NREL
NATIONAL RENEWABLE ENERGY LABORATORY

Pretreatment and Process Hydrolysis

WBS 2.2.3.100

Melvin Tucker

National Renewable Energy Laboratory

2017 DOE Bioenergy Technologies Office (BETO) Project Peer Review

March 9, 2017

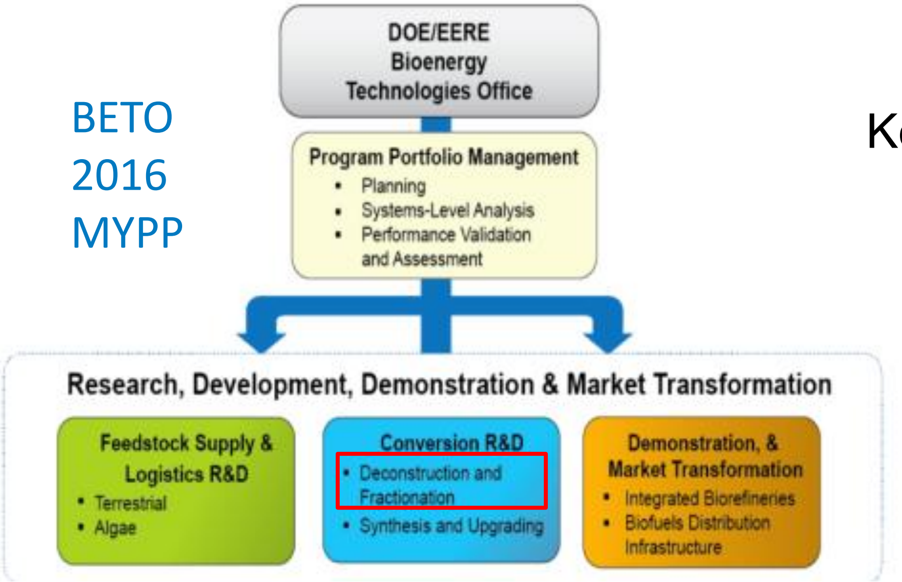
Feedstock-Conversion Interface Consortium

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

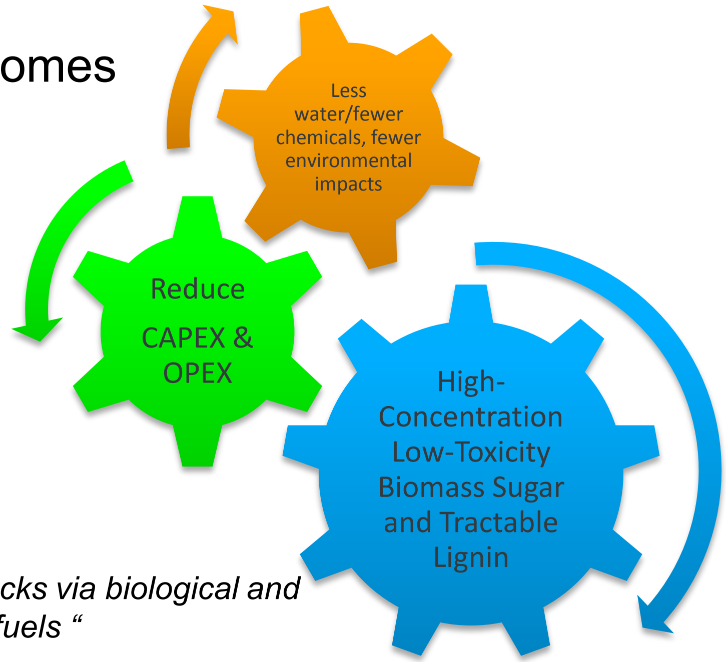
Goal Statement

Develop **scalable deconstruction/fractionation processes** that produce **high-concentration, low-toxicity sugar and tractable lignin streams** at high yields using low enzyme loadings from **relevant feedstock blends and formats** for biological and catalytic upgrading supporting the future biorefinery industry.

BETO 2016 MYPP



Key Outcomes



Relevance to BETO:

- *"Develop commercially viable technologies for converting biomass feedstocks via biological and chemical routes into energy-dense, fungible, finished liquid transportation fuels "*

Relevance to Bioenergy Industry:

- Deconstruction is key to scalable and cost effective upgrading in the biorefinery
  - Lower cost, "cleaner" sugars
  - Tractable, reactive lignin streams
- Enables biochemical utilization of all carbon from sugars and lignin

Quad Chart Overview

Timeline

- Project start date: FY16
- Project end date: FY18
- Percent complete 50%

Budget

	Total Costs FY12 – FY14 ($MM)	FY15 Costs ($MM)	FY16 Costs ($MM)	Total Planned Funding (FY17–Project End Date) ($MM)
DOE-funded	3.8	1.4	1.4	2.8

Barriers

- BETO's 2016 MYPP Barriers:
  - Bt-C: Biomass Recalcitrance
    - Higher recalcitrance of blended feedstocks
  - Bt-E: Pretreatment Costs
    - Lower reactor and OPEX costs
  - Bt-G: Cellulase Enzyme Loading
    - Maintain high yields at ≤ 10 mg protein
- Technical targets:
  - >90% glucose, xylose, and arabinose yields
  - 10 mg protein/g cellulose

Partners/Collaborators

- FCIC-INL
- **NREL BETO Projects: Biochemical Platform Analysis**, Targeted Microbial Development, **Feedstock Process Interface**, Biological Lignin Depolymerization, Lignin Utilization, Bench-Scale Integration, Pilot-Scale Integration
- Other collaborators
  - PNNL
  - Washington State University
  - North Carolina State University
  - Southern Illinois University
  - University of Colorado
  - Co-Optima
  - Agile BioFoundry

Biochemical Conversion Projects—NREL

Enabling/Fundamental Technologies | Synthesis and Upgrading Technology | Process Development | Process integration, Scale-up, Verification

Biochemical Process Modeling and Simulation

Targeted Microbial Development

Enzyme Engineering and Optimization

Biological Lignin Depolymerization

Biological Upgrading of Sugars

Catalytic Upgrading of Biochemical Intermediates

Lignin Utilization

Feedstock-Process Interface

Pretreatment and Process Hydrolysis

Bench-Scale Process Integration

Separations Development and Application

Pilot-Scale Process Integration

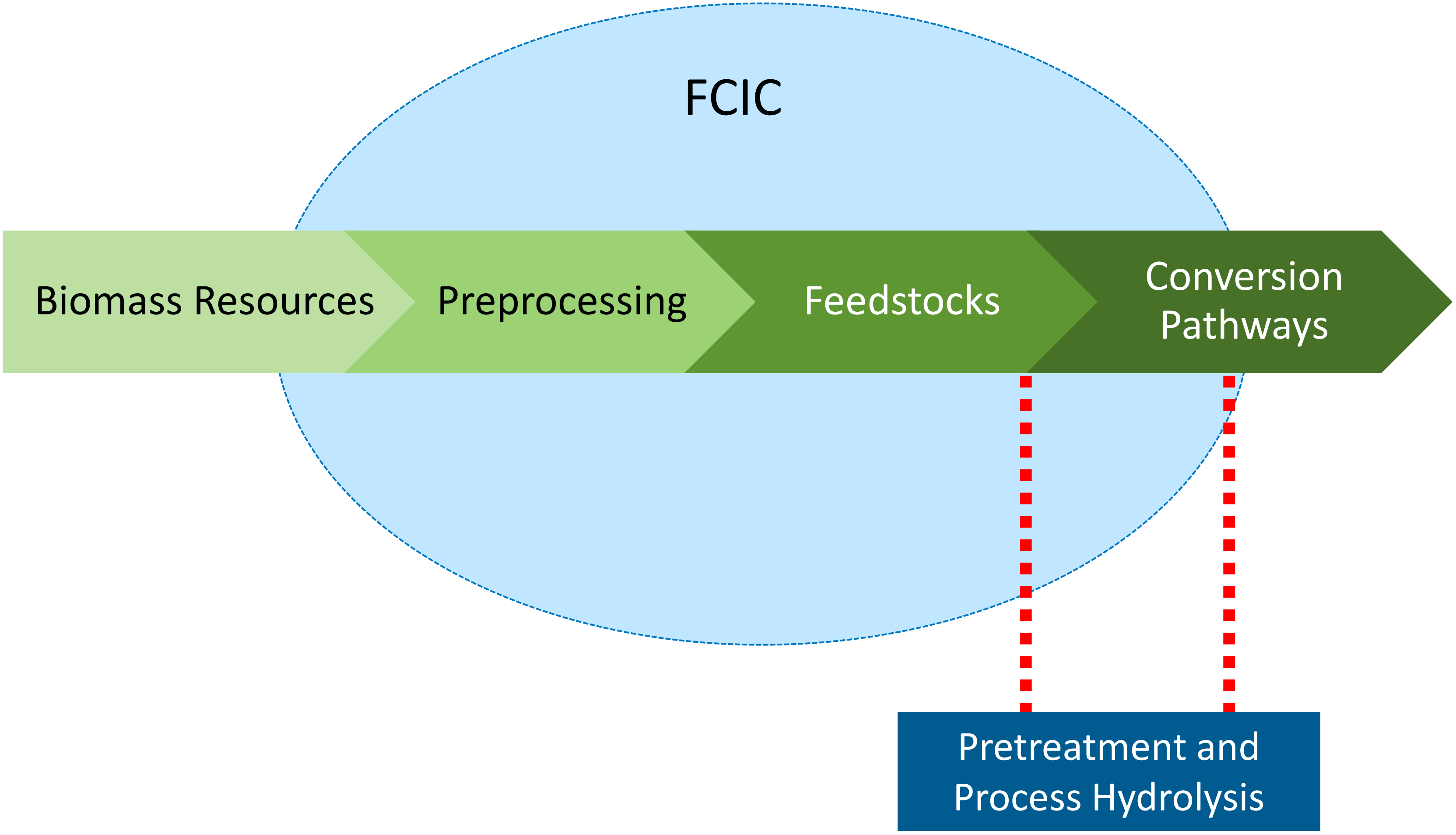
Co-Optima

Agile BioFoundry

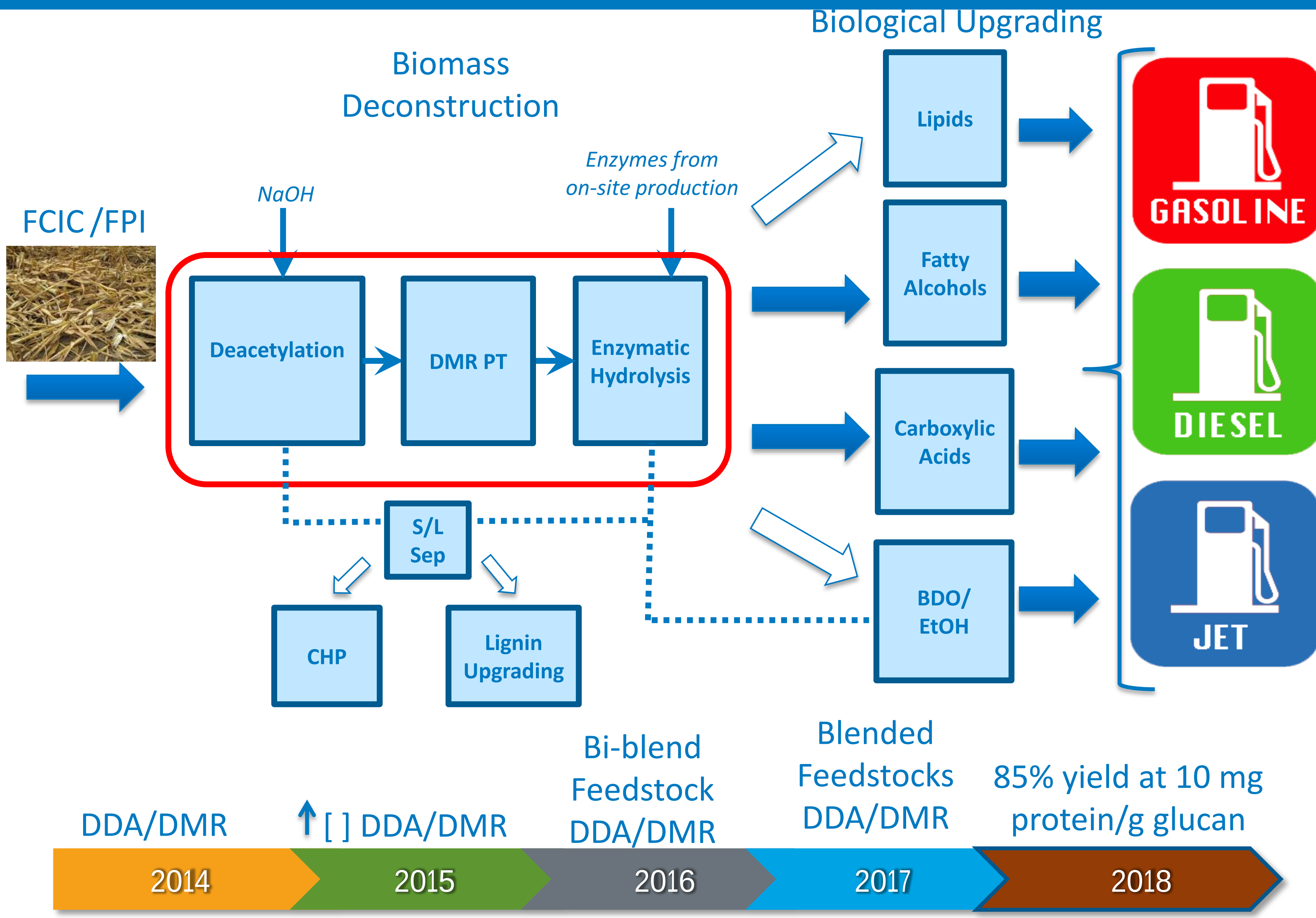
Analytical Development and Support

Biochemical Platform Analysis

Biochemical Conversion Projects—NREL



Project Overview



Project Technical and Management Approaches

Pretreatment and Process Hydrolysis

Approach (Management)

Project Structure

- Pretreatment and Process Hydrolysis
  Mel Tucker
- Low Severity Deconstruction
  Xiaowen Chen
- Deacetylation and Acid Deconstruction
  Erik Kuhn

Create metrified milestones by collaborating with other portfolio projects

Achieve technical targets and adapt to the changing needs of other research teams

Identify process intensification and novel deconstruction approaches

Perform TEA to gauge progress and identify highest impact research needed to meet BETO's 2022 targets and goals

Milestones and Go/No Go Decisions

- FY15: 4 milestones, 1 TEA
- FY16: 4 milestones, 1 TEA, 1 go/no go w/TEA
- FY17: 5 milestones, 1 TEA, 1 go/no go
- FY18: 4 milestones, 1 TEA

Approach (Technical)

Approach:

- High severity thermochemical pretreatments avoided
  - Exotic alloy reactors
  - Production of inhibitors
  - Lignin condensation
- Develop lower severity pretreatments
  - Deacetylation Dilute Acid (DDA)
  - Deacetylation Mechanical Refining (DMR)
- Investigate scalable processes

Challenges:

- Biomass recalcitrance of advanced blended and formatted feedstocks
- Achieve high sugar yields at low enzyme loadings
- Minimize chemical, water and energy usage
- Maintain high lignin quality

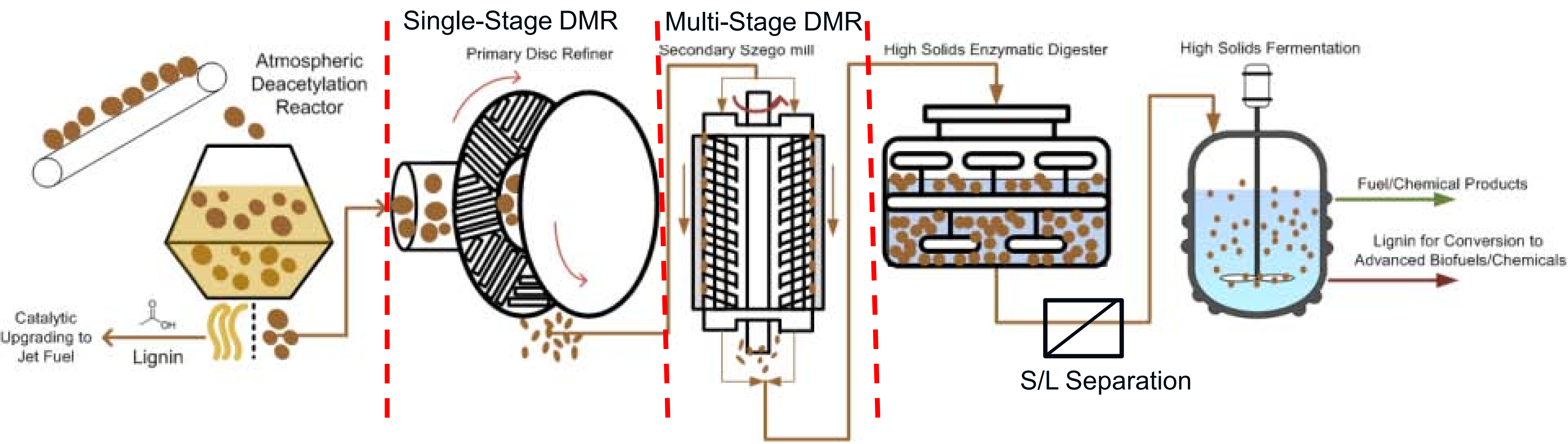
Critical Success Factors:

- Scalable processes
- Processes that decrease:
  - water, chemical and energy use
  - limit wastewater discharges

Technical Accomplishments

Pretreatment and Process Hydrolysis

Deacetylation and Mechanical Refining (DMR) Process versus Dilute Acid Pretreatment



Challenges in Dilute Acid Pretreatment

Inefficient online acid mixing

Inability to Feed!

Steam Blowback!

Accumulation of Coke and Chars

NEW

USED for 3 months

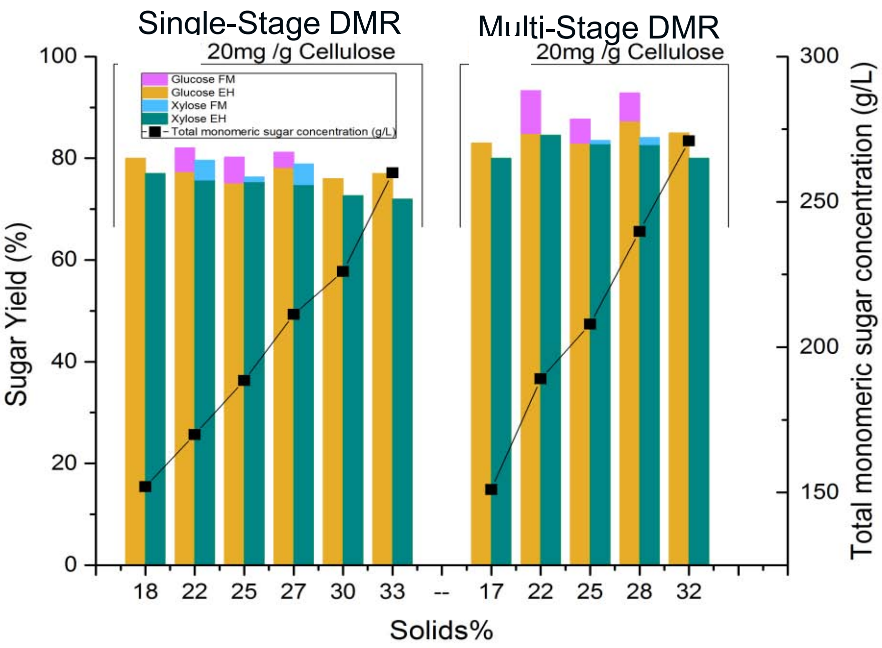
Wearing out of screws

Advantages of DDR/DMR

- Simplified process
- Technologies proven in pulping industry (stranded capital assets)
- Less CAPEX
  - Atmospheric pressure
  - No exotic alloys
- Less maintenance required
- No char production
- More native-like lignin
- Higher solids allows high sugar concentrations

Technical Achievement – **High Solids** Enzymatic Hydrolysis using DDR/DMR Corn Stover Substrates

- **Objective:** Achieve ≥80% monomeric sugar yields at high solids using DMR
- **Approach**: High solids (17% to 33% insoluble solids) enzymatic hydrolysis using DMR
- **Challenges:**
  - High sugar concentrations (500 g/L) for biological conversion
  - Enzymatic Hydrolysis (EH) at 20% TS produces ~150 g/L fermentable sugars
  - Increased energy required for concentration
  - Dilute acid pretreatment limits solids in EH to ~20%
  - Enzyme inhibition for DDA increases above 20% total solids
  - Enzyme loading ≤10 mg protein/g cellulose



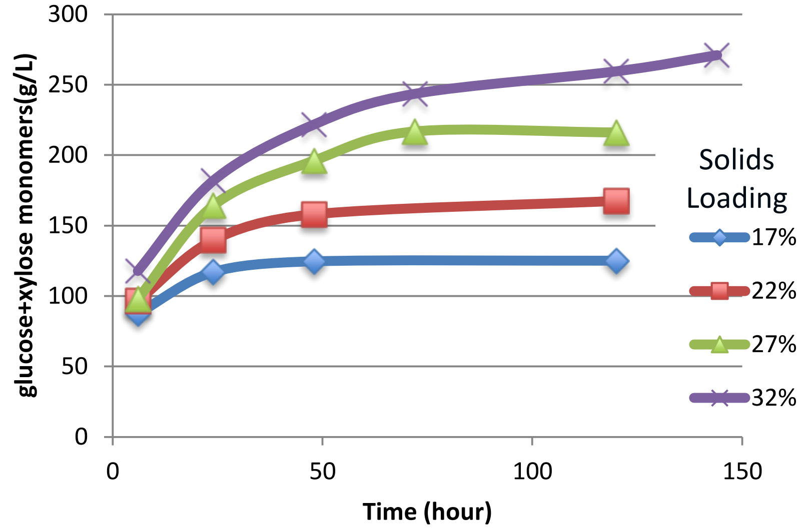
Deconstruction Parameter	DA	DMR
Monomeric Sugar Concentration, g/L	150	270
Concentration Factor to 500 g/L	3.3X	≤2X
Ethanol Titer in Fermentation, g/L	55	86

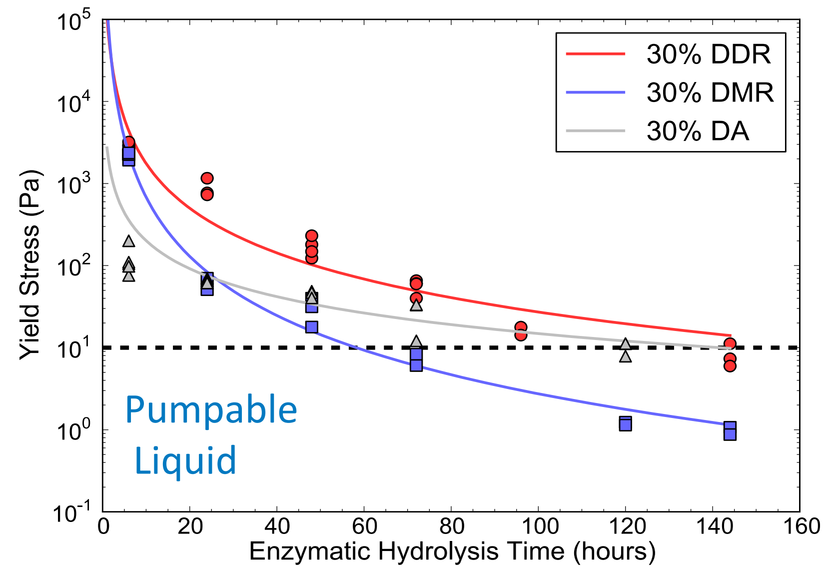
Achievements:

- High monomeric sugar concentration at high yields
  - **270 g/L at 32% TS, 85% glucose yield, 80% xylose yield (FY17 Q1 milestone)**
- Highly fermentable
  - **Ethanol titers of 86 g/L, 90% yield**
- Versus dilute acid, ~150 g/L, higher toxicity, 55 g/L EtOH
- Published in: *Energy and Environmental Science:* 2016,9,1237-1245 DOI: 10.1039/C5EE03718B

Technical Achievement—High Solids Enzymatic Hydrolysis Kinetic and Rheological Studies –DDA and DMR (Achieved FY17 Q1 Milestone)

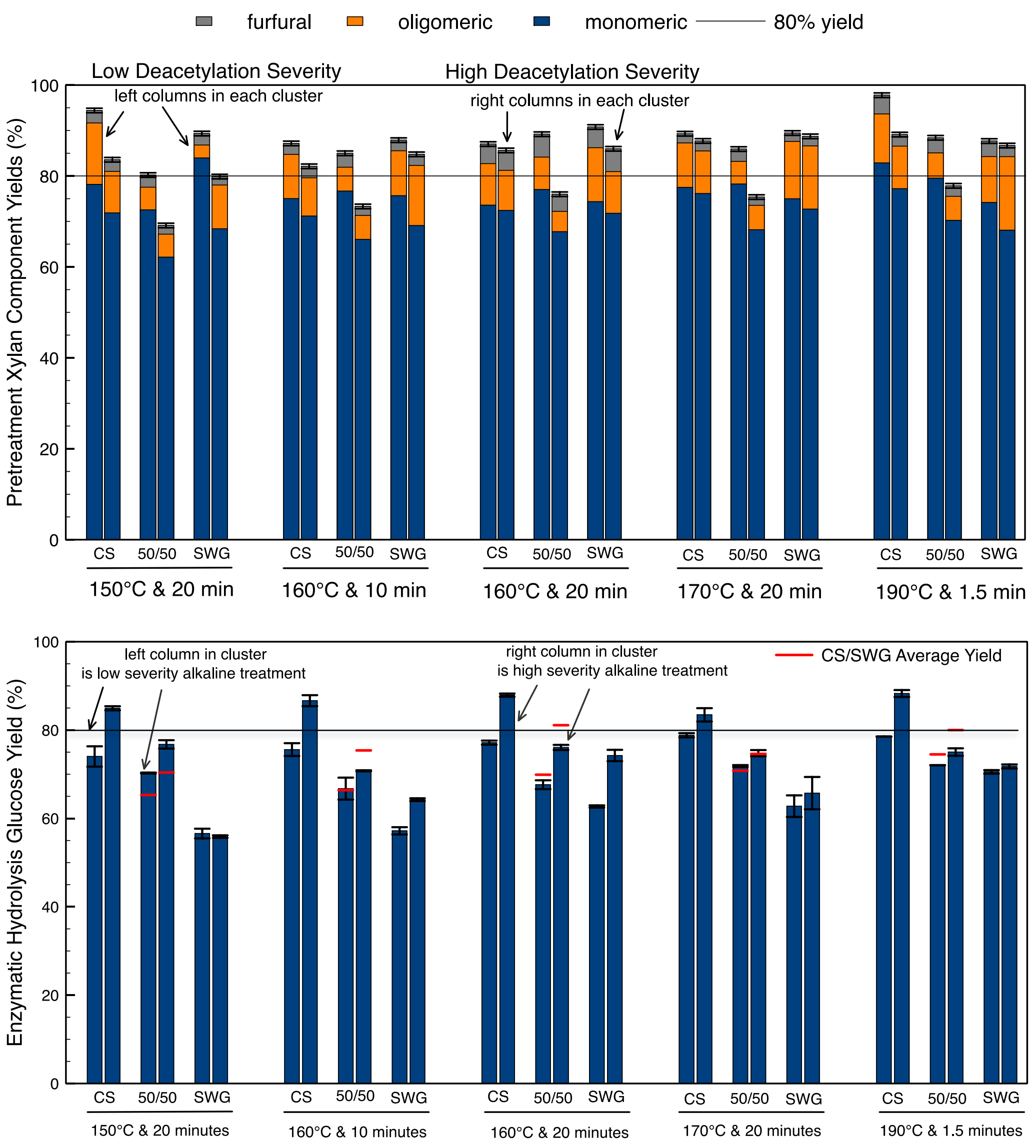
- **Objective:** Determine kinetic and rheological properties of high solids enzymatic hydrolysis reactions.
- **Approach:**
  - Kinetics study (coupled with modeling)
  - Rheology study (yield stress and viscosity coupled with modeling to predict mixing energy)
- **Challenges:**
  - High solids enzymatic hydrolysis results in thick slurries, leading to mixing and pumping issues
  - Feed-back inhibition
- **Achievements:**
  - Kinetics showed no enzyme inhibition for DMR up to 32% insoluble solids
  - Enzymatic hydrolysis of DMR>DDR>DA substrates
  - DMR achieved pumpability at ~60 h





Technical Achievement: Managing Recalcitrance Differences in LCF Blended Feedstocks Through DDA

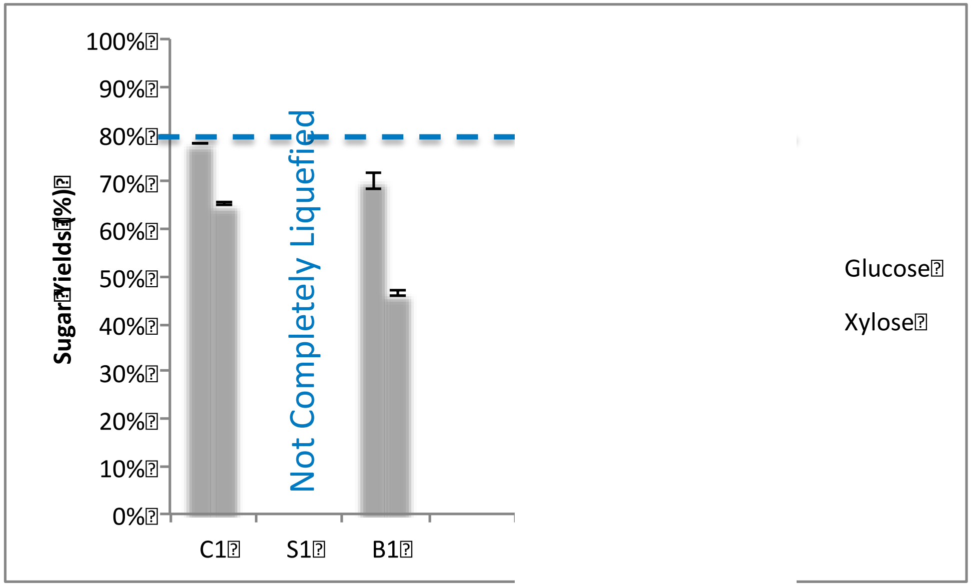
- **Objective:** Achieve 80% monomeric sugar yields on 50/50 switchgrass/corn stover blend using deacetylation/dilute acid pretreatments
- **Approach:** Dilute acid pretreatments on a 50/50 blend and individual feedstocks
  - Two deacetylation severities tested
  - Screening runs of low to high dilute acid pretreatment severities conducted at high solids in 4-L steam explosion reactor
- **Challenges:**
  - More difficult to achieve higher yields due to increased recalcitrance of blend
- **Achievements:**
  - 80% glucose yield at 15 mg/g total protein loading in enzymatic hydrolysis was achieved at 10% solids loadings (Achieved FY16 Q1 milestone).
  - Enzymatic hydrolysis glucose yields of the 50/50 SW/CS blends were the average of the individual feedstocks.
  - FY16 Q2 Go/No Go TEA showed minimal costs with 60% hydrolysis of soluble xylan to monomeric xylose with minimal accessory enzyme addition



Technical Achievement: Managing Recalcitrance Differences in LCF Blended Feedstocks through DMR

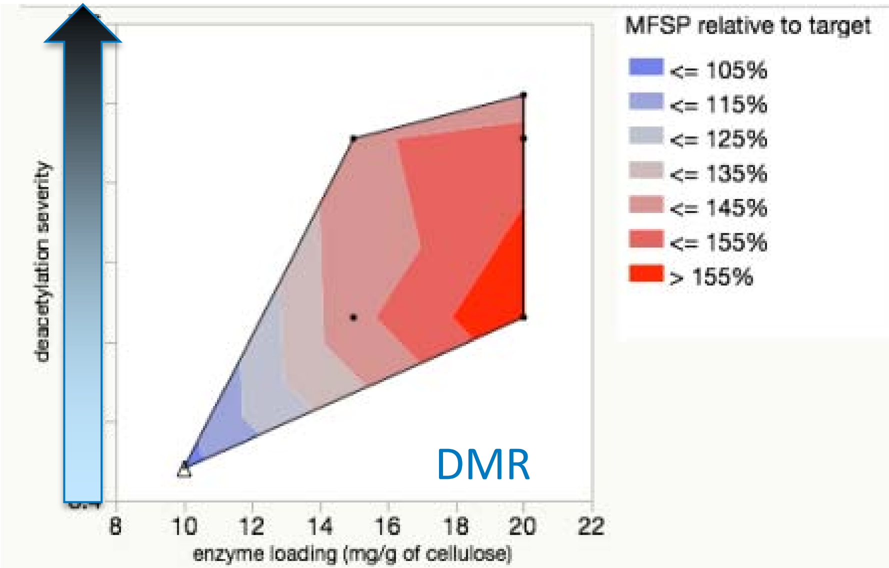
- **Objective:** Achieve 80% monomeric sugar yields on 50/50 switchgrass corn stover blend using deacetylation and mechanical refining
- **Approach:** Increase deacetylation severity
  - Increased sugar yields
  - Solubilizes more sugars into the black liquor
- **Challenges:**
  - Increased recalcitrance
  - Increased variability
- **Achievements:**
  - For 50/50 blend, over 80% glucose yield at 15 mg/g of total enzyme protein loading (Achieved FY16 Q3 milestone)
  - Enzymatic hydrolysis glucose yields of the 50/50 CS/SW blends were the average of the individual feedstocks

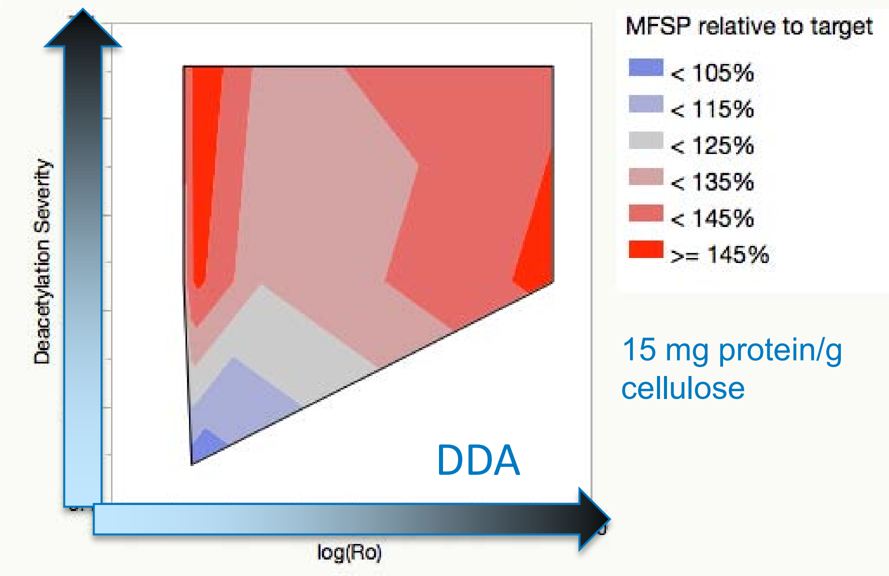
Abbrev.	B	C	S
Feedstock	Blend 50/50	Corn Stover	Switchgrass
Condition:	1		2
Deacetylation Conditions	50kg NaOH/ODMT biomass, 80°C		70kg NaOH/ODMT biomass, 92°C



Technical Achievement: TEA on DDA and DMR using 50/50 Blended Feedstock (Achieved FY16 Q4 Milestone)

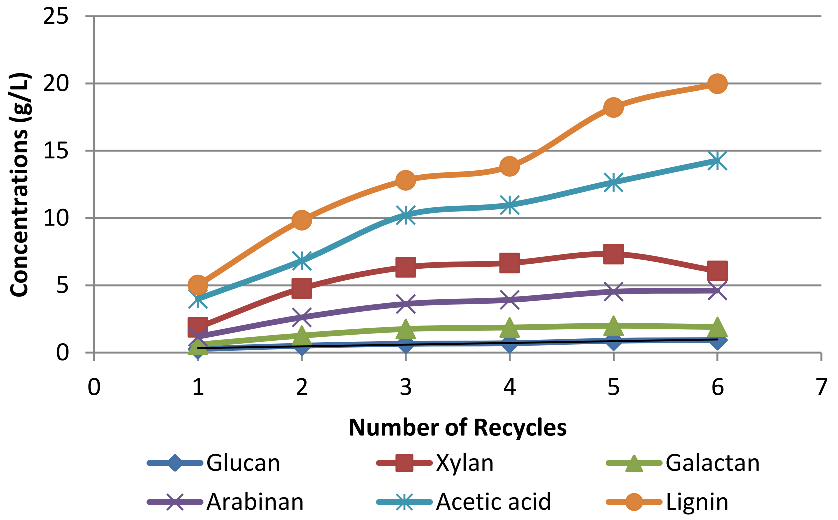
- **Objective:** Evaluate the effects of enzyme loading and process severity on minimum fuel selling price (MFSP)
- **Approach:**
  - FY15 SOT Model (sugar to lipid to fuel) with No Credit from Lignin Co-product
  - 2022 MFSP Target assumed 10 mg/g cellulose enzyme loading and 90% sugar yields for both glucose, xylose, and arabinose
- **Results:**
  - DDA and DMR –similar MFSP due to increased recalcitrance of blended feedstock (without lignin considerations)
  - MFSP for SW>Blend>CS due to lower sugar yields for SW



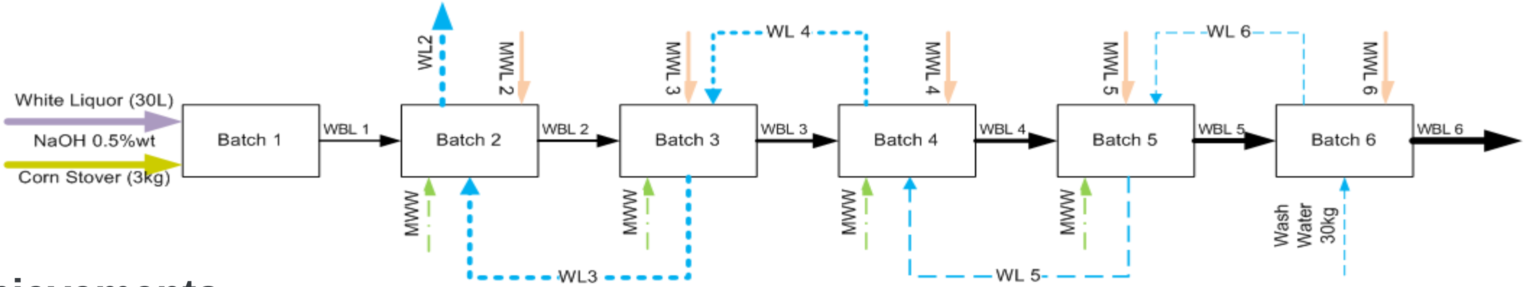


Technical Achievement—Black Liquor Recycling

- **Objective:** Develop black liquor recycle to increase sodium and lignin levels without affecting deacetylation effectiveness, and enzymatic hydrolysis/fermentation performances.
- **Approach:** Simulate continuous counter-current washing and extraction using batch-wise black liquor recycling.
- **Challenges:**
  - Recovery of sodium
  - Decrease xylan losses
  - Decrease water and energy usage
  - Reduce inhibitors



Batch-wise Black Liquor Recycling



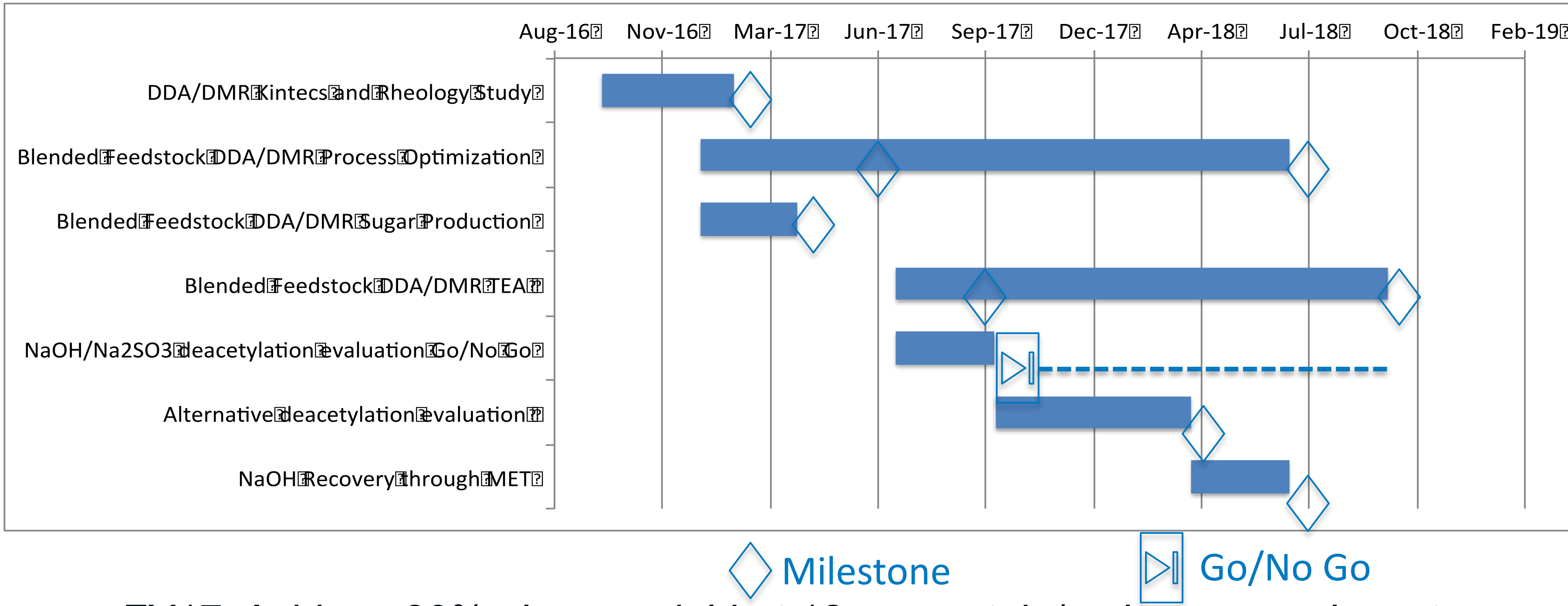
- **Achievements:**
  - 6 cycles: Na:12 g/L, Lignin: 20 g/L, Acetate: 15 g/L
  - Water usage reduction up to 50%
  - No inhibition of enzymatic hydrolysis or fermentation performance
  - TEA showed reduced water, steam, and MFSP with black liquor recycle
  - Achieved FY15 Q3 and Q4 milestones.

Relevance

Develop **scalable pretreatment, deconstruction, and fractionation processes** that decrease conversion costs through production of **low-toxicity, high-concentration sugar and tractable lignin streams** for biological and catalytic upgrading

- Developed processes to deconstruct recalcitrant feedstocks using conventional industrial equipment. This reduces risk and reduces time to commercialization.
  - Stranded capital assets from pulping industry
- Project DMR developments have advanced SOT and positively impacted commercial viability
- Directly supports BETO's 2016 MYPP: *"Develop innovative biomass deconstruction approaches to lower the cost of intermediates…"*
- Collaboration with FCIC and other projects in biochemical conversion portfolio
- Project is at high TRL and is relevant to commercial-scale biorefineries

Future Work



- FY17: Achieve 80% glucose yield at 12 mg protein/g glucan on relevant advanced blended and formatted feedstocks—perform TEA
- FY17 Go/No Go: Decrease xylan losses with NaOH/ Na_2SO_3 deacetylation
- FY18: Achieve 85% glucose yield at 10 mg protein/g glucan on advanced feedstock blends and formats
- FY18 DMR of advanced blended and formatted feedstocks
    - Go/No Go: TEA of continuous deacetylation milestone
    - Complete bench and pilot-scale testing of deacetylation NaOH recycle using microbial electrochemical technologies (MET)

Summary

- **Overview/Approach:**
  - TEA-guided R&D
  - Producing low-cost, low-toxicity, high-concentration sugar syrups and tractable lignin streams at high yields and low enzyme loadings.
- **Results FY15-16:**
  - Achieved 270 g/L monomeric sugars at 32% TS, 85% glucose yield, 80% xylose yield in high solids EH
  - Achieved 80% monomeric sugar yields using DDA and DMR on more recalcitrant 50%/50% corn stover/switchgrass bi-blends
  - Enzymatic hydrolysis of DMR corn stover substrates at 32% insoluble solids achieved pumpability at ~60 h
  - FY16 Q2 Go/No Go TEA showed 60% hydrolysis of soluble xylan to monomeric xylose with minimal accessory enzyme addition and costs
  - MFSP for both DDA and DMR using 50/50 blend showed SW>Blend>CS due to lower sugar yields for SW
  - Separate deacetylation of recalcitrant feedstocks decreased xylan losses and improved pretreatment and EH yields
- **Relevance**: Scalable pretreatment, deconstruction, and fractionation processes that decrease conversion costs through production of low-toxicity, high-concentration sugar and tractable lignin streams for biological and catalytic upgrading essential for viable biorefineries.
- **Critical success factors:** Scalable processes that decrease energy, water, and chemical use and limit wastewater discharges.
- **Future work:**
  - Focus on more recalcitrant LCF feedstocks
    - Optimize DDA and DMR using LCF blended feedstocks
  - Reduce enzyme loadings to 10 mg protein/g cellulose
  - Increase monomeric sugar yields
  - Investigate continuous deacetylation
    - Decrease xylan losses
    - Decrease energy, water, and chemical usage
  - Investigate sodium recycle and recovery using microbial electrochemical technologies (MET)
- DMR process uses existing industrial equipment and is at high TRL

Acknowledgments

U.S. DOE EERE Bioenergy Technologies Office

- Ian Rowe, Technology Manager

NREL

Xiaowen Chen
Erik Kuhn
Ling Tao
MaryKate O'Brien
Todd Vinzant
David Sievers
Ryan Davis
Wei Wang
Hui Wei
Jim McMillan
Ed Jennings
Wes Hjelm
Casey Gunther
Dan Schell
Ryan Ferguson
Joe Shekiro
Peter Ciesielski

Other Collaborators

INL
Allison Ray
Neal Yancey

PNNL
Daiwon Choi
Satish Nune

University of Colorado
Zhiyong "Jason" Ren

North Carolina State University
Sunkyu Park

Southern Illinois University
Yanna Liang

Washington State University
Bin Yang

Andritz Inc.
Marc Sabourin
Thomas Pschorn

Additional Slides

Pretreatment and Process Hydrolysis

Reviewer Comments

Reviewer Comments:

- This is a good project and is a logical follow-up to the pretreatment development work that has gone on for many years at NREL. We see the cost goal for the entire process, including feedstocks cost reductions. It would be nice to see the cost reductions due only to this work.
- A lot of work has been done on using refiners in the industry and the work has not had a lot of success. This research takes a little different approach and may benefit and facilitate advancements in the industry.
- • Overall, this looks like a good project that is working to support BETO goals and provide technology alternatives. There is some concern that irrelevant factors are driving the work (e.g., pulp and paper mills) and that the focus on zymo is not well supported, but it is clearly making good progress.
- • Good progress has been made on a range of conditions and configurations. There is clearly a large body of work behind the figures. Because of the short review time, it was difficult to understand how well the data supported the conclusions of the studies.
- • The project involves next generation pretreatment technologies to reduce potential inhibitors (acetic) and include size reduction (mechanical refining) to improve conversion processes (and lowering cost due to enzyme addition). In addition, the “split stream” approach is important for the coproduction of lower value fuels (from the C6 stream) and higher value chemicals (from the C5 stream).

PI Response to Reviewer Comments

- We appreciate the reviewers’ comments regarding the applicability and progress of the dilute alkali and/or acid pretreatments, and DMR process approaches for producing low cost sugar and reactive lignin streams for bioconversion to intermediates and hydrocarbon fuels covered in this work. The reviewers’ comments concerning pulp industry conversions are appropriate, because of the substantial costs and economic uncertainties associated with pulp mill conversions. However, DOE has engaged in efforts to support re-purposing of pulp and paper facilities as biorefineries, and our pursuit of the DMR technology is related to this purpose.
- The higher sugar yields in both dilute acid pretreatment and the DMR process reported in this project are the direct result of the considerable investments made by DOE in enzyme technology development, incurred by cost sharing with several of the enzyme companies for advanced enzyme technologies. The higher yields possible with the latest enzyme technologies may improve the economics of converting pulp mills to biorefineries. In addition, DOE and DuPont have invested considerable funding in improving Zymomonas to the point where a 30 million gallons/year cellulosic biorefinery in Nevada, Iowa, is possible.
- Our use of Zymomonas as a model organism to test the toxicity of the dilute acid and DMR hydrolyzates produced in this project is based on the extensive knowledge gained at NREL in the past. The Zymomonas organism that will be used in this commercial scale plant is jointly engineered with DuPont scientists. Zymomonas has one of the highest rates of converting sugars to the central metabolite, pyruvate, which can then be used by appropriate, metabolically engineered Zymomonas strains to produce a number of intermediates suitable for upgrading to hydrocarbon fuels, such as butanediol, isoprenes, polyhydroxyalkanoates, etc. If future sugar to intermediates for hydrocarbon production cannot utilize aerobic fermentation processes to lipids because of the very high costs and energy consumption required for aeration, then anaerobic processes will be needed, and Zymomonas has shown promise in anaerobic fermentations.

Publications

- Publications
  - Hongliang Wang *et. al.*, One-Pot Process for Hydrodeoxygenation of Lignin to Alkanes Using Ru-based Bimetallic and Bifunctional Catalysts Supported on Zeolite Y, *ChemSusChem*, 2017, 10.1002/cssc.201700160.
  - Chen, X., *et. al.,* DMR (deacetylation and mechanical refining) processing of corn stover achieves high monomeric sugar concentrations (230 g L-1) during enzymatic hydrolysis and high ethanol concentrations (> 10% v/v) during fermentation without hydrolysate purification or concentration. *Energy & Environmental Science*. 2016,9,1237-1245
  - Chen, X., *et. al.* Techno-economic Analysis of the Deacetylation and Disk Refining Process: Characterizing the Effect of Refining Energy and Enzyme Usage on Minimum Sugar Selling Price and Minimum Ethanol Selling Price, *Biotechnology for Biofuels. 2015 8:173*
  - Chen, X., *et. al.,* Development and characterization of a high-solids deacetylation process. *Sustainable Chemical Processes*, 2016 4:6
  - Chen, X., *et. al.* Improving Sugar Yields and Reducing Enzyme Loadings in the Deacetylation and Mechanical Refining (DMR) Process through Multistage Disk and Szego Refining and Corresponding Techno-Economic Analysis, *ACS Sustainable Chemistry & Engineering.* 2016 4(1), p324-333
  - Kuhn, E., *et. al.* Pilot-Scale Batch Alkaline Pretreatment of Corn Stover, *ACS Sustainable Chemistry & Engineering. 2016 4(3), pp 944-956*
  - Sievers, D., *et. al.* Online residence time distribution measurement of thermochemical biomass pretreatment reactors. *Chemical Engineering Science.* 2016.
  - Yang, H., *et. al.,* Cell Wall Targeted *in planta* Iron Accumulation Enhances Biomass Conversion and Seed Iron Concentration in Arabidopsis and Rice, *Plant Biotechnology Journal*, 2016 DOI: 10.1111/pbi.12557.
  - Wang, H., *et. al.*, "Biomass-derived Lignin to Jet Fuel Range Hydrocarbons via Aqueous Phase Hydrodeoxygenation, *Green Chemistry Communication,* 2015 17, 5131-5135. DOI: 10.1039/c5gc01534k.
  - Wang *et. al.* Biomass-derived Lignin to Jet Fuel Range Hydrocarbons via Aqueous Phase Hydrodeoxygenation, *Green Chemistry. 2015.*
  - Park, J. Chen, X. *et. al.*, Use of mechanical refining to improve the production of low-cost sugars from lignocellulosic biomass. *Bioresources Technology*, 2016 v199, p59-67
  - Ma, R, et. al., Peracetic acid depolymerization of biorefinery lignin for production of selective monomeric phenolic compounds, *Chemistry: A European Journal, 2016 v22 issue 31, p10884-10891*
  - Liang, Y. ,Chen, X. et. al. Conversion of corn stover hydrolysates to acids: comparison between Clostridium carboxidivorans P7 and microbial communities developed from lake sediment and an anaerobic digester. *Biomass Conversion and Biorefinery*, 2016 doi:10.1007/s13399-017-0239-9

(continued on next page)

Presentations

(Publications continued)

- Liang, Y. ,Chen, X. et. al. Sweet sorghum bagasse and corn stover serving as substrates for producing sophorolipids, *Journal of Industrial Microbiology & Biotechnology*, 2016, doi:10.1007/s10295-016-1891-y
- Wei, H., *et. al*., Transgenic Ferritin Overproduction Enhances Thermochemical Pretreatments in Arabidopsis, 2015, *Biomass Bioenergy* 72: 55-64.
- Zeng, Y., *et. al*., In situ Micro-Spectroscopic Investigation of Lignin in Poplar Cell Walls Pretreated by Maleic Acid, *Biotechnology for Biofuels,* 2015 **8**:126, DOI 10.1186/s13068-015-0312-1.
- Wei, H. *et. al*., Identifying the Ionically Bound Cell Wall and Intracellular Glycoside Hydrolases in Late Growth Stage Arabidopsis Stems: Implications for the Genetic Engineering of Bioenergy Crops, 2015, *Frontiers in Plant Science* **6**: 315.
- Sitaraman, H., *et. al*., Multiphysics Modeling and Simulation of High-Solids Dilute-Acid Pretreatment of Corn Stover in a Steam-Explosion Reactor, 2015, *Chemical Engineering Journal* **268**, 47–59
- Wei, H., *et. al*., Feedstock Engineering and Biomass Pretreatments: New Views for a Greener Biofuels Pprocess, 2015, **Direct Microbial Conversion of Biomass to Advanced Biofuels**, Waltham, MA: Elsevier-Science and Technology Books.

Presentations:

- Chen, X. Novel DDR processing of corn stover achieves high monomeric sugar concentrations from enzymatic hydrolysis (230 g/L) and high ethanol concentration (10% v/v) during fermentation. April 28th, 2015
- Kuhn, E. Deacetylation and dilute acid pretreatment of corn stover, swtichgrass, and a stover-switchgrass mix. *38th Symposium on Biotechnology for Fuels and Chemicals.* Oral Presentation. April 26th, 2016.
- Chen, X., *et. al.* Recycling of Dilute Deacetylation Black Liquor to Enable Efficient Recovery and Reuse of Spent Chemicals and Biomass Pretreatment Waste. *38th Symposium on Biotechnology for Fuels and Chemicals.* Poster Presentation. April 25th, 2016.
- Kuhn., E., *et. al.* Pilot scale, continuous alkaline pretreatment of corn stover. *37th Symposium on Biotechnology for Fuels and Chemicals.* Poster Presentation. *April 28th,* 2015.
- Wolfrum, E., *et. al.* Pretreatment reactor scaling: Comparing conversion performance across different pretreatment reactors. *38th Symposium on Biotechnology for Fuels and Chemicals.* Poster Presentation. April 26th, 2016.

Milestone Schedule for Pretreatment & Process Hydrolysis Project

FY15 Milestones

Type	Title/Performance Measure	Due Date
Regular	**Title:** Determine Enzymatic Hydrolysis Glucose and Xylose Yields of DDR solids at High Solids Loadings. **Milestone Description:** Conduct high solids enzymatic hydrolysis on DDR residues produced in a commercial scale disc refiner at 200 kWh/ODMT and Szego milled at 200 kWh/ODMT at solids contents of 22.5, 25, 27.5, 30 wt% insoluble solids in roller bottles with total enzyme loadings of 20mg/g of cellulose.	12/31/2014
Regular	**Title:** Effect of Shear in Steam Explosion Reactor on Pretreatment and Enzymatic Hydrolysis Yields. **Milestone Description:** Perform a set of factorial or surface response experiments consisting of at least 20 bench scale steam explosion pretreatments of corn stover that will maximize pretreatment and enzymatic hydrolysis yields using native and deacetylated corn stover feedstocks over varying acid concentrations, residence times, temperature, and shearing die configurations.	3/31/2015
Regular	**Title:** Determine the Effect of Deacetylation Liquor Recycling on Sugar, Toxicity, and Hydrocarbon Intermediate Yields Using DDR Process Solids and Slurries. **Milestone Description:** Create a deacetylation liquor recycling and chemical makeup process flow diagram and perform recycling of deacetylation liquor up to 5 times using 5 kg corn stover at 10% solids with 0.1N sodium hydroxide at 80C for 2hr in each recycle, draining the liquor using a simple screen solid/liquid separation at the paddle reactor, followed by screw pressing the solids and mixing the recovered squeezed liquor with drained liquor. Dic refine the deacetylated solids from the various recycle experiments. Enzymatically digest the DDR solids at high solids (20 wt% solids or greater).	6/30/2015
Major	**Title:** Assess solid/liquid separation performance of higher severity dilute acidpretreated slurries to Separations Development and Applications Project for Solid/Liquid Separation, Analysis, and Characterization of C5 Sugar Rich Streams. **Milestone Description:** At least five higher severity dilute acid pretreated slurries will be prepared and compositionally characterized by the Analytical Development and Support project (2.5.1.101) and evaluated for their comparative solid-liquid separations performance by the SDA project (2.4.1.101) for possible incorporation into the PSI project's (2.4.1.102) plans for FY17 integrated demonstration.	6/30/2015
Regular	**Title:** Test Six Hemicellulase Enzyme Preparations to Hydrolyze 35% Xylooligomers in Dilute Acid Pretreated and DDR Corn Stover Slurries to Improve Monomeric Xylose Yields. **Performance Measure:** Test at least six commercial and in-house xylanase, xylobiase, hemicellulase and accessory enzyme preparations to hydrolyze at least 35% of the xylooligomers present in dilute acid pretreated and DDR corn stover slurries to improve monomeric xylose yields and to reduce toxicity. Report accessory enzymes needed and monomeric xylose yield performance increases.	6/30/2015
Regular	**Title:** Report in a Techno-Economic Analysis the Effects of Higher Solids Enzymatic Hydrolysis and Deacetylation Liquor Recycling on Sugar and Hydrocarbon Intermediate Production Costs. **Milestone Description:** Construct TEA analysis with Aspen Plus developed models using NaOH and water usage, sugar yields and hydrocarbon intermediate production results from higher solids enzymatic hydrolysis and deacetylation liquor recycle DDR experiments. Prepare a manuscript for submission to a peer reviewed journal for publication of the TEA results.	9/30/2015

FY16 Milestones

TYPE	Milestone Title	Due Date
Regular	SMART GOAL: Identify alkaline preprocessing and acid pretreatment conditions on a blended feedstock to acheive at least 80% glucose yield in enzymatic hydrolysis with <15 mg protein/g glucan enzyme loading using commercially available enzyme preparations. Deliver kg quantities of the EH hydrolyzed slurries and/or alkaline preprocessed and dilute acid pretreatment slurries to the Biological Upgrading of Sugar 2.3.2.105, Lignin Utilization 2.3.4.100, Separations Development and Application 2.4.1.101, and Bench Scale Integration 2.4.1.100 projects	12/31/2015
Regular	Demonstrate feed handling performance in advanced –formulated and pelleted INL feedstocks. Benchmark sugar recovery in preprocessed blended and pelleted materials compared to non-pelleted corn stover using continuous pretreatment systems. Deliver kg quantities of pretreatment slurries to the Biological Upgrading of Sugar 2.3.2.105, Lignin Utilization 2.3.4.100, Separations Development and Application 2.4.1.101, and Bench Scale Integration 2.4.1.100 projectsf Joint milestone Feedstock-Processs Interface 2.2.1.101	3/31/2016
Regular	SMART GOAL: Identify alkaline preprocessing and mechanical refining conditions on a blended feedstock to acheive at least 80% glucose yield in enzymatic hydrolysis with <15 mg protein/g glucan enzyme loading using commercially available enzyme preparations. Deliver kg quantities of the enzymatically hydrolyzed slurries to the Biological Upgrading of Sugar 2.3.2.105, Lignin Utilization 2.3.4.100, Separations Development and Application 2.4.1.101, and Bench Scale Integration 2.4.1.100 projects	6/30/2016
Annual	Perform sensitivity analysis using existing integrated hydrocarbon TEA models and data from the Q1 and Q3 milestones to guide FY17 DDA and DMR optimization studies.	9/30/2016
Go/No Go	Determine if Accessory Enzymes can Remove Microorganism Death and Inhibition of Dilute Acid Pretreated C5 Stream. Perform TEA on costs of accessory enzymes.	3/31/2016

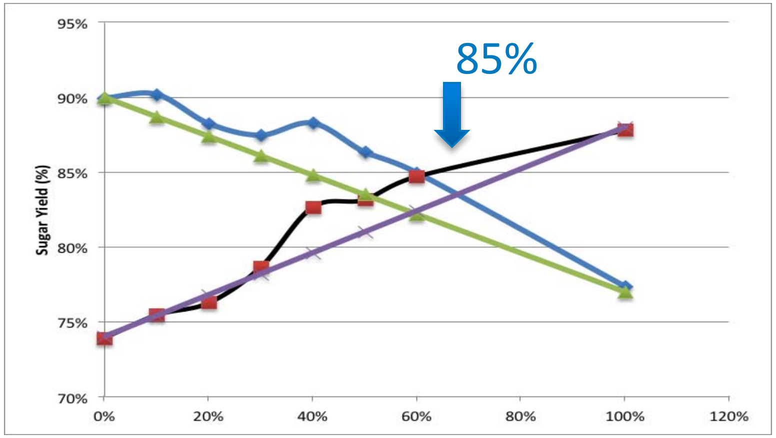
FY17 Milestones for Pretreatment & Process Hydrolysis Project

TYPE	Milestone Title	Due Date
Regular	SMART GOAL: Conduct a thorough investigation into the kinetics and rheological behavior of higher solids (solids>20%) enzymatic hydrolysis using both dilute alkali/acid (DAAD) pretreatment and DMR corn stover slurries. Design and conduct experiments to cover at least three pretreatment types	12/31/2016
Regular	SMART GOAL: Conduct industrial scale DMR of corn stover, switchgrass and one INL advanced tri-blend feedstock using the Andritz 36" disc refiner at a refining energy in the range of 50-200 kWh/ODMT. Produce at least 100 kg of refined biomass for each individual and advanced tri-blend feedstock and conduct enzymatic hydrolysis at the 10 and 100 kg scale. Joint milestone with Feedstock Process Interface (2.2.1.101): FPI will provide at least 100 kg of each individual and tri-blended feedstocks in collaboration with INL. Joint milestone with Biological Upgrading of Sugars (2.3.2.105): Produce at least 200 kg of DMR corn stover whole slurry enzymatic hydrolysate for future fermentations required by BUS. Provide kg quantities of DMR-EH slurries to Catalytic Upgrading of Sugars (2.3.1.101), Bench Scale Integration (2.4.1.100), Lignin Utilization (2.3.4.100), and up to 300 kg for Separations Development and Applications (2.4.1.101).	3/31/2017
Regular	SMART GOAL: Achieve 80% monomeric glucose yield with 12 mg total protein/g glucan (i.e. 10 mg cellulase (CTec3 or other advanced cellulase) plus 2 mg hemicellulase (HTec3 or other advanced hemicellulase)/g cellulose enzyme loading (down from 15) on DDR, DMR, or Dilute Acid Pretreated slurries produced from an INL advanced tri-feedstock blend.	6/30/2017
Major	Perform TEA using an updated FY17 model and dilute acid and DMR data from the Q3 FY17 milestone that achieve >80% monomeric glucose yield with 12 mg cellulase/g cellulose enzyme loading on an INL advanced tri-blend feedstocks.	9/30/2017
Regular	End of 3 Year FY18 Project Key Outcome: TEA of DDA and DMR pretreatment processes on recalciatrant INL advanced tri-blended feedstocks that achieve >85% monomeric glucose yield in enzymatic hydrolysis with 10 mg/g cellulose cellulase and 2 mg/g cellulose hemicellulase loading using commercially available enzyme preparations .	9/30/2018
Go/No Go	Achieve 20% reduction in xylan losses during deacetylation of advanced tri-blended feedstocks.	9/30/2017

Bi-Blend Feedstock Reduces Feedstock Costs But Increases Recalcitrance -DMR

- Objective: Investigating the effects of a 50/50 blend of corn stover and switchgrass on the glucose and xylose pretreatment and enzymatic hydrolysis yields using Deacetylation and Mechanical Refining (DMR) process
- Key findings:
  - Switchgrass require higher deacetylation severity than corn stover.
  - For 50/50 blend, 80% glucose yield at 15mg/g of total enzyme protein loading (DMR).
  - Separate pretreatment followed by mixing may lead to higher sugar yields than pre-mixing

ID	Feedstock	Deacetylation Condition
B2	Blend 50/50	Medium
C2	Corn Stover	Medium
SW3	Switchgrass	High



High solids enzymatic hydrolysis at 20% initial insoluble solids (total solids). The enzymatic hydrolysis was conducted at an enzyme loading of 15 mg total protein/g glucan using a 80/20 mixture of Novozymes Cellic CTec 3/HTec 3 for five days

Future Work to Recycle Sodium using Microbial Electrochemical Technology

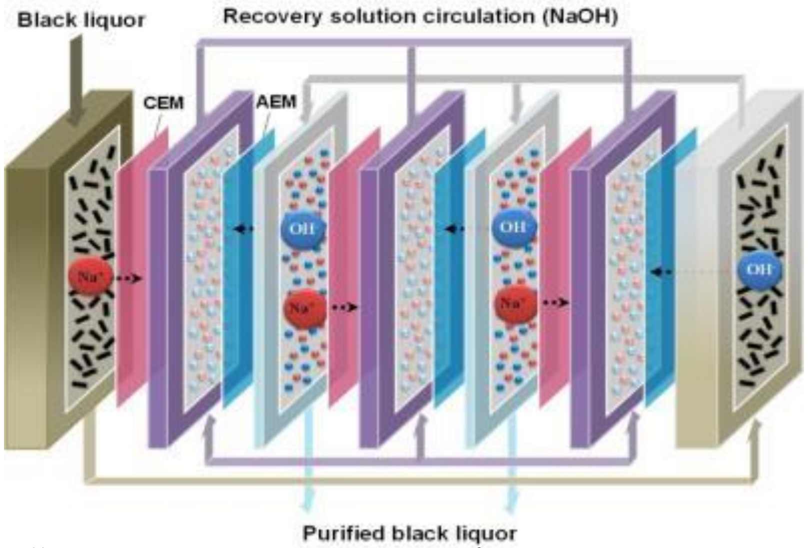
Black Liquor Utilization

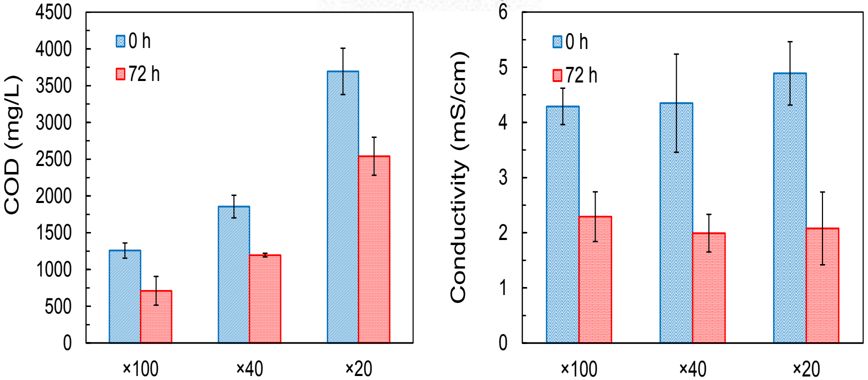
Challenges

- Sodium recovery requires high energy in traditional technology (KRAFT)
- Organics (including lignin, acetate) are burned to generate low value heat streams
- Negative Impact on LCA

Approaches

- Microbial Electrochemical Technology (MET)
  - Generates electricity without burning
  - Recovers Sodium Hydroxide

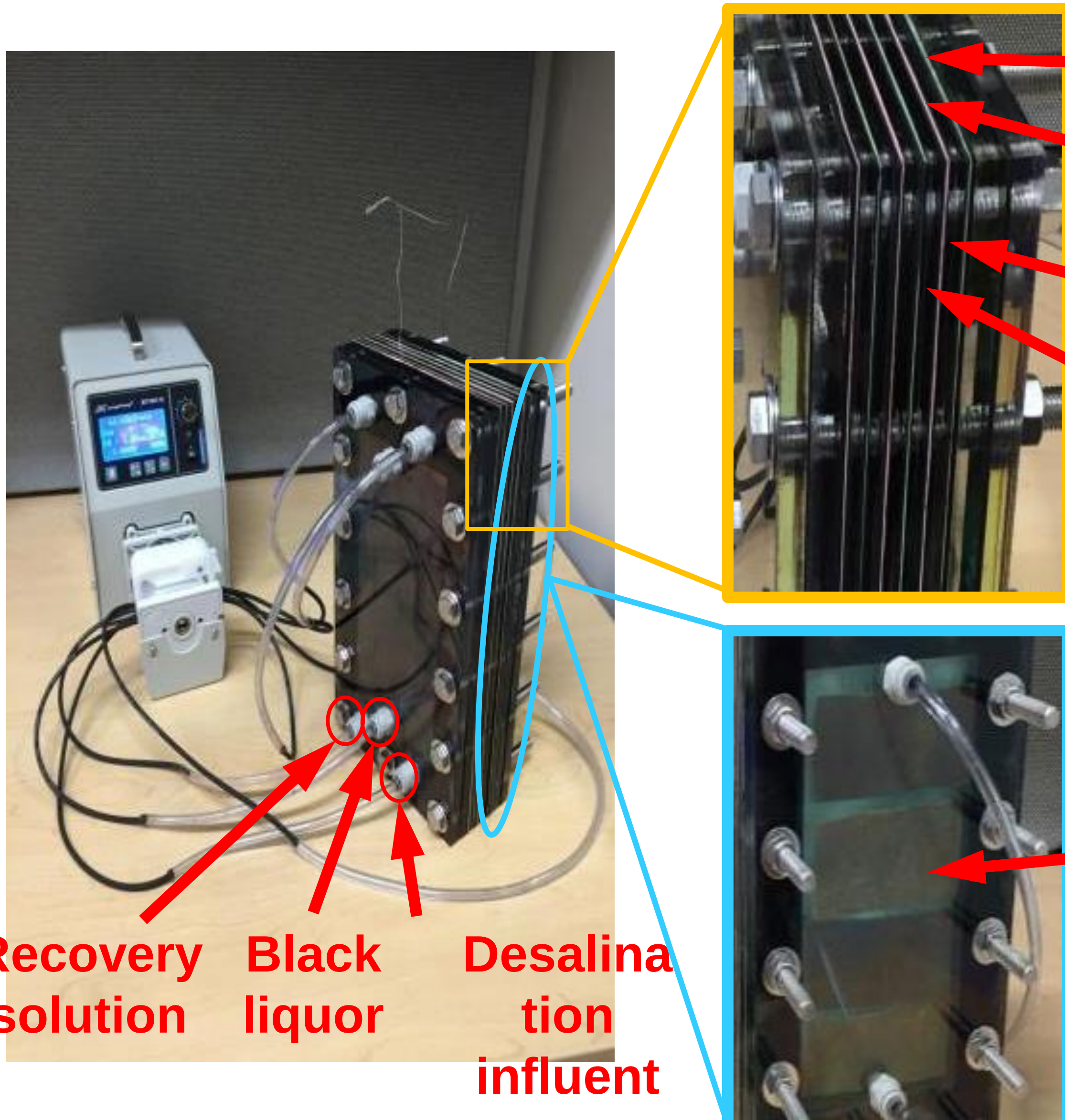




Goals

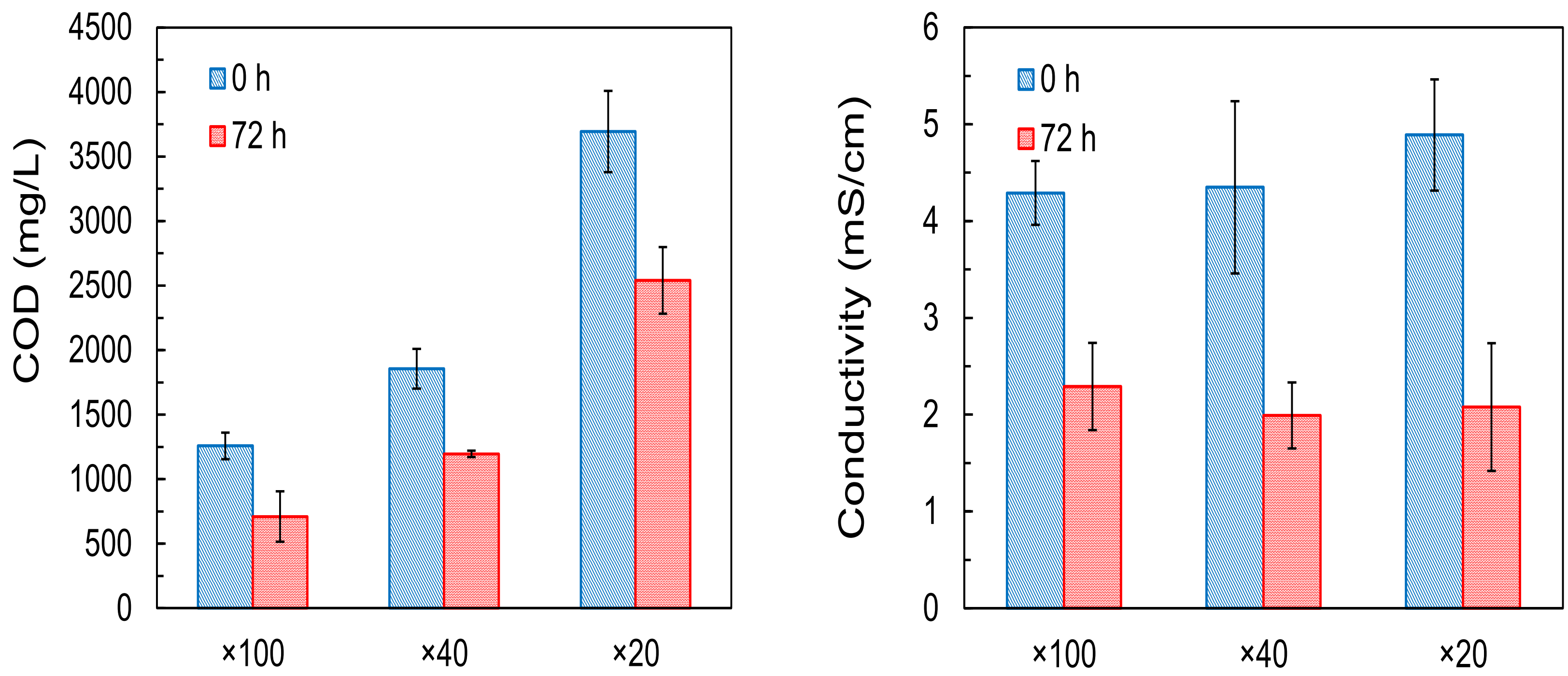
- Recover 80% sodium hydroxide using (MET) by 2022
- Utilize 80% organics in BL

Bench-scale Microbiological Electrochemical Technology Cell



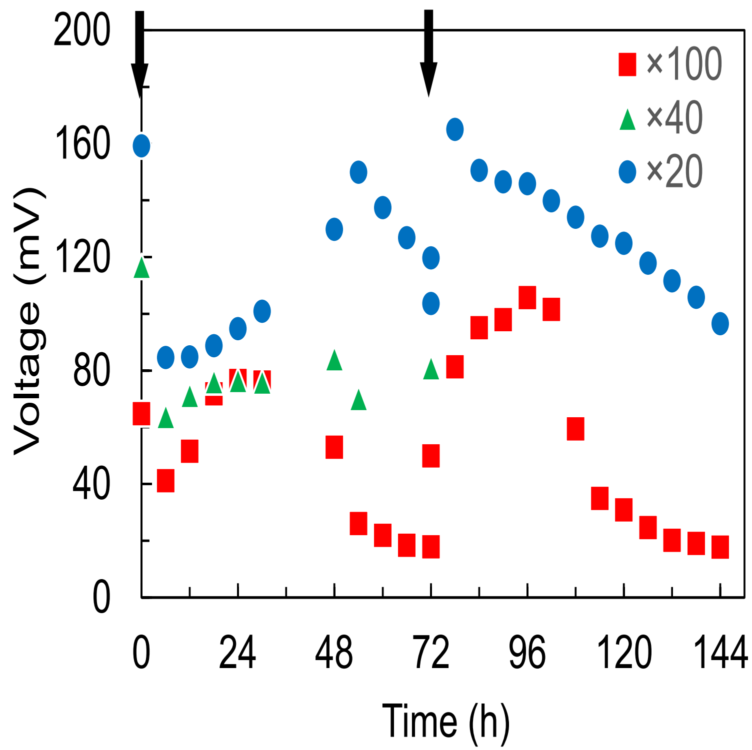
30

Microbial Electrochemical Technology (MET)-Preliminary Experiments



- Black liquor diluted by 100, 40 and 20 times acted as the anolyte of MFCs.
- Cycle time:72 h

Bench-scale MET Preliminary experiments (repeatability)



- Arrows showed the electrolyte renewal

Technical Achievement—TEA on High Solids Enzymatic Hydrolysis of DMR Corn Stover Substrates

Higher solids enzymatic hydrolysis (HSEH) doubles sugar concentration from 150 g/L to 270 g/L:

- Reduces water usage by up to 10%
- Reduces reactor size
- Reduces evaporator size
- Reduces energy of concentration

Achievements

- HSEH could save as much as $33/tonne of sugar (FY15 Q4 milestone report)
- Published in *Energy and Environmental Science:* 2016,9,1237-1245 DOI: 10.1039/C5EE03718B

TEA Analysis of Sodium Recycle

	Without Recycle			With Recycle
Deacetylation TS%	10%	30%	30%	10%
Water for Deacetylation (kg/hr)	666,667	166,667	166,667	166,667
Wash water after deacetylation (kg/hr)	0	563,117	0	67,620
Steam for A200 (kg/hr)	58,764	17,266	17,266	14,870
Total water used for A200 (kg/hr)	728,107	748,949	358,054	404,178
MESP ($/gal)	$2.32	$2.27	$2.21	$2.17