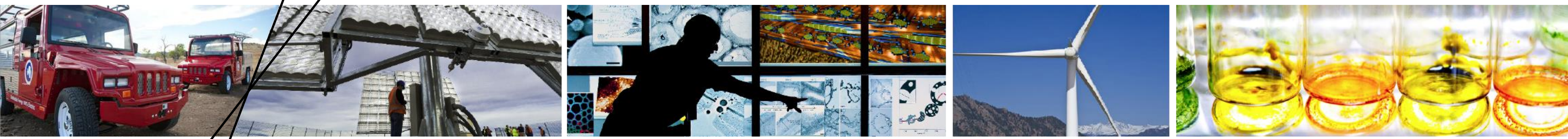
2013 DOE Bioenergy Technologies Office (BETO) Project Peer Review

Pretreatment and Enzyme Hydrolysis



Tuesday, May 21st, 2013

Technology Area Review: Biochemical Conversion

Principal Investigator: David K. Johnson

Organization: National Renewable Energy Laboratory

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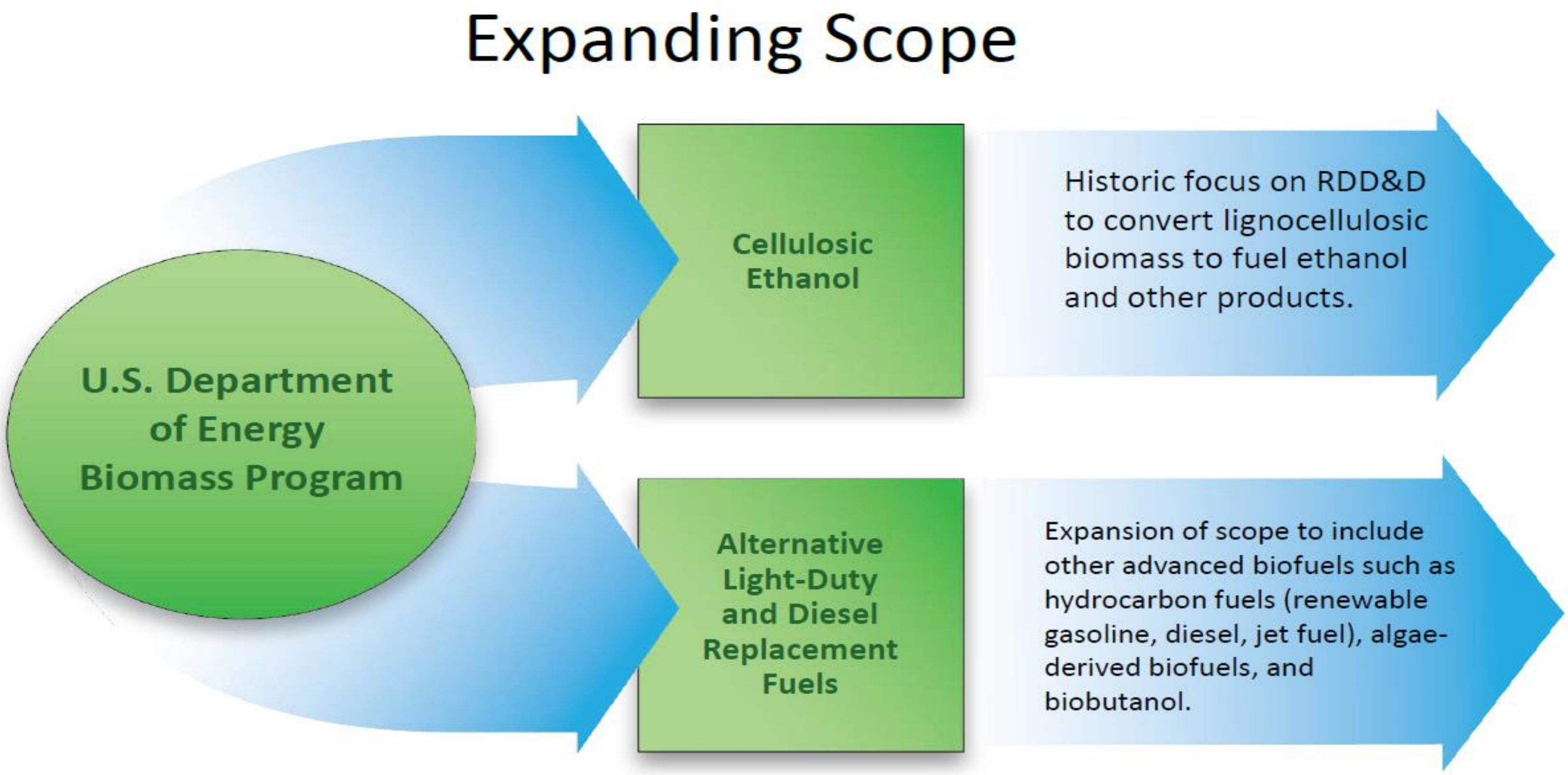
Project Overview

Develop and apply fundamental knowledge of pretreatment and enzymatic hydrolysis concepts in process-relevant contexts to produce lignocellulosic biofuels



Goal Statement

To support the near-term Biochemical Platform cost and process performance goals:

•Generate cost-competitive useable sugars for conversion to cellulosic ethanol by 2012 (corn stover)

- Develop the pretreatment/hydrolysis unit-operations in a process relevant scaleable manner and so that they integrate well with downstream processes
- Reduce estimated mature technology processing costs for converting cellulosic feedstocks to ethanol

Directly support BETO's Multi-Year Program Plan (MYPP) objectives (targets beyond 2012) :

- Produce sugars and other reactive intermediates "to support the 2017 goals for renewable gasoline, diesel, and jet fuel"

Quad Chart Overview

Timeline

- Project start date - 2004
- Project end date - 2017
- Percent complete - 60%

Barriers

- Bt-D Pretreatment Processing
- Bt-E Pretreatment Costs
- Bt-G Cellulase Enzyme Loading

Budget

- Funding for FY11($6.5M / $0)
- Funding for FY12($5.06M/ $0)
- Funding for FY13 ($5.75M / $0)
- Years the project has been funded – 10 years/ $5.1M average annual funding.

Partners

- Subcontracts
  - Auburn University, U. Georgia CCRC, Oklahoma State University, Baylor University, Virginia Tech University, U. Colorado, U. North Dakota, Colorado School of Mines, NIST, Washington State University
- Other Collaborations
  - North Carolina State University, Pennsylvania State University, U. Toronto, Andritz, IdeaCHEM,
- The project is managed under the Biochemical Platform at NREL

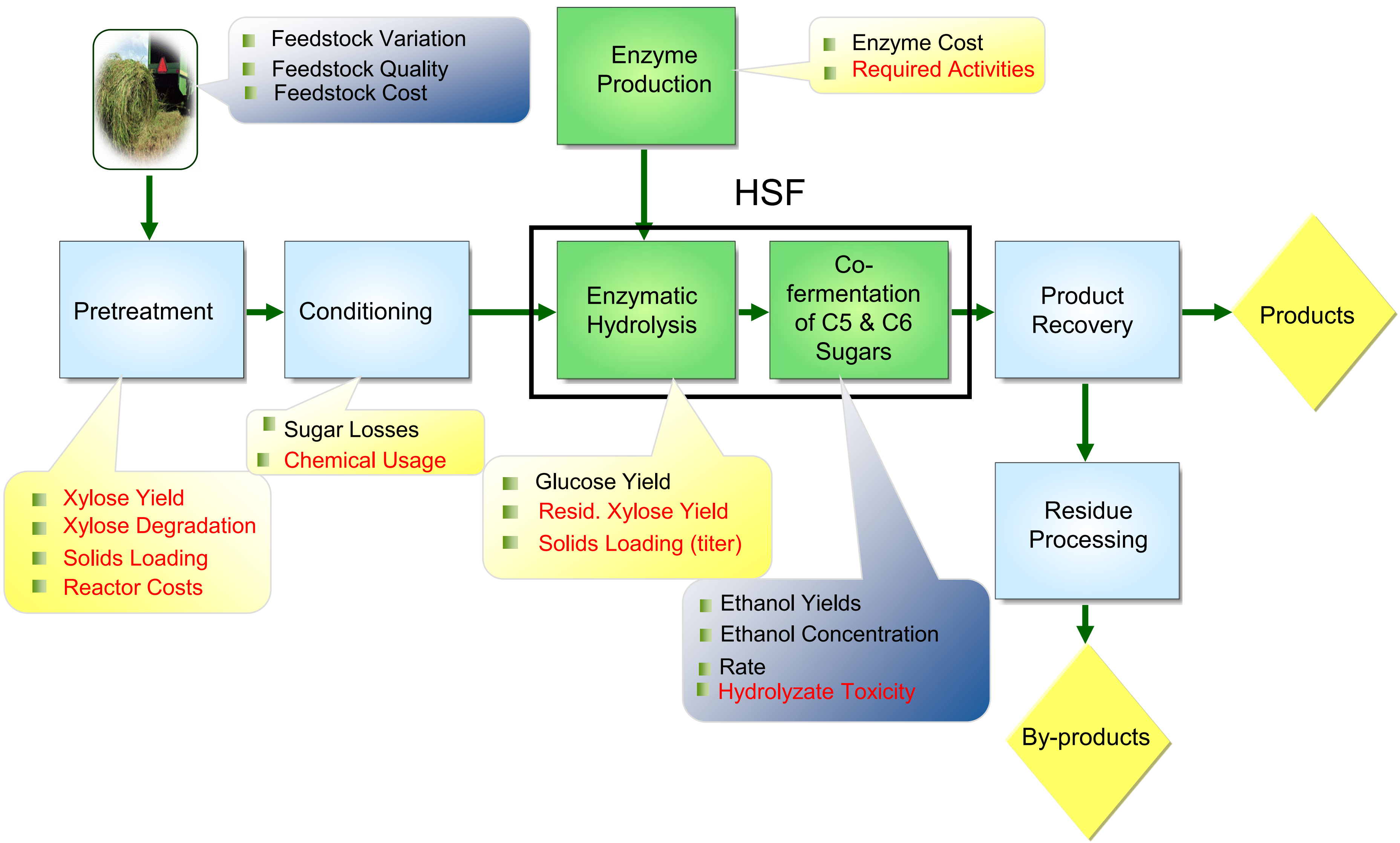
Approach

- Transition from knowledge development to unit-operation applications

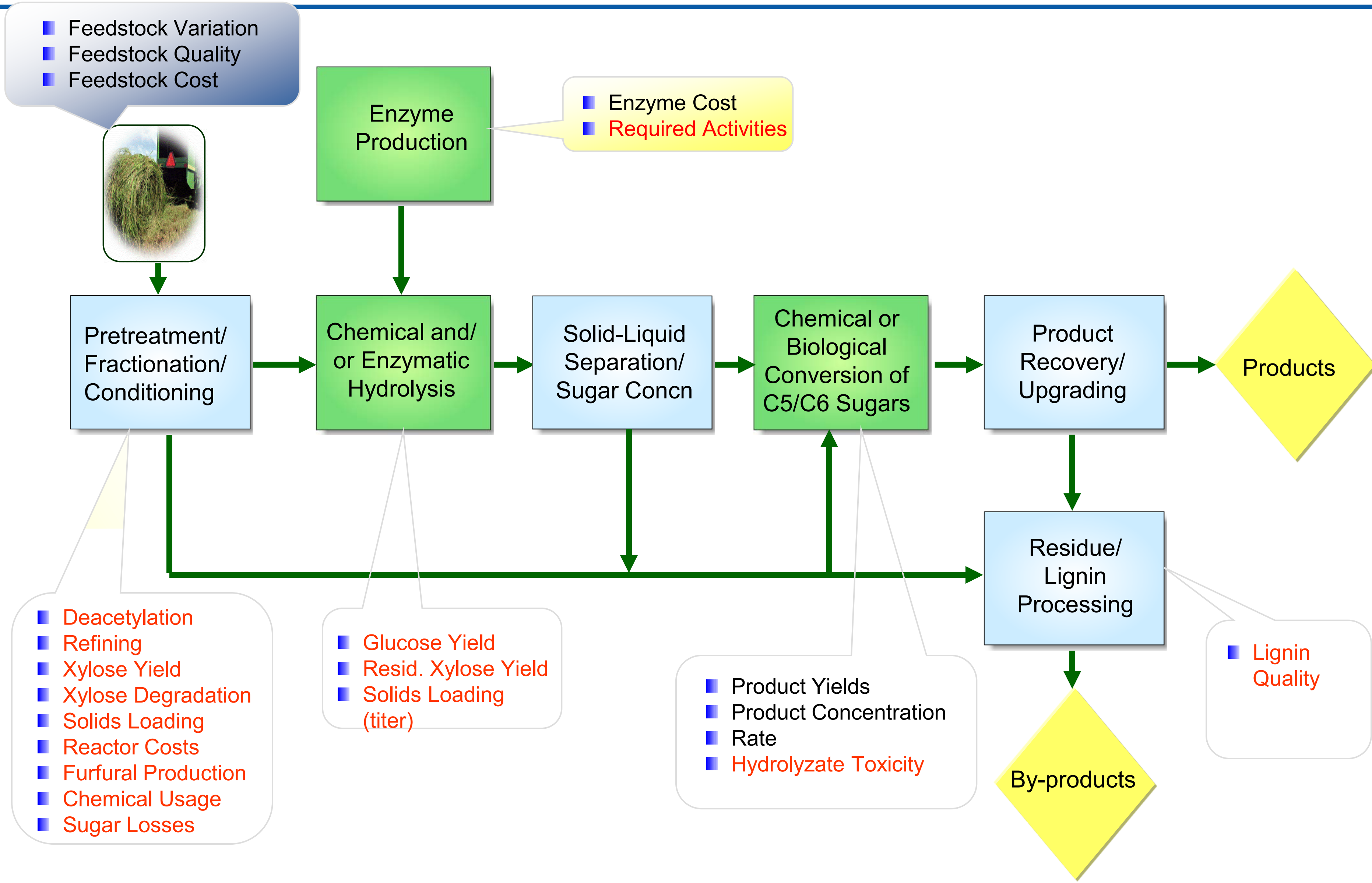
Knowledge Development	Applications
• Impact of pretreatment parameters on monomeric xylose yields, inhibitor formation, and overall fermentation yields from high solids, unwashed slurries	• Selection of pretreatment configuration for 2012 integrated biochemical conversion of corn stover to ethanol, including TEA considerations to achieve 2012 ethanol cost targets.
• Identification of approaches for conversion xylooligomers / residual xylan to monomer	• Use of selected component enzymes to achieve xylooligomer saccharification
• Rheological tools and methods for enzymatic hydrolysis slurries	• Application of rheological findings for bench-scale / pilot-scale high-solids saccharification reactor design
• Identification of inhibitors and characterize effects on biological and chemical processes	• Mitigate toxicity of high-concentration hydrolyzates
• Investigate deoxygenation routes • Investigate pathways to increase carbon chain length	• Develop strategies for converting sugars into hydrocarbon fuels and intermediates

- Management plan has well-defined performance targets leading to 2012 and 2017/2022 integrated process demonstrations
  - Detailed Annual Operating Plan (AOP) at Subtask Level
  - 10-12 milestones/deliverables per year
  - Tracking of results in annual State of Technology (SOT) updates

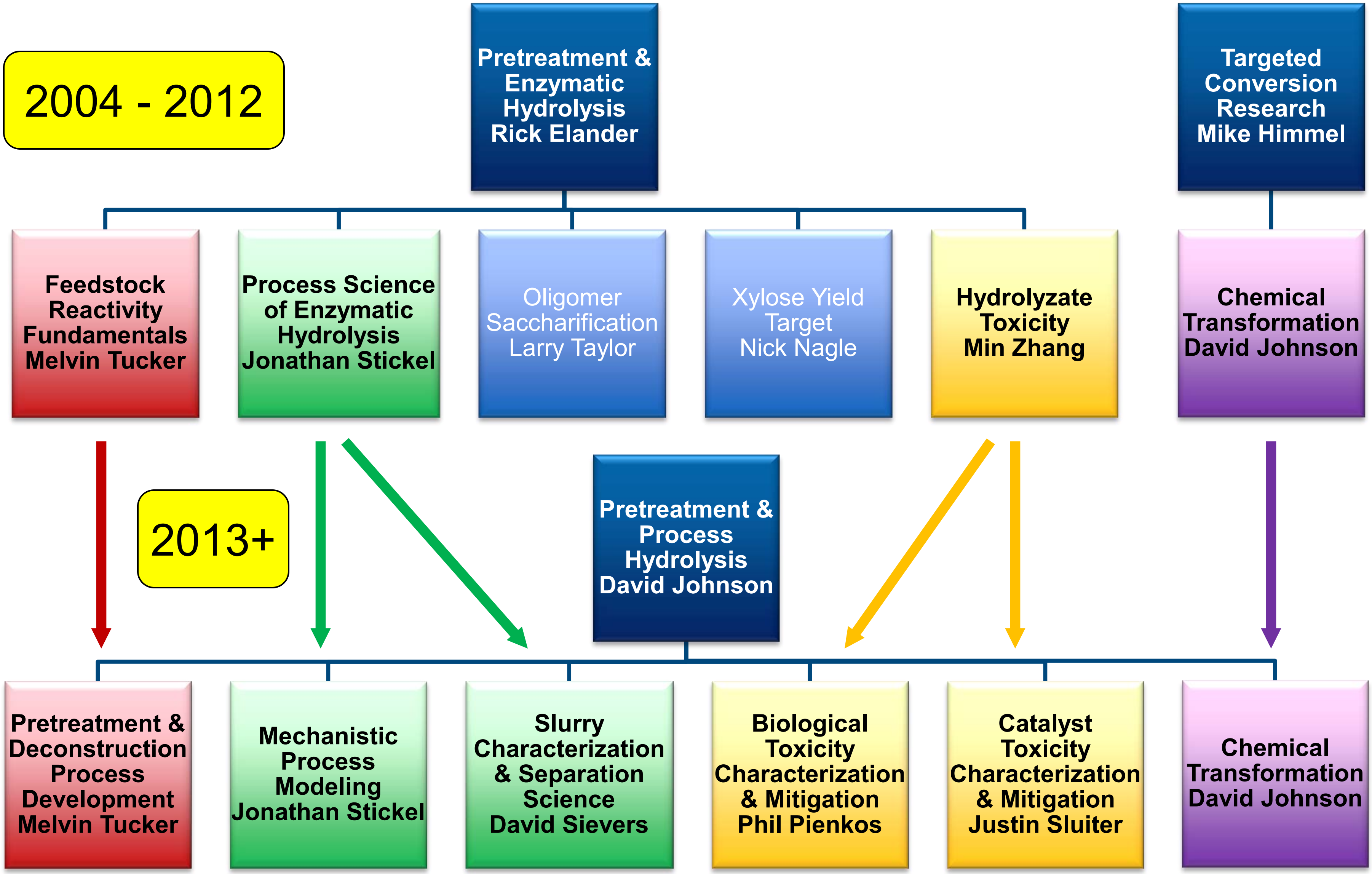
Biochemical Platform Process Impacts – Cellulosic Ethanol



Biochemical Platform Process Impacts – Advanced Biofuels



Task Structure



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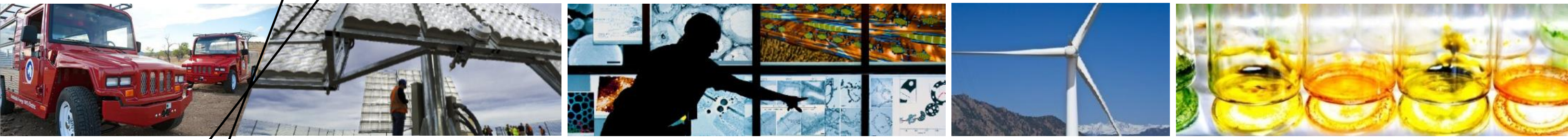
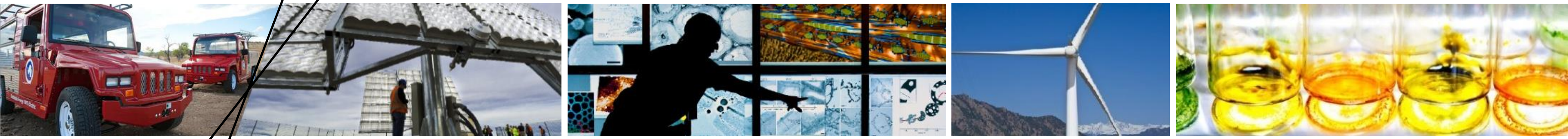
Technical Accomplishments/Progress/Results



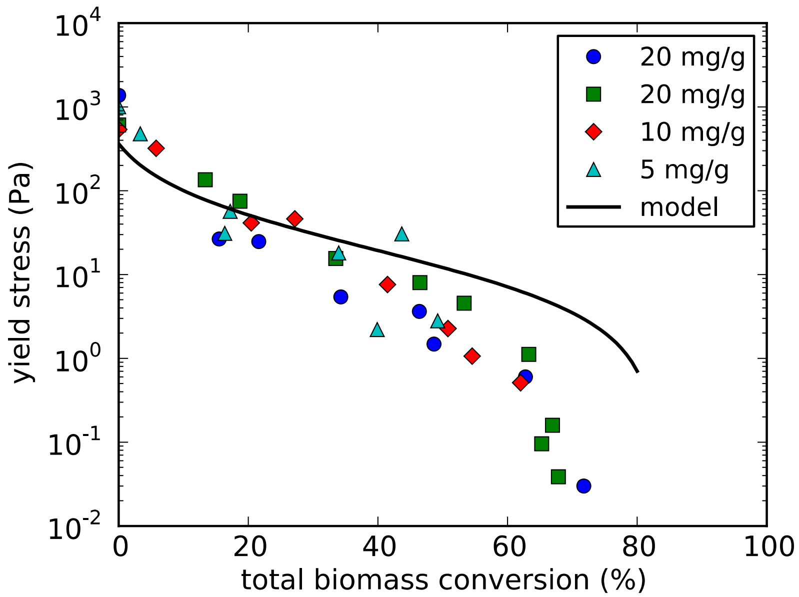
- Accomplishments supporting the successful 2012 cellulosic ethanol demonstration runs
- Progress aimed at further reducing the cost of converting biomass to sugars
- Progress in support of producing hydrocarbons from biomass-derived sugars



Accomplishments supporting the successful 2012 cellulosic ethanol demonstration runs

Characterization of Process Slurries

- Pretreated slurry rheology characterized
- Enzymatic hydrolysis rheological behavior characterized over reaction time
- Data used to specify enzymatic hydrolysis reactor vessels/agitators and pumps/pipelines in NREL's pilot plant
- Equipment successfully delivered performance to meet 2012 demonstration run goals



Deacetylation and Lower Severity Pretreatment of Corn Stover

- Pre-process with 0.1M NaOH, 80°C to remove acetate prior to pretreatment
- Deacetylation enabled use of lower severity pretreatment conditions with lower furfural generation, high enzymatic digestibility, and reduced hydrolyzate toxicity to achieve high fermentation yields and ethanol titers
- Deacetylated corn stover pretreated 150°C, 0.5% acid, as digestible as control CS pretreated 170°C, 1.0% acid, but furfural yield only 2% vs 10%
- Equipment incorporating deacetylation and acid impregnation was scaled-up in support 2012 cellulose ethanol demonstration runs

2000 L Deacetylation/Impregnation Mix Tank

Continuous Dewatering Screw Press

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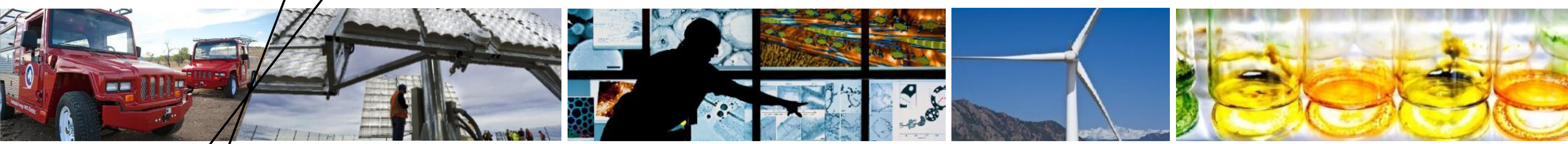


Progress aimed at further reducing the cost of converting biomass to sugars

Saccharification of Xylooligomers

Identifying barriers to maximizing xylose yields

- In FY11 a series of xylooligomers with 4-O-methyl-α-glucuronic acid residue (MeGlcA) identified as most abundant pool of remaining convertible xylose
- X-MeGlcA and X-X-MeGlcA predominant species. Persist despite thermochemical oligomer hold, or enzymatic hydrolysis
- FY12 D milestone: achieve 50% conversion of 4-O-methyl-α-glucuronic acid-substituted xylooligomers to monomeric xylose from relevant pretreatment liquid
- Of four tested enzymes GH67s from *A. niger* and *Geobacillus stearothermophilus* exceeded target reductions of X-MeGlcA and X-X-MeGlcA

Composition of Deacetylated Hydrolyzate

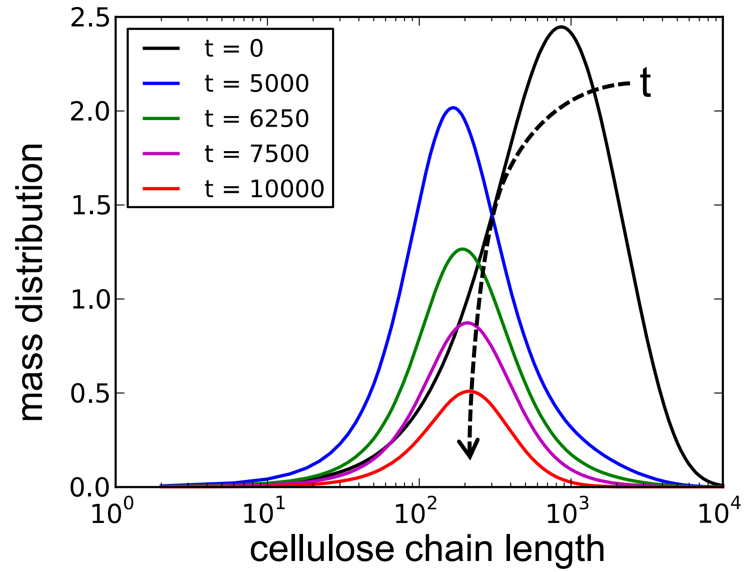
	Concentration (mg/mL)
Glucose	1.8
Celloligomers	3.8
Xylose	0.8
Xylooligomers	15.2

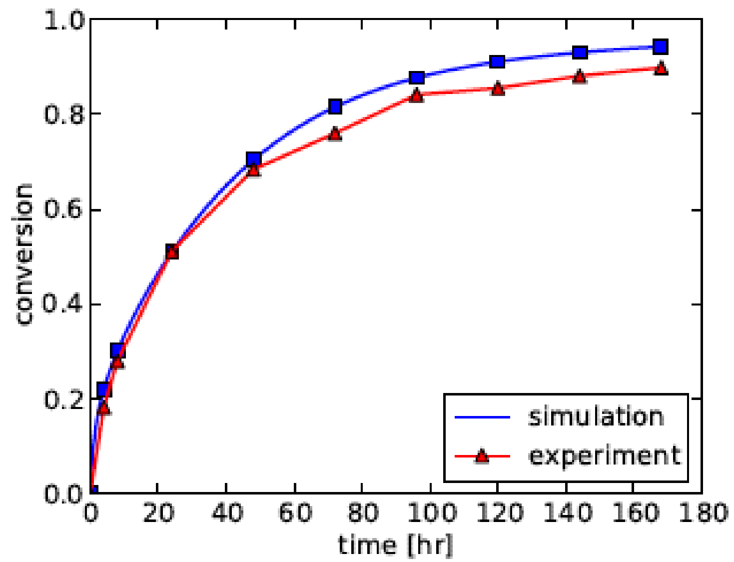
Conversion of Xylooligomers using Alpha-Glucuronidases

α-glucuronidase	*X-X-MeGlcA*	*X-MeGlcA*
$GH115_B$	3	18
$GH67_C$	0	15
$GH67_G$	77	87
$GH67_A$	96	83
No α-glucuronidase	2	6
no enzyme	0	0

Modeling of Enzymatic Hydrolysis Kinetics

- Models incorporating the independent action of different enzyme types (EG, CBH, βG) used to predict the population distribution of cellulose
- Simulation closely predicts actual hydrolysis conversion over time
- Useful for predicting conversion yields in process simulation based on enzyme cocktail and feedstock properties

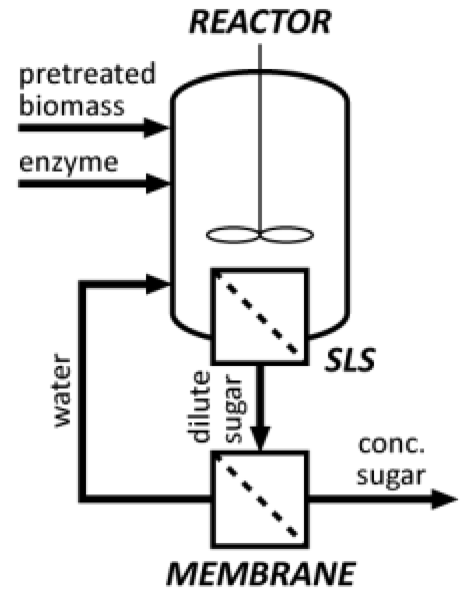


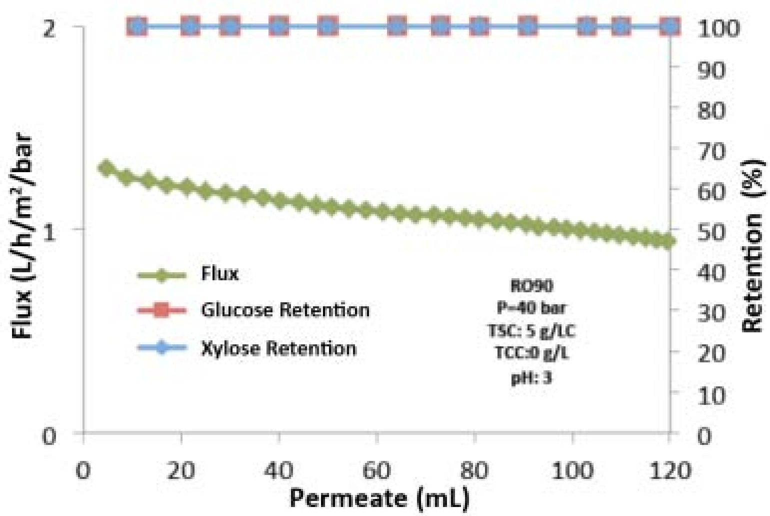


FY11 D-Milestone, "Incorporate additional phenomena in the enzymatic hydrolysis kinetics model and perform model validation with experimental data"

Separations Development for Continuous Enzymatic Hydrolysis

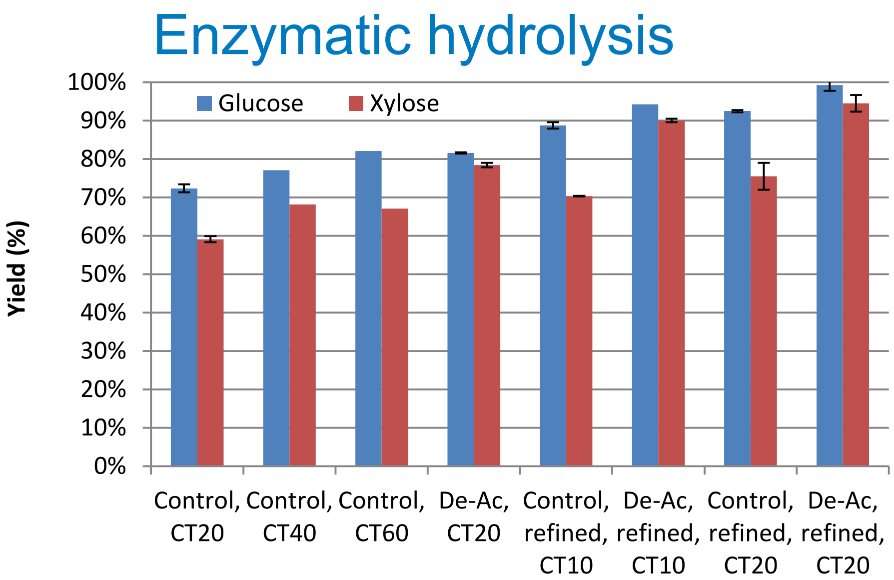
- Solid-liquid separation (SLS) to remove products: reduce product inhibition and increase productivity
  - Development and experimentation planned for FY13 through FY15
- Sugar product concentrated to prepare sugar stream for variety of fuel pathways.
  - High sugar retention demonstrated with nanofiltration membrane

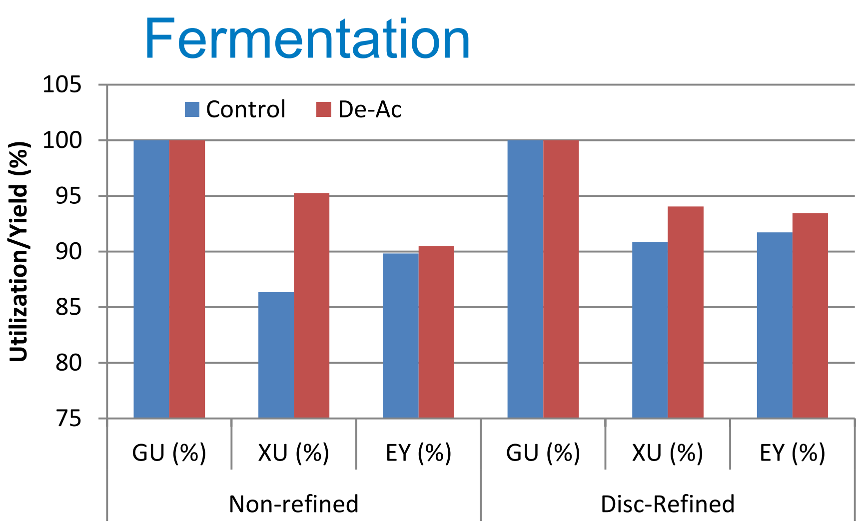




FY13 D-Milestone, "Develop membrane-based water removal (sugar concentrating) process technology."

Deacetylation and Mechanical Refining with Dilute-Acid Pretreatment



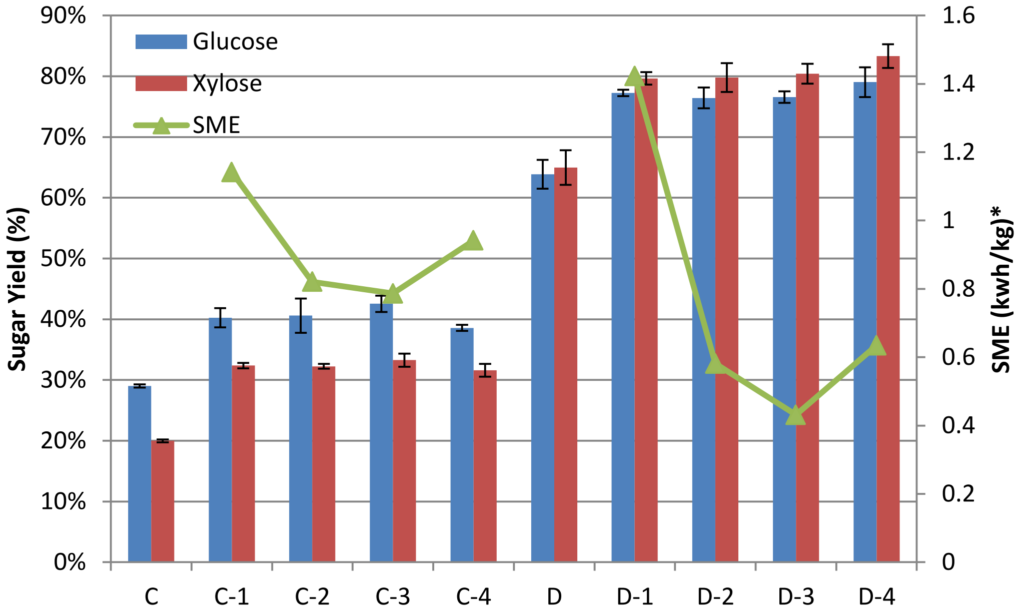


•GU=Glucose Utilization, XU=Xylose Utilization, EY=Ethanol Yield

- Pre-process corn stover with 0.1M NaOH at 80 °C for 2h to remove acetate prior to pretreatment
- Post-pretreatment mechanical refining requires less energy, and increases cellulose accessibility
- Deacetylation and mechanical refining significantly enhance enzymatic digestibility of low severity dilute-acid pretreated corn stover (150 °C, 0.5% acid, 20 min)
- Deacetylation significantly increases xylose utilization in fermentation and improves ethanol yield and titer
- Deacetylation and mechanical refining can reduce the minimum ethanol selling price

Deacetylation and Mechanical Refining without Dilute Acid Pretreatment

A Coperion ZSK-25 twin screw, co-rotating extruder from IdeaCHEM, Rapid City, SD



- C-control, non-extruded; C1-C4- control, extruded
- D-deacetylated, non-extruded; D1-D4-deacetylated, extruded
- Total Enzyme loading=26mg/g cellulose.
- SME = Specific Mechanical Energy (kWh/kg)

Mechanical refining with deacetylation could potentially replace acid pretreatment to achieve high sugar yields and produce a clean sugar stream without degradation products.

Similar results obtained with PFI mill and disk refiner

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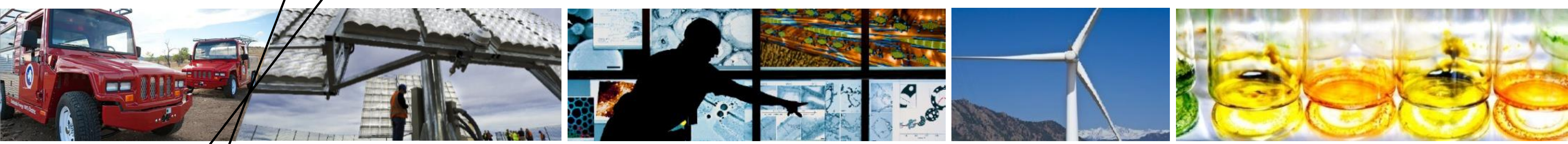
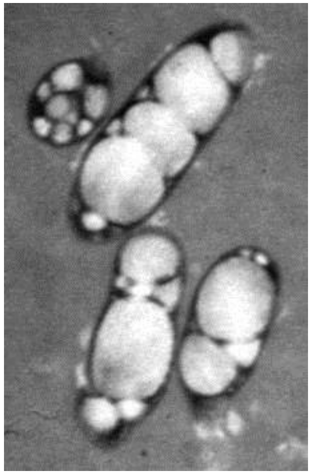
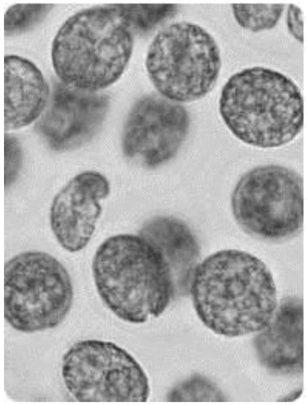


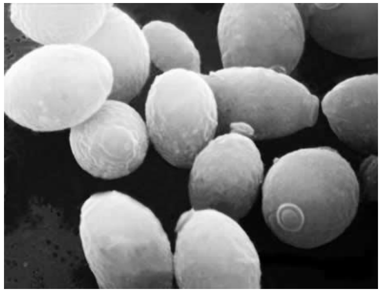
Progress in support of producing hydrocarbons from biomass-derived sugars

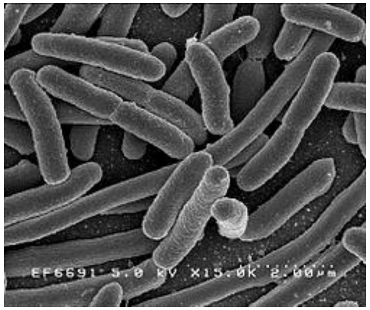
Identification of Inhibitors to Fuel Producing Organisms

- Identify specific inhibitors and assess impacts on HC producing microorganisms
- Ultimately develop strategy to mitigate their impact on the biological processes involved.
- Identify production pathways and relevant strains
  - Fatty Acid Pathway
  - Isoprenoid Pathway
  - Polyhydroxybutyrate Pathway
  - Polyketide Pathway
- Determine growth characteristics using acetate w/ or w/o sugars
- Develop high throughput inhibition assay
- Implement high throughput method to generate inhibitor profiles.



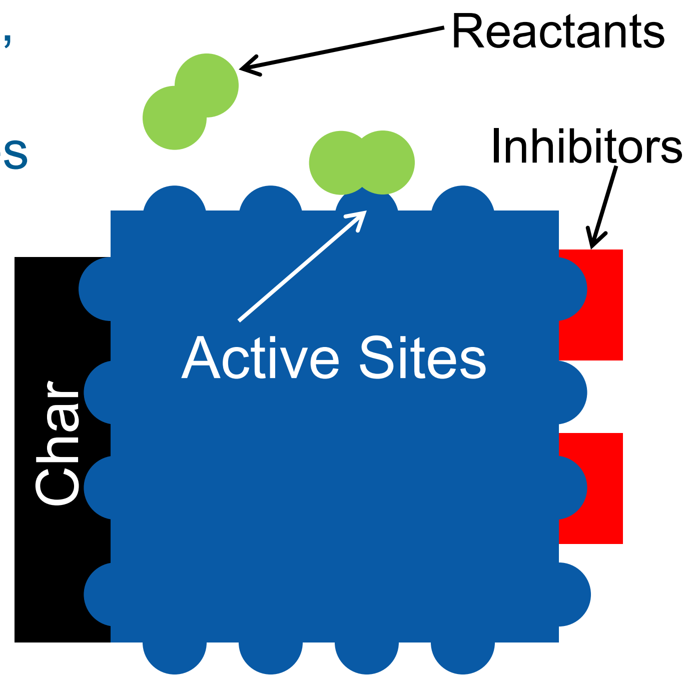






Prediction of Catalyst Deactivation

- Analysis for fermentation processes have been focused on carbohydrates and biological inhibitors.
- Many potential catalytic inhibitors are not routinely or robustly measured by current methods.
- Need to learn how biomass pre-processing, pretreatment, and hydrolysis should be optimized for catalytic conversion processes

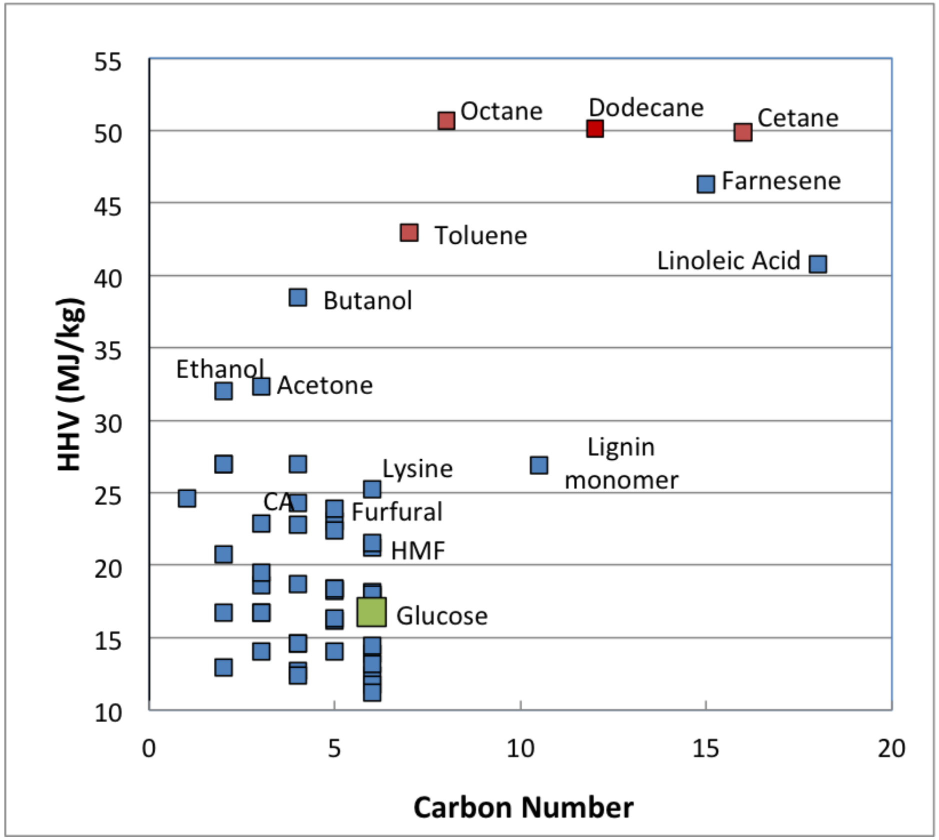


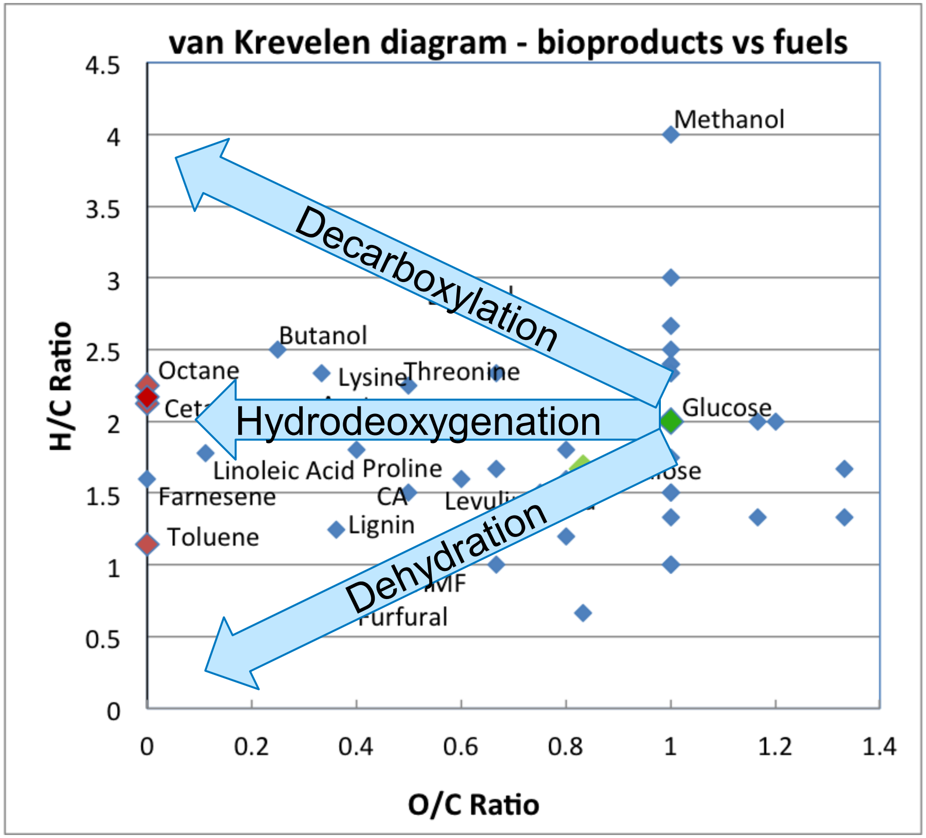
This task is developing and adapting methods for routine quantification of potential catalyst inhibitors.

- ICP for detection of known toxic elements (i.e. sulfur)
- LC/MS & GC/MS for quantification of potential char producing, non-carbohydrate components (i.e. phenolic or aldehydes)

Production of Hydrocarbon Fuels from Biomass-derived Components

- Increase energy content - Decrease oxygen content
- Increase carbon chain length – favors jet and diesel over gasoline

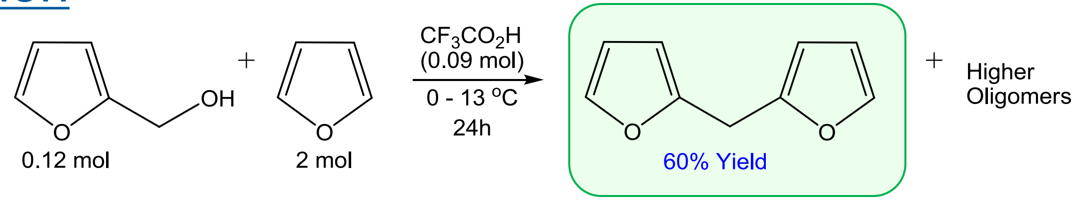




Top Value Added Chemicals from Biomass Volume I - Results of Screening for Potential Candidates from Sugars and Synthesis Gas (2004). Top 30 chemicals + others

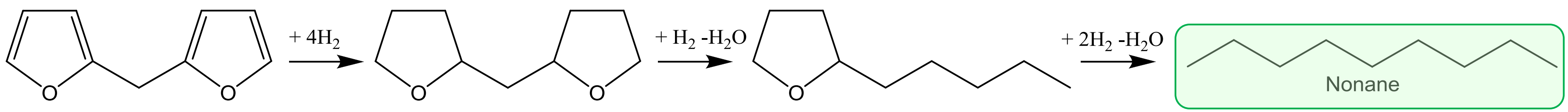
Furans to Hydrocarbons

Dimerization



[Schwenter *et al. Chem. Eur. J.* **2000**, *6*, 4091-4103]

Hydrodeoxygenation

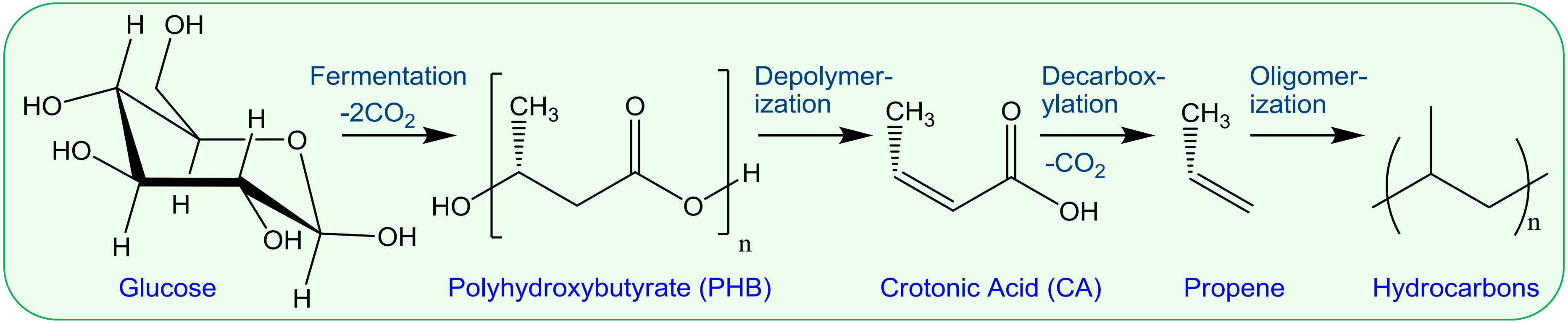


- Can a furfural-based dimer be produced and hydrodeoxygenated to alkanes
- Moderate yields of C7 - C9 alkanes were made from furfuryl alcohol – furan dimer
- With much higher yields it could be possible to produce HC fuels from furfural

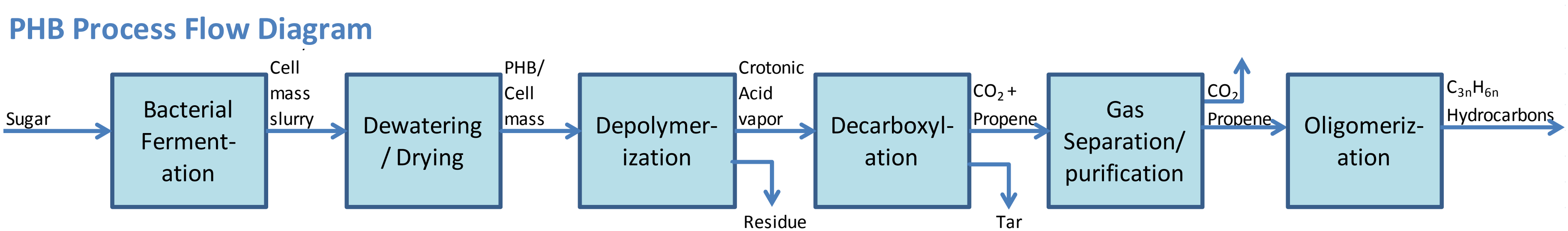
Catalyst	T (°C)	t (h)	Oxygenates %	Heptane %	Octane %	Nonane %
Pt/C	165-300	1	60	5	6	5
	300	4	54	12	23	11
Pt/C Pt/Al_2O_3	300	3	76	1	4	2
	300-350	5	37	13	28	11

2012 D-Milestone. Evaluate experimentally the transformation of furfuraldehyde into a mixture of aliphatic hydrocarbons

Polyhydroxyalkanoates to Hydrocarbons



- Fermentation gives solid product containing up to 80% PHB
- Depolymerization and decarboxylation can be combined to give propene a gaseous product in 70-80% molar yield
- Known technology would be used to separate propene and CO_2
- Propene oligomerization using known technology to give HC fuels



Polyhydroxyalkanoates to Hydrocarbons

- Can PHB be converted to propene in a simple reaction system
- A simple heated system without catalyst or solvent was shown to convert the intermediate CA to propene and CO_2
- Commercial PHB and PHB in bacteria cells also converted to propene
- Less costly process without prior PHB isolation appears possible

Substrate	Temperature (°C)	Pressure (psig) *	Propene Yield (molar %)	CO_2 Yield (molar %)
CA	350	23 ± 3	19 ± 3	15 ± 7
CA	400	76 ± 2	77 ± 3	74 ± 3
PHB	350	24 ± 1	22 ± 3	20 ± 2
PHB	400	71 ± 7	70 ± 7	78 ± 6
C. Necator 75% PHB	400	28	69	118

Reaction Time 15 min; At least 5 replicate reactions except for C. Necator
* Autogenic pressure at room temperature

2012 D-Milestone. Identify process conditions to achieve simultaneous depolymerization and thermocatalytic conversion of a PHA into a hydrocarbon)

Relevance

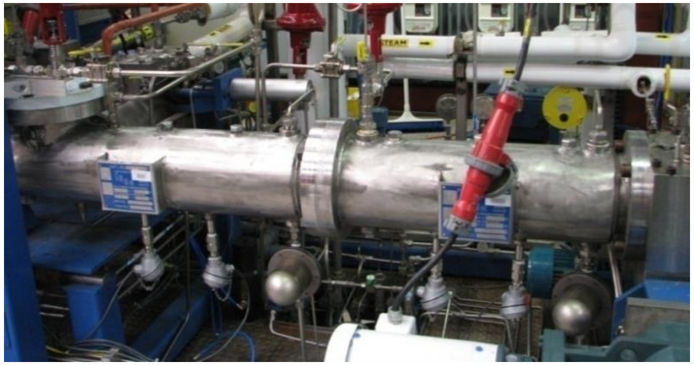
- Task goals have been and will continue to be clearly tied to well-established Biochemical Platform technical and economic goals
  - Sugar yields from pretreatment and enzymatic hydrolysis unit operations
  - Relevant processes, equipment, and mode of operation taken to larger scale
  - Considers impacts on downstream operations - sugar concentration, inhibitor formation, lignin quality
- Findings and know-how were directly utilized in 2012 biochemical State-of-Technology process demonstrations
- Transitioning to MYPP goal of by 2022, achieving the overall Program performance goal of $3 per GGE ($2011), based on data at the integrated pilot scale

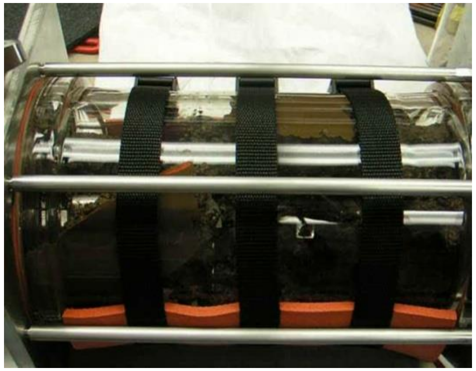
– By 2017, validate integrated production of a hydrocarbon fuel or blend stock from cellulosic biomass via a biological or chemical route at integrated bench-scale

– Develop commercially viable technologies for reducing the processing cost of converting biomass feedstocks into energy dense fungible, liquid transportation fuels, bioproducts, and chemical intermediates

Critical Success Factors

Factors	Strategy to Overcome
Technical	• Develop and apply knowledge on how to improve pretreatment AND enzymatic hydrolysis as a SYSTEM to saccharify biomass – Coordinated development to achieve BETO conversion targets – Well-defined technical targets and schedule to achieve overall targets
Business/ Market	• Numerous collaborative projects and proposals are leveraging core programmatic pretreatment/enzymatic hydrolysis capabilities and expertise • Prototype process equipment design and testing (vendor market) • Publications/outreach to disseminate R&D findings
Regulatory	• High solids loadings and concentrations to reduce water usage • Provide data and samples to evaluate wastewater treatment and solids disposal





Challenges

- Development of scaleable unit operations producing soluble sugar/carbon streams for cost-effective biological/chemical production of hydrocarbons from biomass
  - Will require further reduction in cost of producing sugars
  - Further reduction in formation of degradation products in pilot-scale, continuous pretreatment systems
  - Integration of sugar production with organisms and catalysts for producing new fuels possibly involving new separations and concentrating processes
  - Pretreatment must be coordinated with production of lignin-derived products
- Achieving process performance targets for new fuels at process relevant scale probably with new reactor systems

Future Work – Reduced Sugar Costs

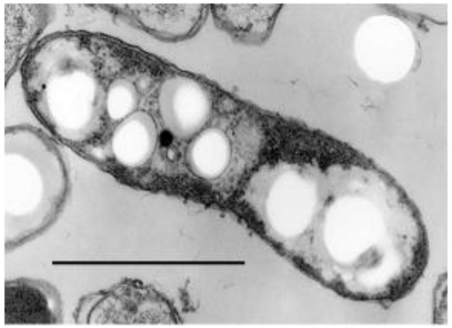
- Pretreatment and Deconstruction Process Development
  - Optimize / scale-up mechanical refining of deacetylated feedstock from low severity or no acid pretreatments
  - Optimize furfural production and recovery in reactor flash or separate two stage reactor processes

- Slurry Characterization & Separation Science
  - Further develop separation methods to enable continuous enzymatic hydrolysis
  - Develop methods for characterizing wet-granular slurries and correlate with pretreatment performance

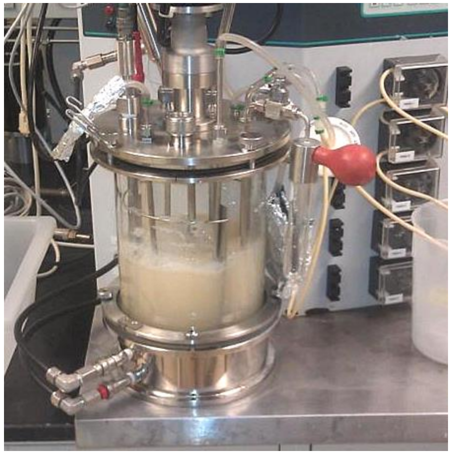
- Mechanistic Process Modeling
  - Parameter estimation and experimental validation of model describing cellulose enzymatic hydrolysis kinetics
  - Continue development of CFD for mixing biomass slurries and couple with reaction kinetics models

Future Work – Hydrocarbon Fuels

- Biological Toxicity Characterization & Mitigation
  - Perform inorganic and detailed organic analysis of saccharified slurries to identify potential inhibitors and relevant concentration ranges.
  - Identify top three inhibitors for select microorganisms.



- Catalyst Toxicity Characterization & Mitigation
  - Develop small scale catalyst bed for testing individual analytes to determine magnitudes of catalyst poisoning
  - Evaluate methods for removal or mitigation of the inhibitors in hydrolyzates prior to catalytic upgrading



- Chemical Transformation
  - Further develop processes for oligomerization of furfural derived chemicals and their hydrodeoxygenation to hydrocarbons
  - Scale-up conversion of PHB to propene and demonstrate production of PHB on biomass hydrolyzates
  - Perform TEA of furfural and PHB processes

Summary

- Work in the Pretreatment and Enzymatic Hydrolysis Task was critical to achieving 2012 technical performance and ethanol cost targets
  - Identification of pretreatment configuration for 2012 integrated biochemical conversion of corn stover to ethanol
  - Scale-up of deacetylation and its impact on decreased severity pretreatments with lower acid loadings, decreased neutralization chemicals, and improved enzyme digestibilities
  - Identification and mitigation of the fermentation inhibitors formed in pretreatment

- Work in Task is transitioning to achieving the 2022 goal of $3 per GGE ($2011), for hydrocarbon fuel or fuel blend stock from cellulosic biomass
  - Modifications to pretreatment aimed at lowering sugar production costs
  - Evaluation of hydrolyzate toxicity towards new hydrocarbon and intermediate producing organisms (and in the near future catalysts)
  - Evaluation of processes for converting sugars and furfurals into hydrocarbons

Acknowledgements

- Subtask Leaders
  - Steve Decker
  - Nick Nagle
  - Philip Pienkos
  - Jonathan Stickel
  - David Sievers
  - Justin Sluiter
  - Melvin Tucker
  - Min Zhang
- Staff
  - B. Adney
  - G. Beckham
  - S. Black
  - B. Bray
- Staff (continued)
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  - X. Chen
  - C. Dibble
  - J. Farmer
  - M. Franden
  - A. Griggs
  - S. Habas
  - W. Hjelm
  - D. Humbird
  - T. Johnston
  - E. Kuhn
  - J. Lischeske
  - W. Michener
- Staff (continued)
  - A. Mittal
  - L. Moens
  - A. Mohagheghi
  - H. Pilath
  - M. Resch
  - J. Shekiro
  - D. Sievers
  - D. Templeton
  - T. Vinzant
  - W. Wang
  - J. Wolfe

Funding

- US DOE EERE BioEnergy Technology Office

Additional Slides

Previous Reviewers Comments

2011 Biochemical Platform Peer Review

Comment	Response
Comprehension of all parts of the project at once is difficult. Periodic economic evaluations of contemplated process additions might provide a framework for this. The milestones are well defined however no risk mitigation was addressed.	We attempt to include understanding of the process implications of pretreatment approaches and conditions across relevant downstream conversion elements. This clearly includes the impact on enzymatic hydrolysis, including digestibility of cellulose and any remaining insoluble xylan and/oligomeric soluble xylose after pretreatment. Not so directly, it also includes understanding the impact of pretreatment upon fermentation of released sugars, especially as related to the presence of compounds solubilized from biomass during pretreatment and enzymatic hydrolysis, as well as generated via undesirable sugar degradation reactions during pretreatment. All of these factors are well understood in terms of their impact on overall process economics, which is routinely evaluated via annual State of Technology updates as well as by specific technoeconomic impact studies that are incorporated into many task milestones. These process economic evaluations are a key risk mitigation strategy to focus efforts on the most economically important factors.
This research has a long history in trying to understand dilute acid-enzyme hydrolysis kinetics and increase in sugar yields. It seems that NREL has for years been doing the same thing. That being said, however, it's clear that the difference in the latest research is that they are doing the work at larger scale which brings new equipment changes and different technical challenges.	In regard to the continued use of dilute-acid pretreatment as the "standard" pretreatment method and the incremental progress related to that technology. We agree that our R&D plan is focused on incremental improvements, especially for xylose yields, as much is already well understood about dilute acid pretreatment mechanisms and reaction kinetics. The reviewers correctly pointed out the challenge of translating these improvements to pilot-scale, continuous systems and the drive toward lower acid usage levels by the use of Fe salts and other approaches, which can help achieve these incremental improvements at reaction conditions that are better suited towards conditions that are more practical for implementation at this scale of operation.

Previous Reviewers Comments

2011 Biochemical Platform Peer Review

Comment	Response
Success will ultimately depend on the translation of this research in a scaled up demonstration and application. Consideration of water treatment and recycling could add to the challenges of process integration.	Reviewers noted that the application of much of the presented work in scale-up applications was a critical success factor, with a specific mention of water recycling/treatment. Such factors are specifically being addressed within the Biochemical Processing Integration Task, which was presented later in the review meeting. We strive for well-integrated activities across this task and the Biochemical Processing Integration Task, as that becomes the ultimate proving ground of process concepts in a truly integrated process.

Publications and Presentations: FY2011-13

Publications

Kamireddy, S. R.; Li, J.; Tucker, M.; Degenstein, J.; Ji, (2013). Effects and Mechanism of Metal Chloride Salts on Pretreatment and Enzymatic Digestibility of Corn Stover. Industrial and Engineering Chemistry Research. Vol. 52(5), 6 February 2013; pp. 1775-1782; NREL Report No. JA-5100-58138. http://dx.doi.org/10.1021/ie3019609

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Chen, X.; Tao, L.; Shekiro, J.; Mohagheghi, A.; Decker, S.; Wang, W.; Smith, H.; Park, S.; Himmel, M. E.; Tucker, M. (2012). Improved Ethanol Yield and Reduced Minimum Ethanol Selling Price (MESP) by Modifying Low Severity Dilute Acid Pretreatment with Deacetylation and Mechanical Refining: 1) Experimental. Biotechnology for Biofuels. Vol. 5, August 2012; 10 pp.; NREL Report No. JA-5100-55366. http://dx.doi.org/10.1186/1754-6834-5-60

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Publications and Presentations: FY2011-13

Publications (continued)

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Publications and Presentations: FY2011-13

Publications (continued)

Dibble, C. J.; Shatova, T. A.; Jorgenson, J. L.; Stickel, J. J. (2011). Particle Morphology Characterization and Manipulation in Biomass Slurries and the Effect on Rheological Properties and Enzymatic Conversion. Biotechnology Progress. Vol. 27(6), November/December 2011; pp. 1751-1759; NREL Report No. JA-5100-51065. http://dx.doi.org/10.1002/btpr.669

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Publications and Presentations: FY2011-13

Publications (continued)

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Helm, R. F.; Jervis, J.; Ray, W. K.; Willoughby, N.; Irvin, B.; Hastie, J.; Schell, D. J.; Nagle, N. (2010). Mass Spectral Analyses of Corn Stover Prehydrolysates to Assess Conditioning Processes. Journal of Agricultural and Food Chemistry. Vol. 58(24), 22 December 2010; pp. 12642-12649; NREL Report No. JA-5100-51595. http://dx.doi.org/10.1021/jf1031197

Zhu Y., Malten M., Torry-Smith M., McMillan J.D., Stickel J.J. (2010). Calculating sugar yields in high solids hydrolysis of biomass. Bioresour. Technol., 102:2897–2903.

Numerous oral and poster presentations have been made at ACS and AIChE Annual Meetings, annual Symposia on Biotechnology for Fuels and Chemicals, and annual meetings of the Society of Rheology.

Supplementary Slides

Milestone Schedule for Pretreatment and Enzyme Hydrolysis Task

FY 2012

Type	Title	Due Date
E	Identify gene targets for improved resistance to acetate and furfural	10/31/11
D	Select overall pretreatment configuration for 2012 integrated biochemical conversion of corn stover to ethanol, including technoeconomic considerations of any pre-processing and post-processing steps being used to achieve 2012 pretreatment and enzymatic hydrolysis yield targets.	12/31/11
D	Implement coupled computational fluid dynamics and kinetics simulations for enzymatic hydrolysis at moderate solids loadings and validate against appropriate experimental data.	03/31/12
D	Parametric study using continuous, pilot-scale pretreatment reactor system on corn stover to determine operating conditions that achieve overall process yields of 90% of monomeric xylose with ≤5% conversion to furfural	05/31/12
E	Determine pore-size distribution of milled and pretreated biomass (likely corn stover and pine). Determine convective and diffusive transport of solutes (salts, sugars, and macromolecules) in packed beds of the biomass.	06/30/12
E	Produce at least 50 kg (dry) of pretreated un-deacetylated and deacetylated corn stover feedstocks in a continuous pretreatment reactor for commercial scale disk refining. Determine pretreatment and enzymatic digestibility yields, feedstock reactivities, and refining energies at various refining conditions.	06/30/12
E	Identify limit products which act as barriers to achieving 90% glucan conversion and 80% xylan conversion in 15-30% total solids pretreated corn stover digestions.	07/15/12
E	Knockout and overexpression of furfural toxicity related genes in Zymomonas mobilis strains and evaluate their impact on furfural toxicity.	07/15/12
D/E	Achieve 50% conversion of 4-O-methyl-alpha glucuronic acid-substituted xylooligomers to monomeric xylose from relevant pretreatment liquid	08/31/12
D/E	Evaluate impact of candidate genes related to acetate and furfural toxicity contribution to xylose fermentation of Zymomonas mobilis strains	09/30/12

FY 2013

Type	Title	Due Date
D	Develop membrane-based water removal (sugar concentrating) process technology	02/28/13
E	Report kinetics of depolymerization and decarboxylation reactions involved in conversion of polyhydroxybutyrate to propene	03/31/13
E	Evaluate various mathematical modeling approaches for dilute-acid pretreatment of lignocellulosic biomass and select one that includes the most relevant chemical and physical mechanisms	03/31/13
E	Assess the possibility of utilizing acetate in hydrolysate/slurry along with sugars or pre-hydrolysate liquor from biomass deacetylation with hydrocarbon producing organisms	03/31/13
E	Demonstrate experimentally a new pathway from a furfuraldehyde to a liquid hydrocarbon with a chain length of C9 or higher	06/30/13
E	Develop experimental and data analysis methods for routinely measuring the rheological properties of biomass slurries by means of large amplitude shear rheometry	06/30/13
E	Investigate various co-catalysts in hydrothermal, dilute acid, and alkali pretreatment for the production of furfural/HMF for upgrading to C-10 to C-20 hydrocarbons	07/31/13
D	Scale up promising biomass hydrolysis/deconstruction technology to a continuous reactor to produce intermediates for supply to the Chemical Transformation subtask and other stakeholders for conversion to hydrocarbon fuel molecules.	08/31/13
D	Demonstrate production of a hydrocarbon fuel intermediate from a process relevant lignocellulosic derived sugar stream	09/30/13
D	Utilize existing software and develop additional computational tools to enable full-scale simulation of a mixing and reacting enzymatic hydrolysis reactor	09/30/13
D	Identify inhibitors and their impact on model hydrocarbon fuel and/or intermediate-producing microorganisms in hydrolysate liquor and/or saccharified slurry from dilute acid pretreatment	09/30/13
D	Develop a method for quantification of organic inhibitors to catalytic conversion present in dilute acid pretreatment liquors	09/30/13

Development of Pretreatment Process

Lower cost of producing sugars

- Lower reactor costs
  - Decreased pretreatment severity and temperature
- Lower chemical usage and costs
  - Decreased sulfuric acid and NH_4OH usage
- Decrease inhibitors in fermentation
  - Deacetylation decreased acetate in fermentation
  - Low severity pretreatments lowered salts, furfural, HMF concentration
- Mechanical refining improves enzymatic digestibility of low severity pretreatments
  - Bench scale: PFI mill, blender, disk refining, twin screw extruding
  - Industrial scale: Andritz 36-inch disk refiner, Szego mill
- Recover furfural and acetate in reactor flash for downstream conversion
- Developed new process of deacetylation of corn stover followed by mechanical refining for high enzymatic digestibility WITHOUT pretreatment
  - Sugar yields 80% although with slightly higher enzyme dosage

Develop processes for low cost reactive lignin

- Low severity pretreatment lessen lignin condensation
- Deacetylation provides source of acetate and soluble lignin

Support of FY12 SOT Demonstration

Biochemical Platform Conversion Targets

	2007	2008	2009	2010	2011	*2012 Target*	*2012 SOT*
Minimum Ethanol Selling Price ($/gal)	**$3.64**	**$3.57**	**$3.18**	**$2.77**	**$2.56**	***$2.15***	***$2.15***
Feedstock Contribution ($/gal)	$1.12	$1.04	$0.95	$0.82	$0.76	*$0.74*	*$0.83*
Conversion Contribution ($/gal)	$2.52	$2.52	$2.24	$1.95	$1.80	$1.41	*$1.32*
Yield (Gallon/dry ton)	69	70	73	75	78	*79*	*71*
Feedstock							
Feedstock Cost ($/dry ton)	$77.20	$72.90	$69.65	$61.30	$59.60	*$58.50*	*$58.50*
Pretreatment							
Solids Loading (wt%)	30%	30%	30%	30%	30%	*30%*	*30%*
Xylan to Xylose (including enzymatic)	75%	75%	84%	85%	88%	*90%*	*81%*
Xylan to Degradation Products	13%	11%	6%	8%	5%	*5%*	*5%*
Conditioning							
Ammonia Loading (g per L hydrolysate liquor)	12.9	12.9	9.8	4.8	3.8	*4.8*	*1.6*
Hydrolysate solid-liquid separation	Yes	Yes	Yes	Yes	Yes	*No*	*No*
Xylose Sugar Loss	2%	2%	2%	2%	1%	*1%*	*0%*
Glucose Sugar Loss	1%	1%	1%	1%	1%	*0%*	*0%*
Enzymes							
Enzyme Contribution ($/gal EtOH)	$0.39	$0.38	$0.36	$0.36	$0.34	*$0.34*	*$0.36*
Enzymatic Hydrolysis & Fermentation							
Total Solids Loading (wt%)	20%	20%	20%	17.5%	17.5%	*20%*	*20%*
Saccharification Mode	Washed-solids	Washed-solids	Washed-solids	Washed-solids	Washed-solids	*Whole-slurry*	*Whole-slurry*
Combined Sacchrifcatn & Fermentatn Time (d)	7	7	7	5	5	*5*	*5*
Corn Steep Liquor Loading (wt%)	1%	1%	1%	1%	0.25%	*0.25%*	*0.25%*
Overall Cellulose to Ethanol	86%	86%	84%	86%	89%	*86%*	*74%*
Xylose to Ethanol	76%	80%	82%	79%	85%	*85%*	*93%*
Arabinose to Ethanol	0%	0%	51%	68%	47%	*85%*	*54%*

Feedstock Deacetylation and Pretreatment Scale-Up

- Lower-severity pretreatment conditions (in conjunction with feedstock deacetylation) were demonstrated in continuous pilot scale reactors
- Higher enzymatic digestibilities of pretreated corn stover and ethanol yields from xylose enabled cost target achievement
- Strong collaboration with Biochemical Processing Interface Task enabled scale-up of deacetylation and pretreatment unit operations for the 2012 cellulosic ethanol demonstration runs in the 1 ton/day continuous pretreatment reactor

200 kg/day Continuous Horizontal Reactor

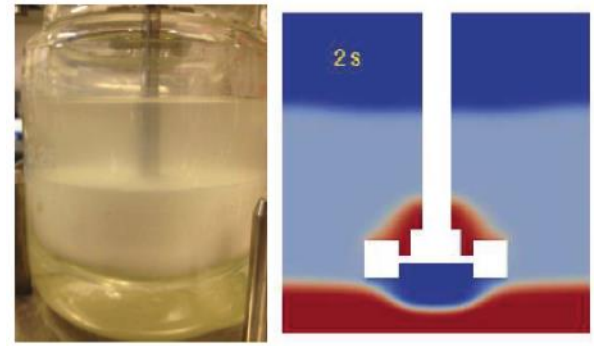
1 ton/day Continuous Horizontal Reactor

Effect of Inhibitor Removal on Ethanol Production

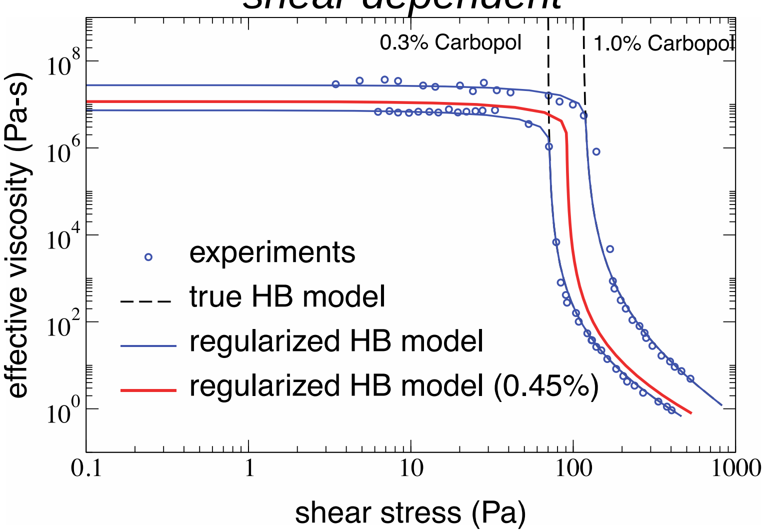
- Deacetylation with lower pretreatment temperature/acid loading allows for more complete xylose utilization and higher final ethanol titers of ~70 g/L (at 19.2% TS loading)

Computational Fluid Dynamics of Biomass Slurries

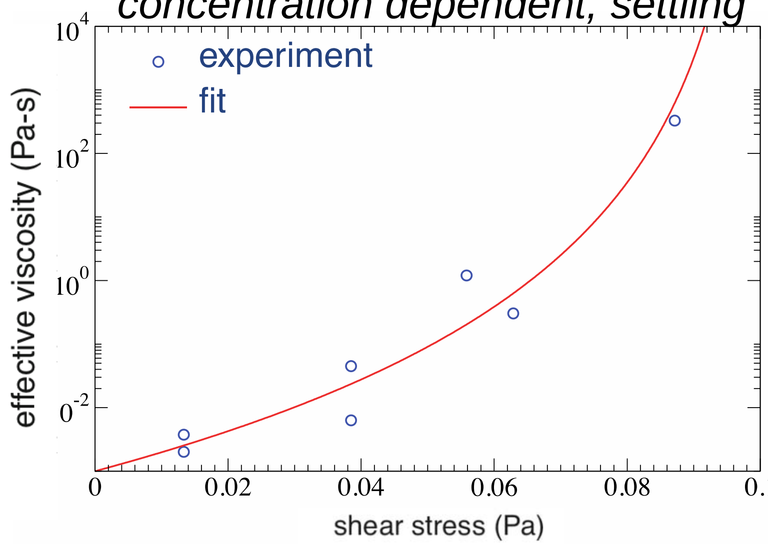
- Two CFD approaches to predict rheological behavior of enzymatically hydrolyzing biomass slurries in scaled biorefinery equipment
- Enzymatic hydrolysis reactors, fermenters, pumps, pipelines, and conveyors are some applications



<u>Yield-Stress Model</u>
for high solids concentration:
shear dependent



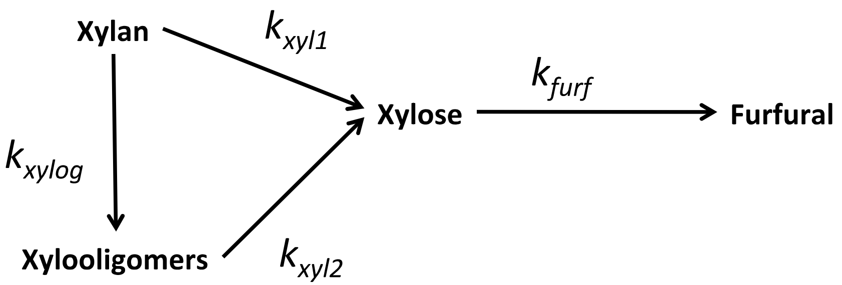
<u>Viscosity Model</u>
for low solids concentration:
concentration dependent, settling



FY12 D-Milestone, “Implement coupled computational fluid dynamics and reaction kinetics simulations for the enzymatic hydrolysis of cellulose at dilute to moderate solids concentrations and validate against appropriate experimental data. ”

Multi-Physics Pretreatment Model

- Developing a model coupling mass transfer, heat transfer, phase transition, and reaction kinetics
- Literature indicates no other existing pretreatment model includes all these phenomena in one model



Model	Mass Transfer	Chemical Reaction	Heat Transfer	Phase Transition
Kazi et al. (1997)	✓(D)	×	×	×
Kim and Lee (2002)	✓(D)	×	×	×
Jacobson and Banerjee (2006)	✓(D)	×	×	×
Gustafson et al. (1983)	✓(D)	✓	×	×
Costanza et al. (2001)	✓(D)	✓	×	×
Mittal et al. (2009)	✓(C)	✓	×	×
Griggs and Kuhn (2011)	✓(C)	✓	×	×
Abasaeed et al. (1991)	×	✓	✓	×
Xu et al. (2010)	✓(C)	×	✓	✓

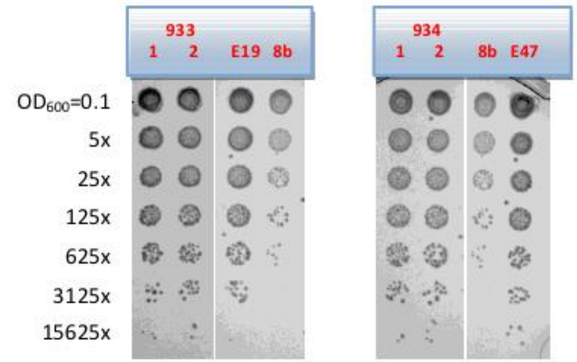
FY13 E-Milestone, “Evaluate various mathematical modeling approaches for dilute-acid pretreatment of lignocellulosic biomass and select one that includes the most relevant chemical and physical mechanisms.”

Improving Tolerance to Inhibitors

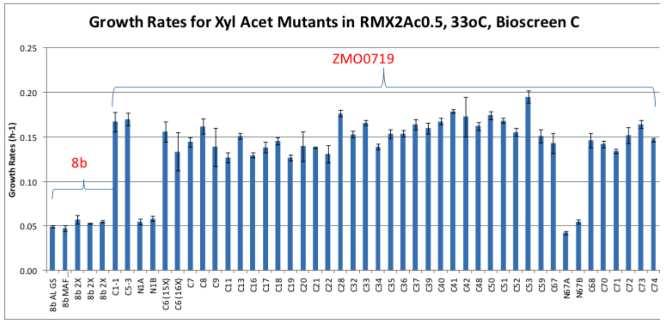
Genes related to toxicity in *Zymomonas mobilis*

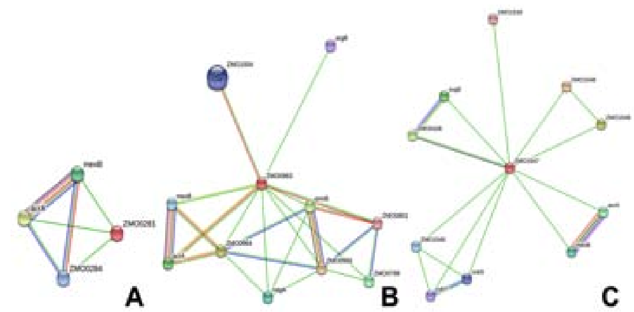
- Identified and tested top candidate genes for resistance to furfural and acetate through both screening and selection of mutant library, and transcriptomics study
  - 3 genes conferring advantages over parental strains to acetate tolerance using xylose substrate and 6 genes related to improved furfural resistance
- Developed and improved synthetic genomics tools: NGS, RNA-seq, knockin-knockout gene testing methods and regulatory promoter development.
- Evaluated hydrolysate toxicity to support Xylose Yield subtask’s D-milestone
  - Discovered new inhibitors in the hydrolysate - work in progress for confirmation.

Furfural Tolerant Mutants



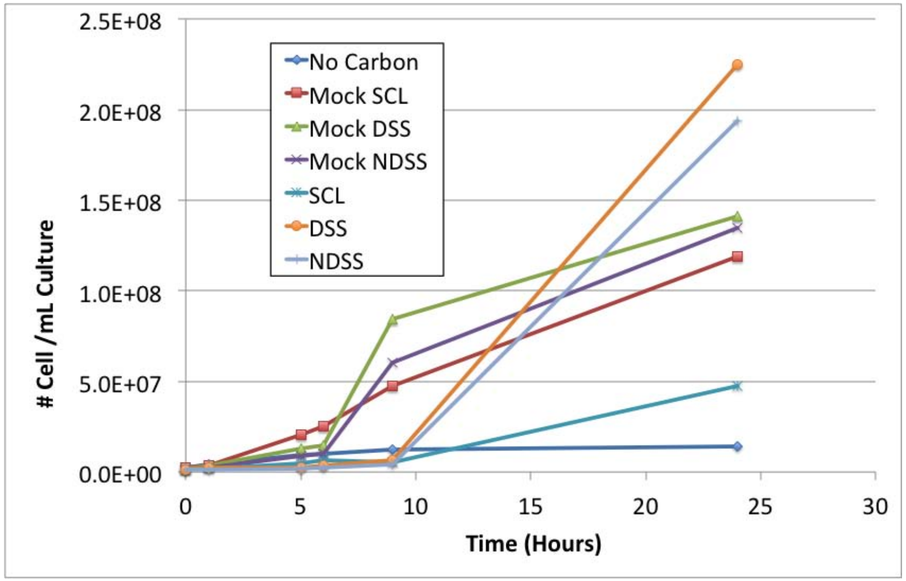
3 g/L Furfural RMG Plate

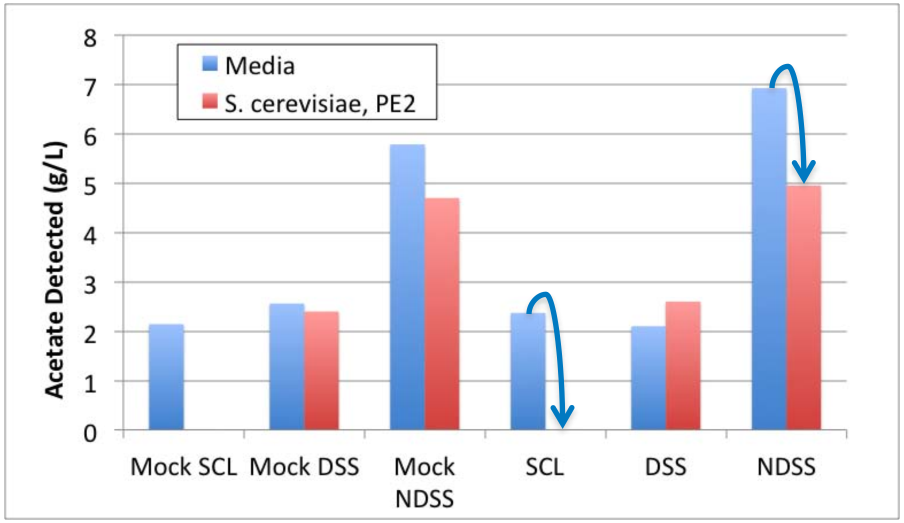




Acetate Utilization

- Assess possibility of utilizing biomass deacetylation liquor with acetate-assimilating hydrocarbon producing organisms
  - Test 8 microbial strains capable of producing hydrocarbon via variety of pathways
  - Evaluate growth in acetate w/ or w/o sugars and in spent caustic liquor and saccharified slurries
  - *Saccharomyces cerevisiae* PE-2 identified as most robust strain
    - Growth and acetate utilization in medium supplemented with either liquor or slurry.
- Oleaginous yeast and algae also show promise for acetate utilization

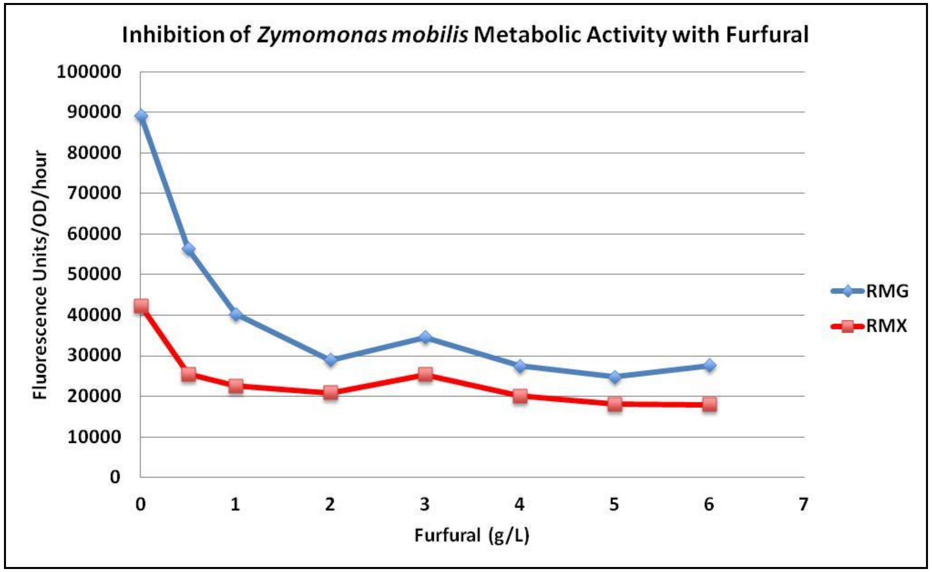


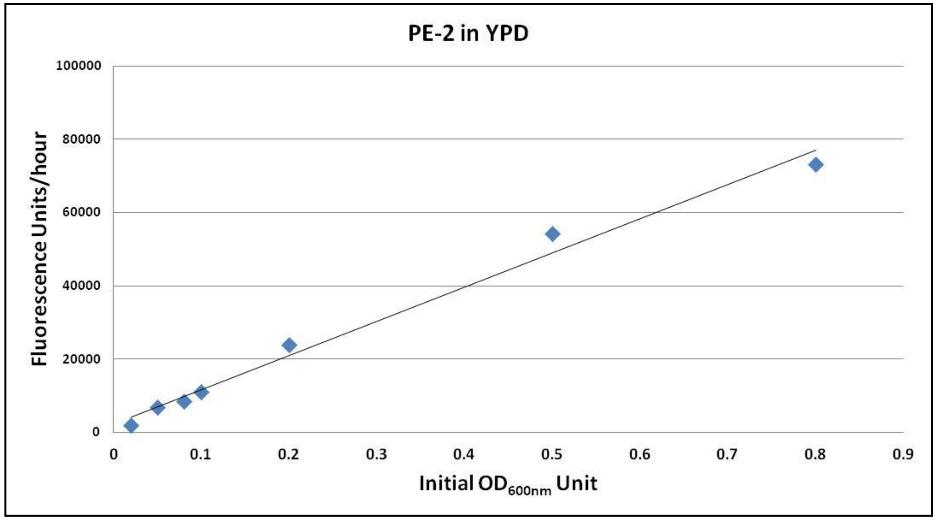


2013 E-Milestone. Assess the possibility of utilizing acetate in hydrolysate/slurry along with sugars or pre-hydrolysate liquor from biomass deacetylation with hydrocarbon producing organisms

Inhibitor Identification

- Identify inhibitors and their impact on model hydrocarbon fuel and/or intermediate-producing microorganisms in hydrolysate liquor and/or saccharified slurry from dilute acid pretreatment
- Develop high throughput assay that allows rapid measurement of toxic response for yeast and bacteria, aerobes and anaerobes
- Identify the top three toxicity contributors for 3 model hydrocarbon fuel and/or intermediate-producing microorganisms in a process relevant hydrolyzate and/or saccharified slurry
- Development of Alamar Blue fluorescent microtitre plate assay is nearly complete





Furans to hydrocarbons

- Synthesis of furan oligomers to C10 – C20 units
- HDO of furan oligomers to C10 – C20 hydrocarbons (gasoline, diesel, jet fuel)
- Develop fuel pathways using furans from dilute acid pretreatment flash condensate
- Develop improved HDO catalysts
  - High activity (bimetallic nanocatalysts)
  - Hydrothermally stable
  - Acid-resistant (e.g. carboxylic acids)