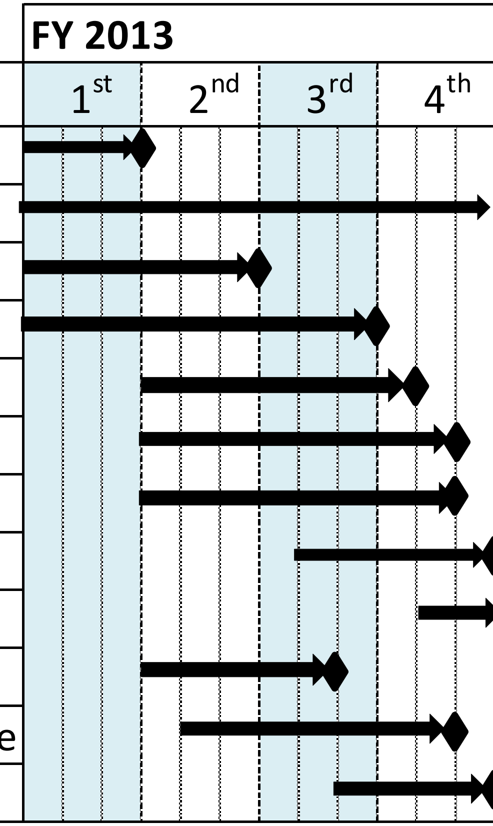
- Transitioning from cellulosic ethanol to hydrocarbon (HC) work
- Continue work relevant to HC production
  - Develop analytical methods for HC-based production processes
  - Advance understanding of pretreatment and enzymatic hydrolysis, particularly at pilot scale—understand needs and economic tradeoffs for different HC processes
- Begin new work on HC processes
  - Understand aeration performance and cost
  - Test HC-producing microbes



Current/Future Work

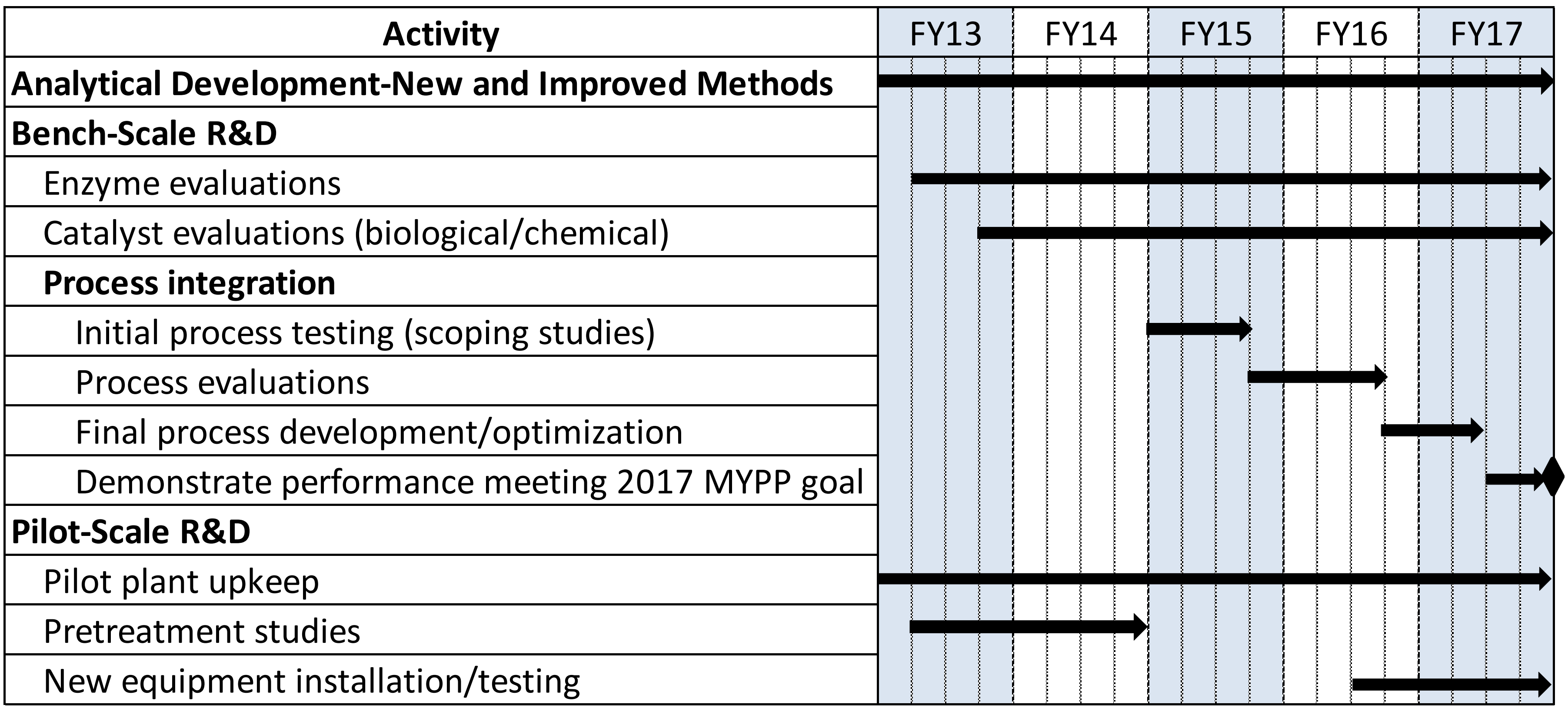
FY13 Project Schedule/Milestones

Area	Activity
Transition activities	Impact of deacetylation on process performance
	Publish cellulosic ethanol work
HC relevant	Evaluate storage conditions on sample composition
	New HPLC method for xylo-oligomers
	Evaluate performance of new enzymes
	NIR model for pretreated corn stover slurry composition
	Residence time distributions in pilot scale reactors
	Impact of feedstock pre-processing during pretreatment
	Pretreatment pilot-scale kinetics studies
HC processes	Evaluate aeration at large scale (literature search)
	Understand large-scale aeration: cost versus performance
	Evaluate performance of new HC-producing organisms



Future Work-High Level Gantt Chart

- Analytical method development and improvement
- Bench-scale process integration work focused on identifying and testing a process that meets the 2017 MYPP goal
- Pilot-plant work with near term focus on pretreatment optimization in collaboration with other NREL task and maintaining pilot plant capabilities



Summary

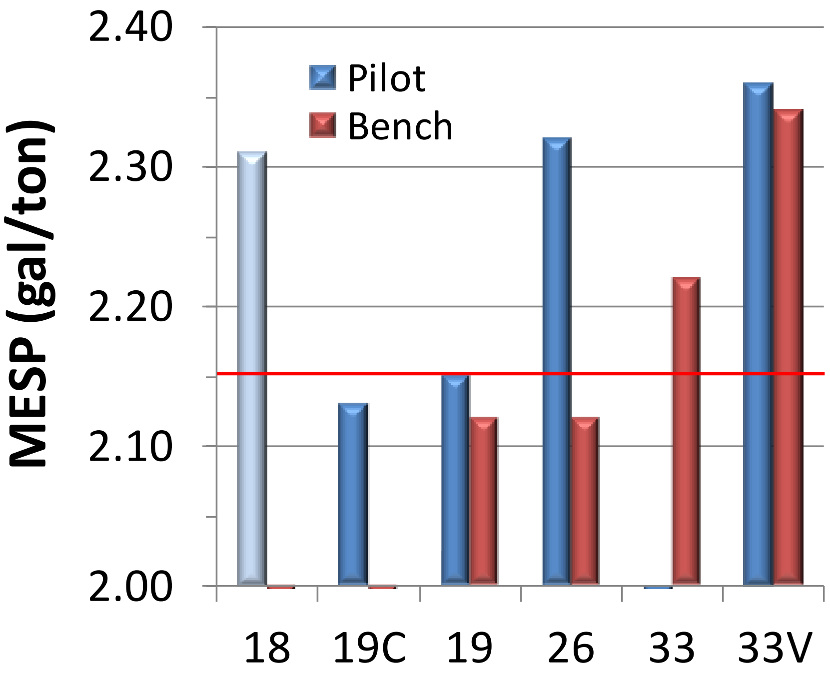
Project Objectives

- Produce integrated process performance data that when combined with a cost estimate achieves BETO's MYPP cost targets
- Advance understanding of key issues/challenges affecting process performance and cost to enable/inform the biomass industry
- Develop new and improved analytical techniques and provide analytical standards and reference materials to industrial and academic researchers
- Make findings available by presenting and publishing

Summary

Key Accomplishments

- Continued deployment of wet-chemical and rapid biomass analytical methods
- Developed genomically-integrate *Z. mobilis* strain able to effectively convert arabinose to ethanol
- Demonstrated integrated performance at bench- and pilot-scale meeting the MYPP 2012 cellulosic ethanol cost target



Summary

Current/Future Activities

- Research direction and activities guided by technoeconomic analysis (TEA)
- TEA facilitates development of detailed annual operating plans focused on MYPP goals
- Near term (1-3 years) work will investigate options for HC production processes
- Mid term goal (2017) to achieve interim HC fuel cost target
- Long term goal to demonstrate pilot-scale integrated performance meeting 2022 cost target

Team Members

NATIONAL RENEWABLE ENERGY LABORATORY

Pilot Plant Operations/Analysis

- Alex Chapeaux
- Nancy Dowe
- Rick Elander
- Jody Farmer
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- David Templeton
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- Ed Wolfrum

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Other Contributors, Partners

- University of Louisville, Eric Berson
- Colorado State University, Ranil Wickramasinghe
- Hazen Research, Inc., Brian Copper
- MAST Center
- ORNL, Abhijeet Borole

Biocatalyst Developers

- Novozymes
- DuPont

Questions?

Additional Slides

Previous Review Comments

- Comment: Approach to process configuration work is not novel. Separate C5 and C6 fermentation has been discussed for many years and I am surprised this was even mentioned in the presentation for the reviewers.
- Response: The separate C5/C6 concept was originally proposed in the early 1980's when different organisms were needed for the different sugars as cofermenting organism were not yet available, but the concept has not been rigorously investigated. The concept is interesting since enzymatic cellulose conversion yields can be increased compared to whole slurry processes because enzyme inhibition is significantly reduced with no sugars being initial present in the system. Furthermore, better conversion of C5 sugars can be achieved because sugars concentrations, and thus product concentrations, are lower in the C5 stream leading to higher conversion yields. However, a solid-liquid separation step is required and the associated cost must be offset by better conversion yields. The intent of this work was to understand which process was economically better prior to performing the pilot-scale integrated runs in 2012.

Previous Review Comments

- Comment: The PDU should be performing more continuous and integrated processes at the pilot scale, NOT at the bench scale as mentioned by the presenter. A greater use of the PDU could be achievable by industry should the operating costs of the PDU be lowered. Even large companies like Mitsubishi have been astounded at what it costs to operate the NREL PDU. This needs to be addressed in a VERY serious manner.
- Response: Pilot scale runs are very time and resource (labor and money) intensive and so bench-scale work is a very effective tool for screening and identifying conditions for pilot runs. During bench-scale work performed in FY11 and early FY12, we identified the best strategy to employ in the pilot runs and were able perform 6 runs in the latter half of FY12 that produced data meeting the 2012 cellulosic ethanol cost target. The cost of operating our facility is significantly higher than cost of academic-based work. However, we believe our rates are similar to most industrial-based facilities and other national laboratories.

Publications

- Jennings, E., Schell, D.J. 2011, “Conditioning of dilute-acid pretreated corn stover hydrolysate liquors by treatment with lime or ammonium hydroxide to improve conversion of sugars to ethanol,” Bioresourc. Technol. 102, 1240-1245
- Grzenia, D., Wickramasinghe, R, Schell, D.J. 2012. “Fermentation of reactive-membrane-extracted and ammonium-hydroxide-conditioned dilute-acid pretreated corn stover.” App. Biochem, Biotech. 166, 470-478.
- Vicari, K., Tallam, S., Shatova, T., Joo, K., Scarlata, C., Humbird, D., Wolfrum, E., Beckham, G. 2012. “Uncertainty in techno-economic estimates of cellulosic ethanol production due to experimental measurement uncertainty.” Biotechnol. Biofuels 5:23.
- Grzenia, D., Schell, D.J., Wickramasinghe, R, 2012. “Membrane extraction for detoxification of biomass hydrolysates.” Bioresourc. Technol. 111, 248-254.
- Borole, A.P., Hamilton, C.Y., Schell, D.J. 2013. “Conversion of residual organics in corn stover-derived biorefinery stream to bioenergy via a microbial fuel cell.” Env. Sci. Technol. 47, 642-648.
- Katahira, R., Sluiter, J., Schell, D.J., Davis, M. 2013. "Degradation of carbohydrates during dilute sulfuric acid pretreatment can interfere with lignin measurements in solid residues." J. of Agricul. Food Chem. 61, 3286-3292.
- Sluiter, AS., Sluiter, J., Wolfrum, E. 2013. “Methods for Biomass Compositional Analysis,“ in *Catalysis for the Conversion of Biomass and Its Derivatives*, Max Planck Research Library for the History and Development of Knowledge, Proceedings 2.” Berlin: Edition Open Access (2013), ISBN 978-3-8442-4282-9.

FY 11 Presentations

- Templeton, D., Yen, H., Sharpless, K.E., Wolfrum, E. 2010 “Compositional analysis of biomass reference materials: Results from an interlaboratory study “, oral presentation at the International Chemical Congress of Pacific Basin Societies (Pacifichem 2010) , Honolulu, HI.
- Templeton, D.W., Scarlata, C.J., Sluiter, J.B, Yen, J.H., Sharpless, K.E., Wolfrum, E.J. 2011 “Biomass compositional analysis of feedstock materials. Oral presentation at the 33rd Symposium on Biotechnology for Fuels and Chemicals. Seattle, WA.
- Chapeaux, A., Dowe, N., Schell, D.J. 2011. “Performance of a Separate Hemicellulosic and Cellulosic Stream Process Design for Producing Ethanol from Lignocellulosic Biomass.” Poster presentation at the 33rd Symposium on Biotechnology for Fuels and Chemicals. Seattle, WA.
- Dowe, N., Chapeaux, A., Humbird, D., Jennings, E.W., and Schell, D.J. 2011. “Performance and Economics of Three Process Configurations for Production of Ethanol from Dilute Acid Pretreated Corn Stover.” Poster presentation at the 33rd Symposium on Biotechnology for Fuels and Chemicals. Seattle, WA.
- Chambliss, K., Sevcik, R.S., Hyman, D.A., Scarlata, C.J., Pohl, C. 2011. “Rapid HPAE-PAD determination of sugars in liquid process samples: Inter-laboratory comparion of analytical performance for the CarboPac SA10 stationary phase.” Poster presentation at the 33rd Symposium on Biotechnology for Fuels and Chemicals. Seattle, WA.
- Hyman, D.A, Scarlata, C.J. 2011. “Analysis of carbohydrates in pretreated biomass hydrolyzate liquor: A comparison between two HPLC methods.” Poster presentation at the 33rd Symposium on Biotechnology for Fuels and Chemicals. Seattle, WA.
- Sluiter, A., Payne, P., Wolfrum, E. 2011. “Using NIR/PLS for the rapid analysis of acid pretreated slurries.” Poster presentation at the 33rd Symposium on Biotechnology for Fuels and Chemicals. Seattle, WA.
- Sluiter, J. 2011. “Biomass compositional analysis - Summative mass closure and method uncertainties.” Poster presentation at the 33rd Symposium on Biotechnology for Fuels and Chemicals. Seattle, WA.
- Chou, Y.-C., Mohagheghi, A. Zhang, M. 2011. “Construction and evaluation of glucose/xylose/arabinose co-fermenting *Zymomonas mobilis* strains” Poster presentation at the 33rd Symposium on *Biotechnology* for Fuels and Chemicals. Seattle, WA.

FY12 Presentations

- Templeton, D., Scarlata, C., Sluiter, J., Crocker, D., Payne, C., Wolfrum, E. 2012 “Long-term variability in bagasse compositional analysis.” Poster presentation at 34th Biotechnology Symposium for Fuels and Chemicals. New Orleans, LA.
- Gomes, A., Santa Anna, L., Tavares, R., Araujo, V., Templeton, D. 2012 “Comparative Characterization Study of Components of Sugarcane Bagasse and Corn Stover.” Poster presentation at 34th Biotechnology Symposium for Fuels and Chemicals. New Orleans, LA.
- Jennings, E., Schell, D., Dowe, N., Peterson, D. 2012 “Production of monomeric and oligomeric glucose during enzymatic hydrolysis of dilute-acid pretreated corn stover”. Poster presentation at 34th Symposium on Biotechnology for Fuels and Chemicals. New Orleans, LA.
- Yat-Chen, C., Linger, Z., Yang, Z., Mohagheghi, A., Zhang, M. 2012 “Genetic improvement and evaluation of arabinose utilizing *Zymomonas mobilis* strains in pretreated corn stover hydrolysate.” Poster presentation at SIM 2012 Annual Meeting and Exhibition. Washington, DC.
- Schell, D.J. 2012 “Progress toward Sustainable Biofuels — Pilot-Scale Demonstration of Integrated Cellulosic Ethanol Production” Oral presentation at the 2012 Annual AIChE Meeting. Pittsburgh, PA.

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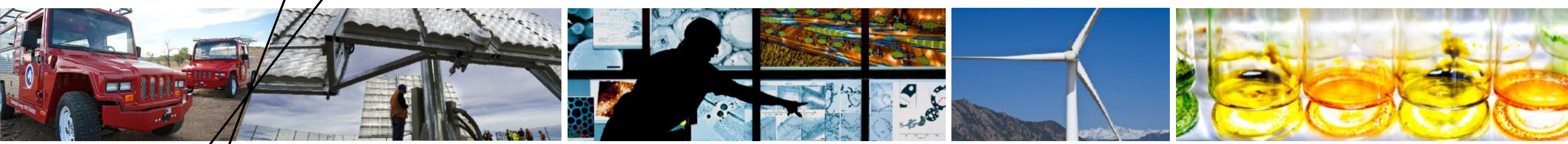


Technical Accomplishments Details

Additional Analytical Development Work

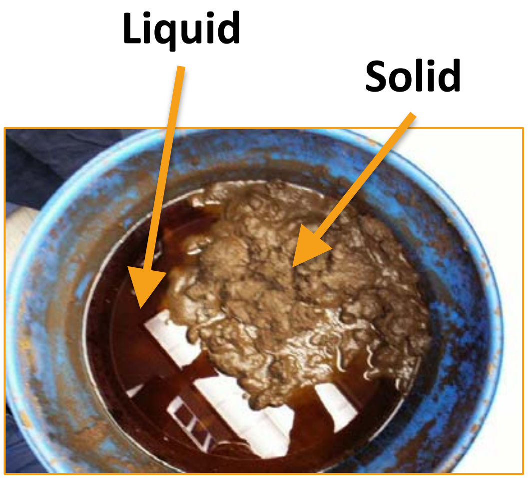
Scientific Data Management System (SDMS)

- New system developed by BPI to support NREL Biomass Program needs for data management and storage
- It has been replicated (with attribution!) for use by:
  - The Sustainable Algal Biofuels Consortium (SABC)
  - The Algal Testbed Private-Public Partnership (ATP^3)
  - Internal NREL algae researchers
- The code is all open source
  - Java, Google Windows Toolkit (GWT), Spring Security, Hibernate, mySQL
  - Codebase available for free on GitHub

Measurement Uncertainty

- Researchers from ***MIT's David H. Koch School of Chemical Engineering Practice*** and NREL studied the effect of uncertainty in primary analytical measurements on the uncertainty in calculated yields and the Minimum Ethanol Selling Price
- One measurement, the Fraction Insoluble Solids (FIS) of pretreated slurries drives xylose, glucose, and ethanol yield uncertainties; these yield uncertainties drive MESP uncertainty
- New FIS method under development using Automated Solvent Extraction system to reduce variability of this measurement, but good sampling technique is necessary to reduce errors

"Uncertainty in Techno-Economic Estimates of Cellulosic Ethanol Production due to Experimental Measurement Uncertainty", K. Vicari et al., Biotechnology for Biofuels. 5:23 (2012).

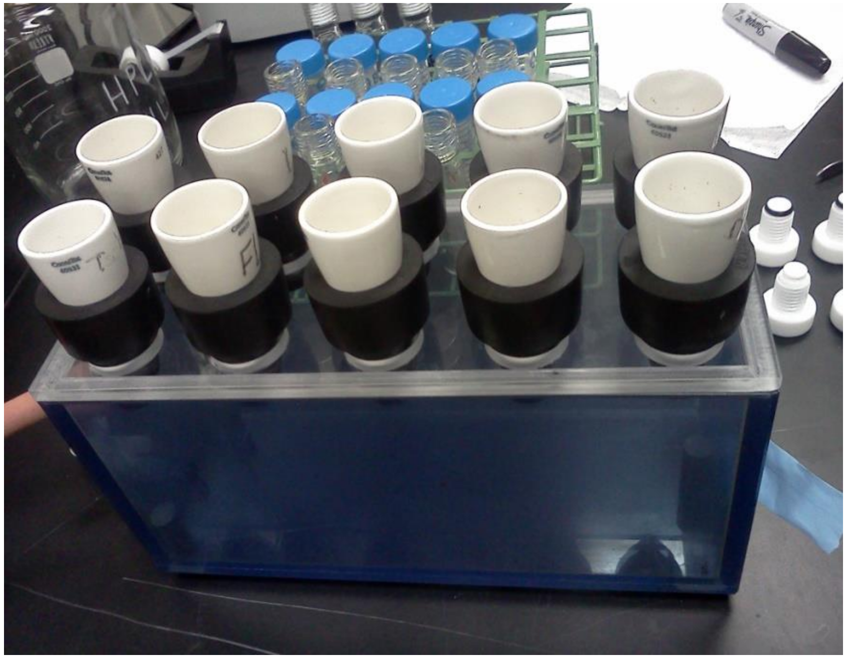


Increasing Solids Analysis Throughput

- Developed a higher-throughput biomass compositional analysis method for both feedstocks and washed pretreated solids
- New method has precision and accuracy equivalent to traditional method, with two- to three-fold increase in sample throughput that will significantly improve our productivity

Key Improvements:

- Combined water & ethanol extraction
- Automated the acid-soluble lignin measurement
- Smaller biomass sample needed for analytical hydrolysis
- Custom-designed filtration apparatus



NREL
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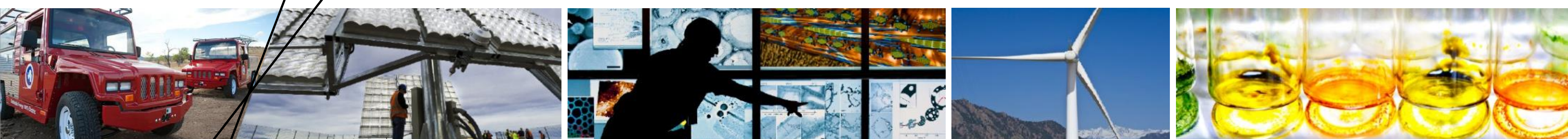
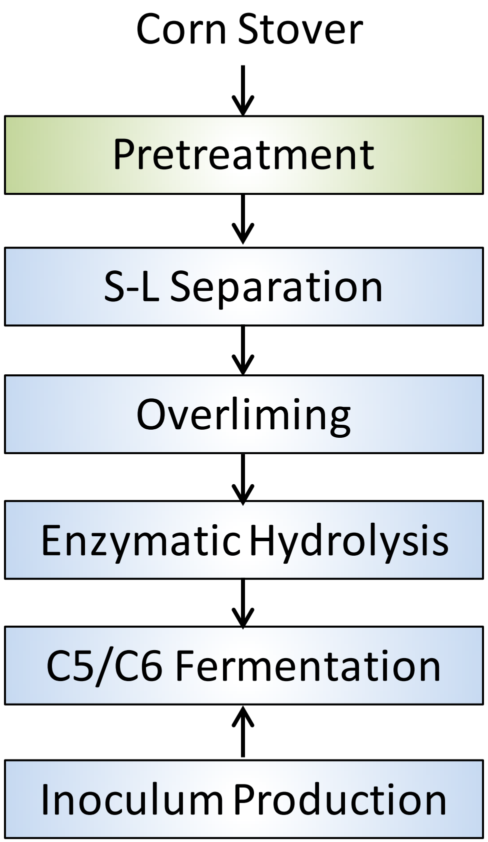


Integrated Bench-Scale Run Details

Equipment and Methodologies

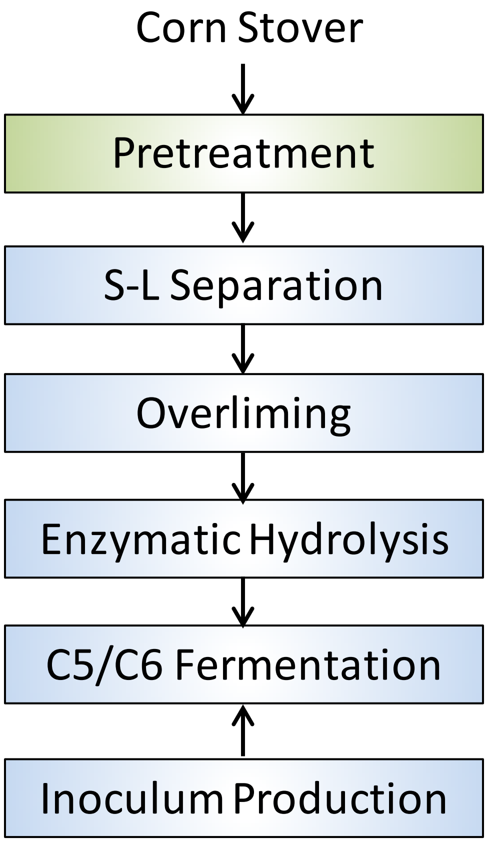
Bench-Scale Methodology



Up until 2008 used 30-40 dry kg/h Vertical Pretreatment Reactor (VtR)

- Corn stover acquired from a farm in Wray, CO
- Pretreatment carried out pilot-scale reactors

Bench-Scale Methodology



Post 2007 used 5 dry kg/h Horizontal Pretreatment Reactor System

- Operating conditions evolved over time

Bench-Scale Methodology

Corn Stover

↓

Pretreatment

↓

S-L Separation

↓

Overliming

↓

Enzymatic Hydrolysis

↓

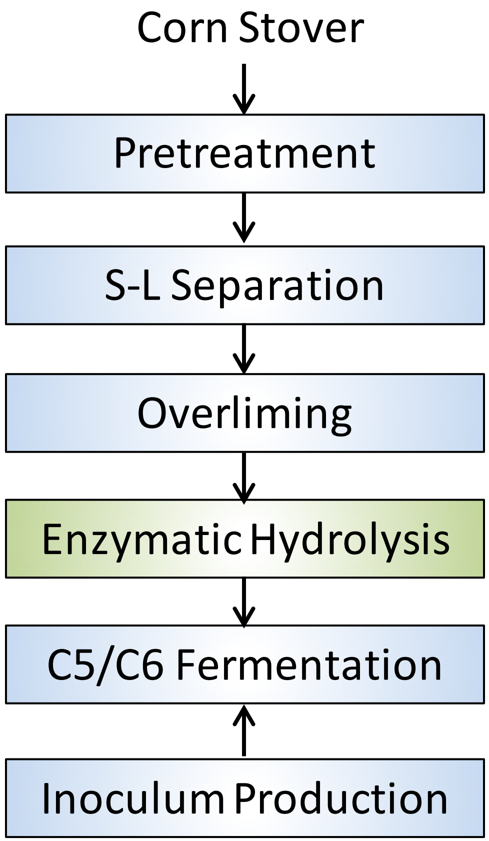
C5/C6 Fermentation

↑

Inoculum Production

7.5 L Perforated Bowl Centrifuge

Bench-Scale Methodology

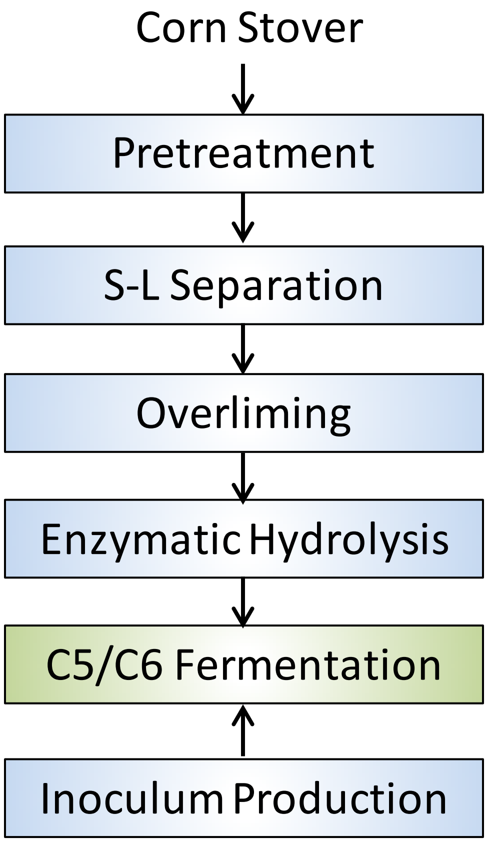


Bench-Top Shaking Incubator

- Solids loading fixed at 20% (w/w) total solids
- Enzyme loading fixed at 40 mg protein*/g cellulose
- Temperature 45°-50°C, pH initially set to 5.0-5.2
- Enzyme package changed with time

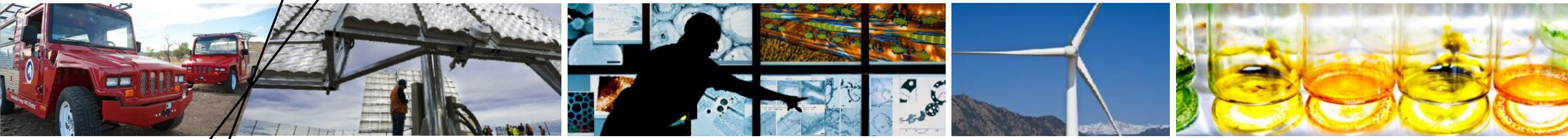
*BCA protein assay

Bench-Scale Methodology



500-mL Fermentors

- Two cofermenting *Zymomonas mobilis* strains tested
- Temperature 33°C, pH controlled at 5.8 with NH_4OH
- Media: 5 g/L yeast extract, 1 g/L KH_2PO_4 or 0.25% Corn Steep Liquor
- Initial cell density ~ 0.5 g/L (dry basis, cell paste or 10% v/v transfer)



Technical Accomplishments Details

All Pilot Plant Run Results

Summer 2012

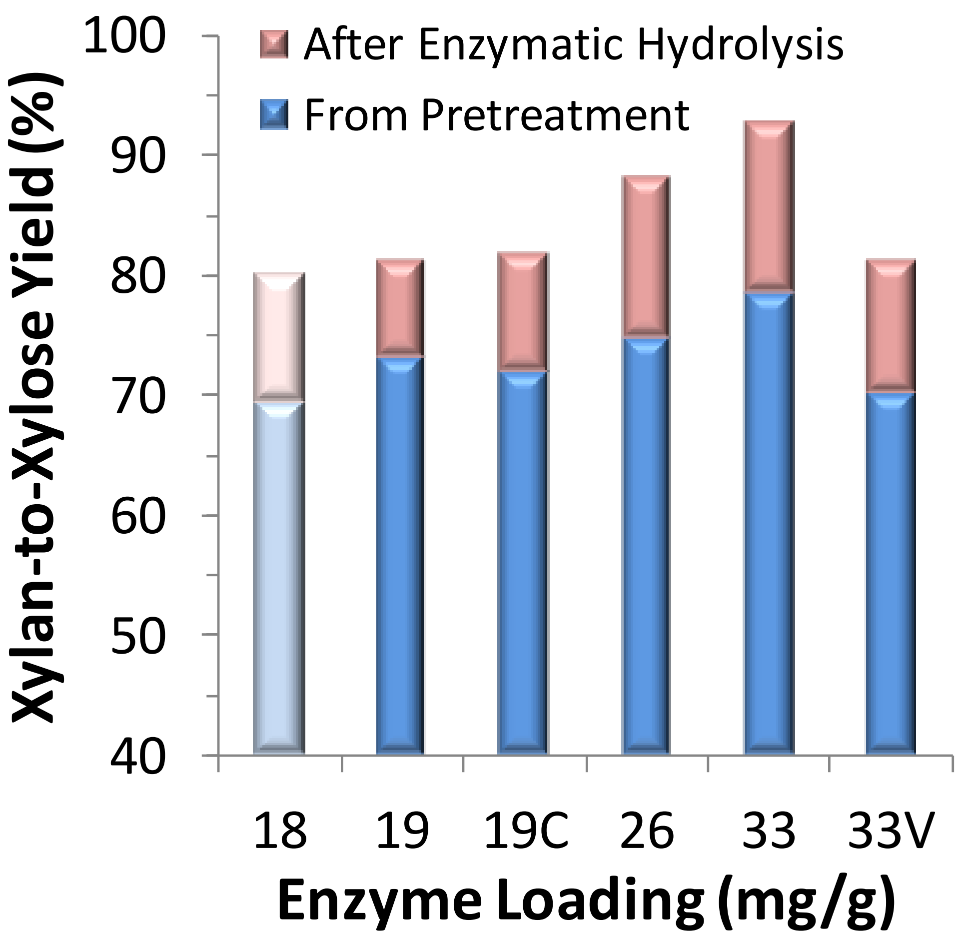
Variable Pilot Plant Run Conditions

Run #	1	2	3	4	5	6
Feedstock	Unt	Deace	Deace	Deace	Deace	Deace
Pretreatment Reactor	HzR	HzR	HzR	HzR	HzR	VtR
Enzyme Type	CTec2	CTec1	CTec 2	CTec2	CTec2	CTec2
Enzyme Loading (mg/g)	18	19	19	26	33	33
Code	18	19C	19	26	33	33V

Unt-Untreated stover
Deace-Deacetylated stover

HzR-Horizontal Reactor
VtR-Vertical Reactor

Xylan Conversion Yield after Enzymatic Hydrolysis

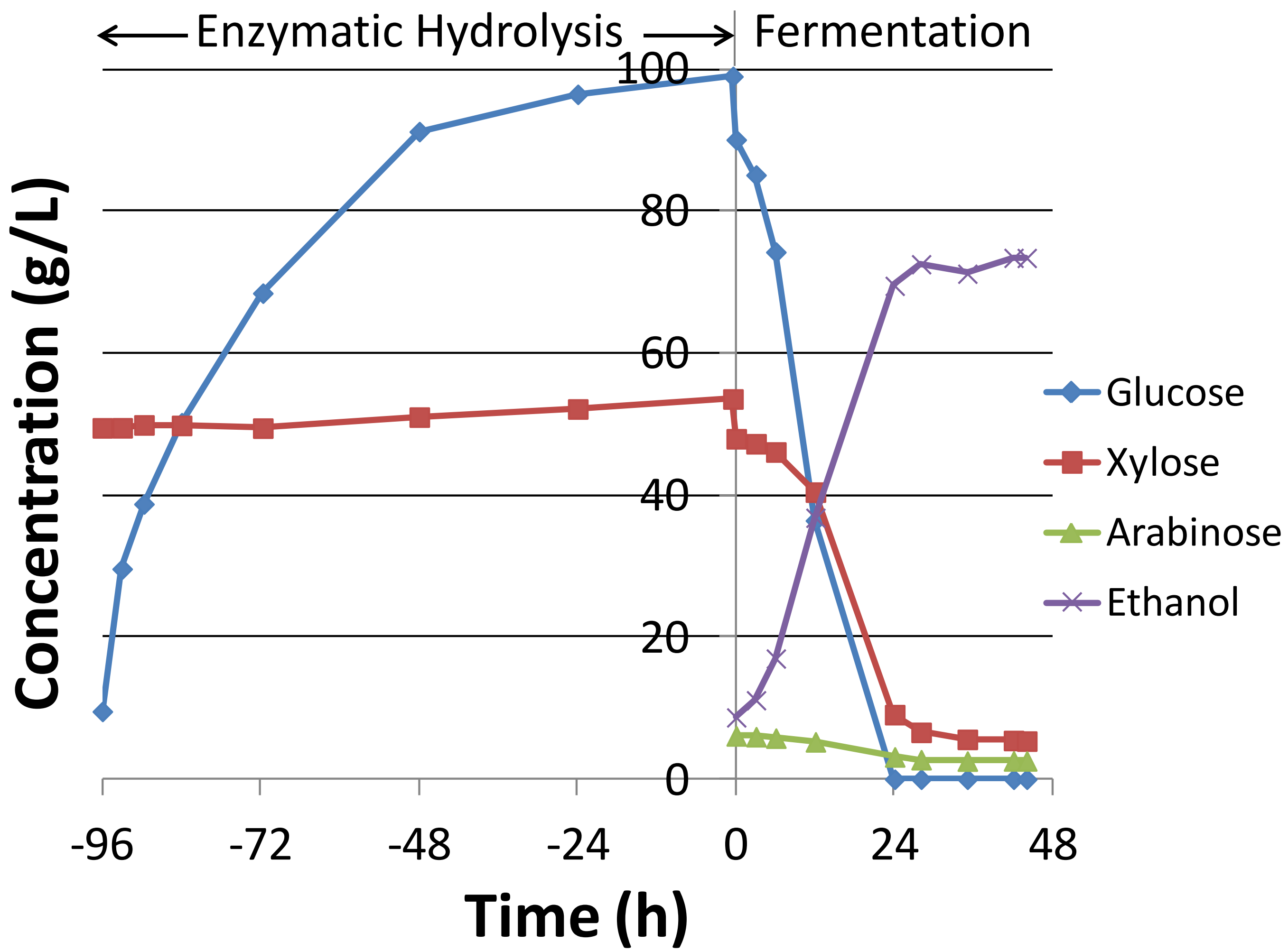


ıt xylose yields were
ınsistent on
ɪ stover (Deace) in
treatment reactor

ers and residual
converted to xylose
matic hydrolysis

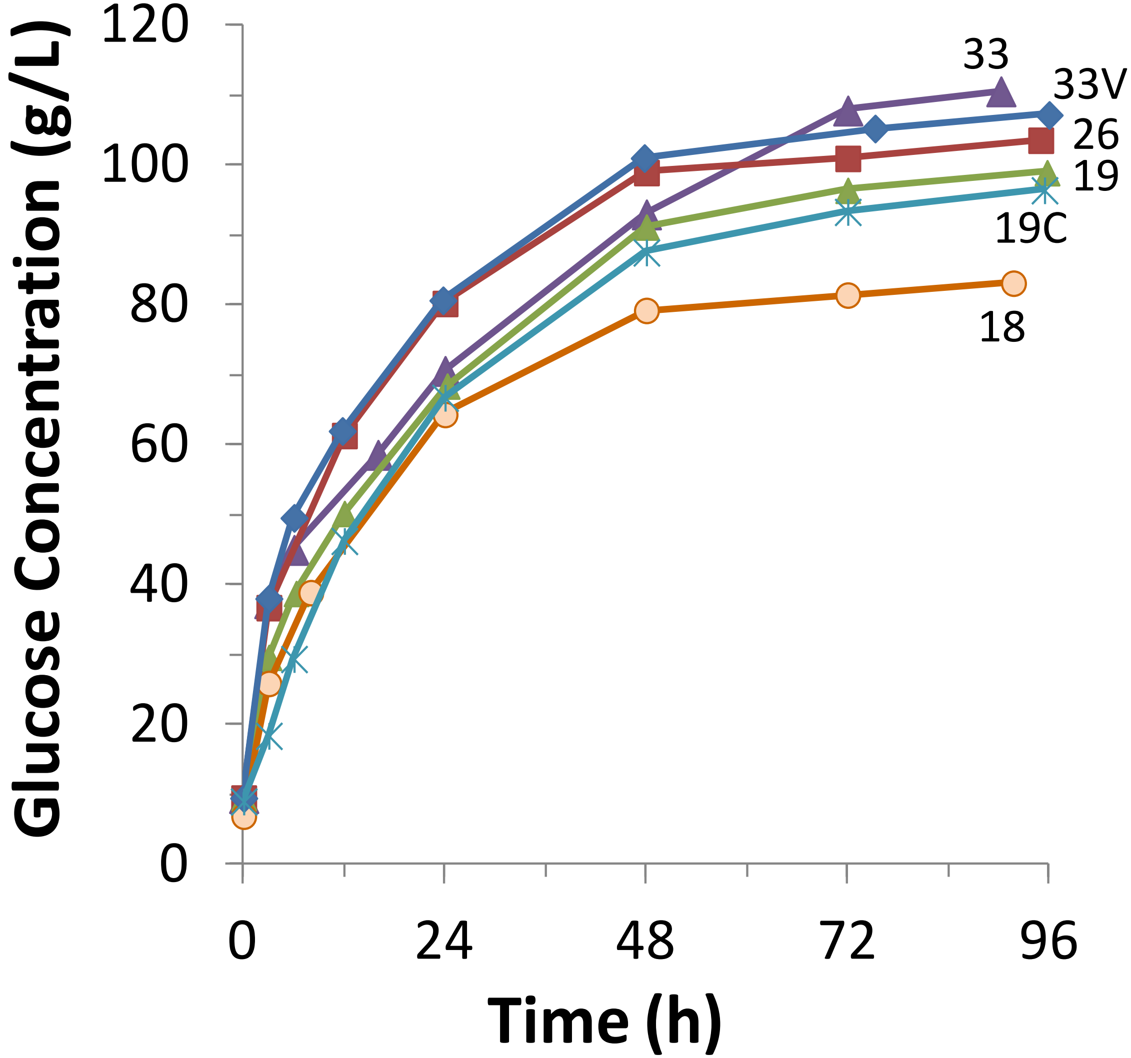
nditions in the VtR
evere than in the HzR
by the lower yields

Representative Component Concentration Profiles

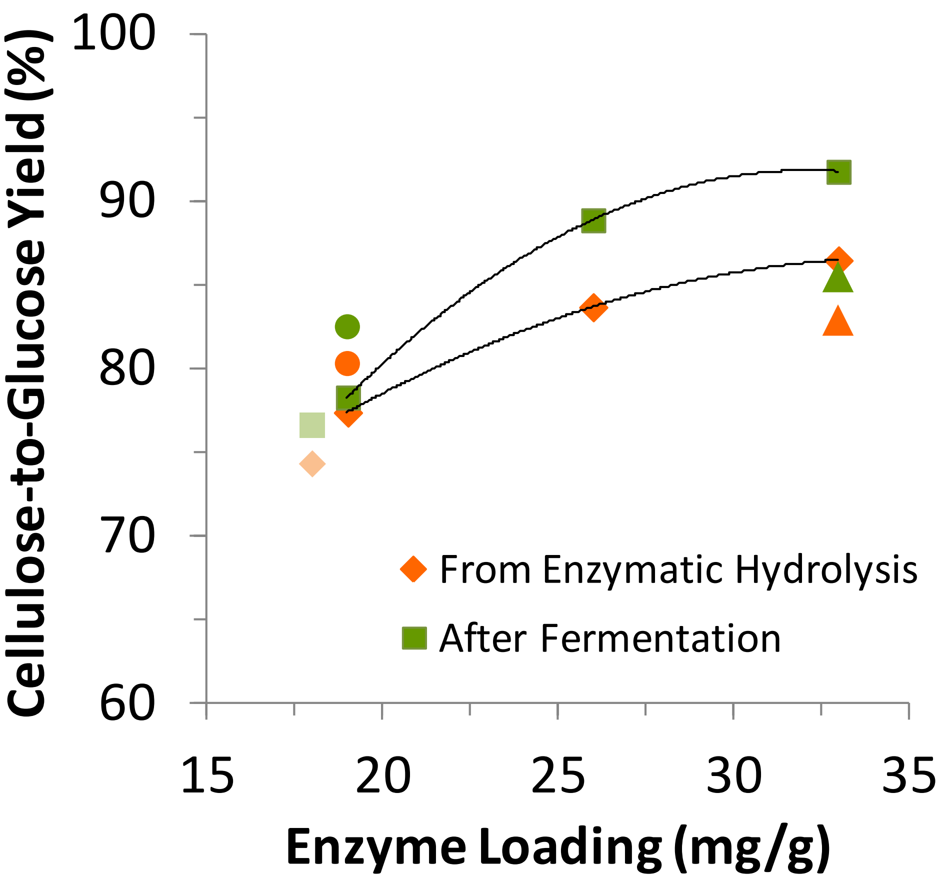


For run performed with 19 mg enzyme/g cellulose

Glucose Production during Enzymatic Hydrolysis

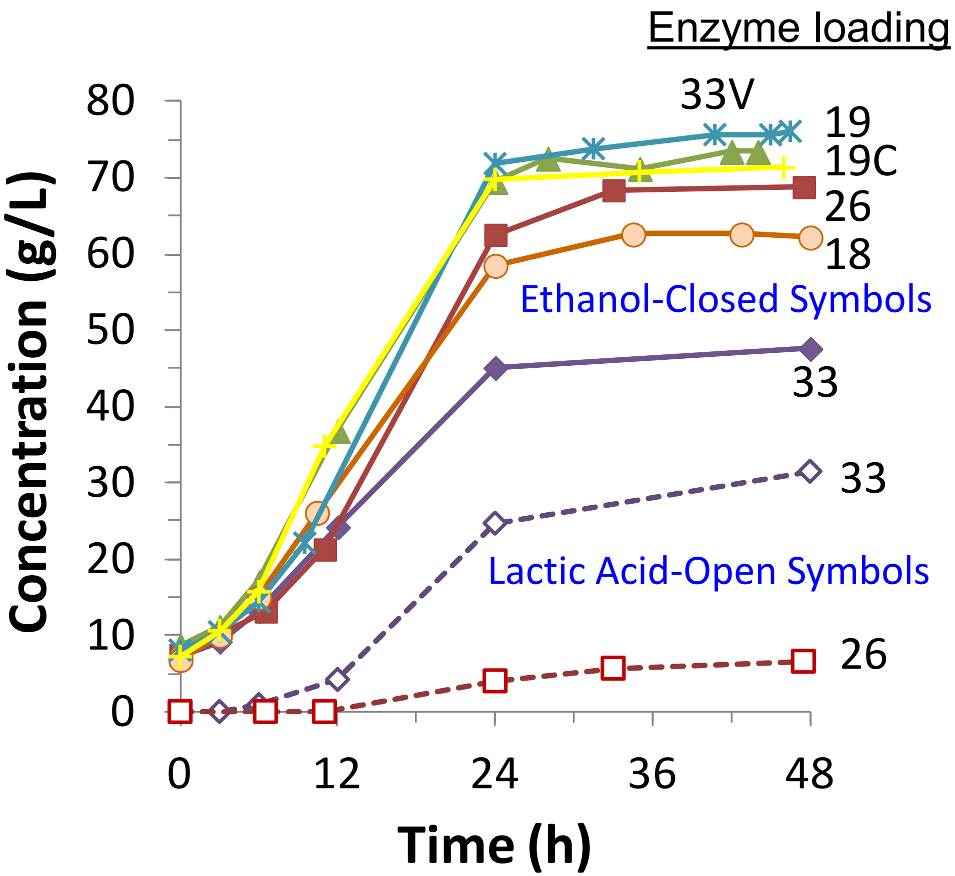


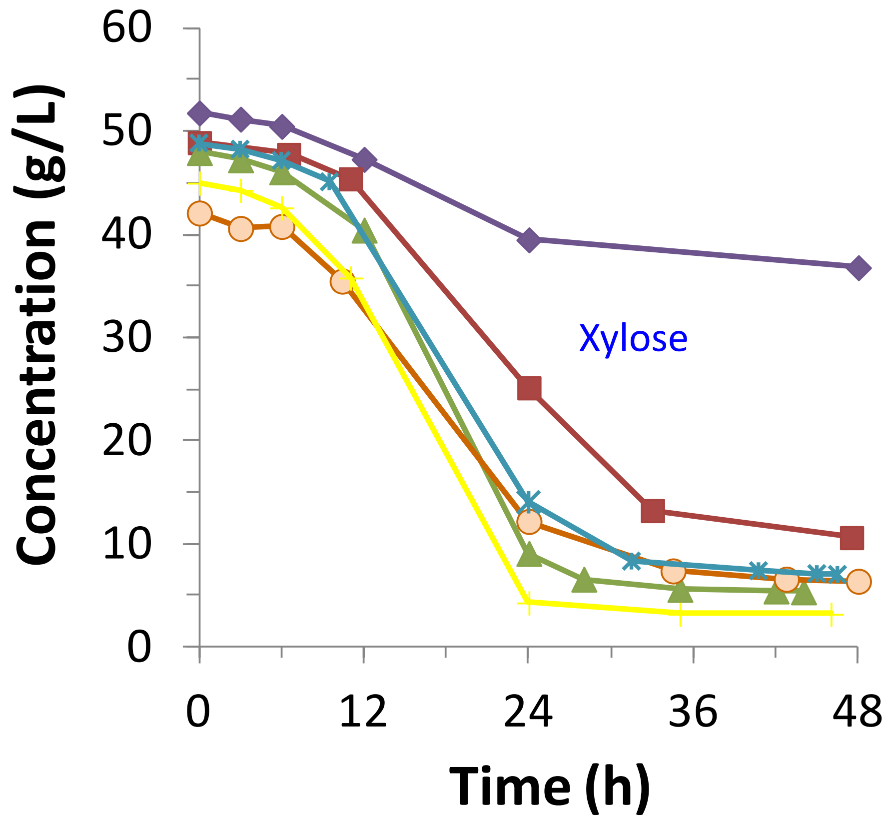
Enzymatic Cellulose Conversion



- Gluco-oligomers and residual cellulose were converted to glucose during fermentation
- Lower yields for VtR-treated stover due to the less severe pretreatment conditions
- Lower yields for untreated stover suggests that the deacetylation process enhanced cellulose conversion

Component Profiles during Fermentation





Contamination was a problem in early runs (33 and 26 mg/g enzyme loadings) and was later eliminated by better handling of the inoculation process.

Fermentation Process Yields