2013 DOE Bioenergy Technologies Office (BETO) Project Peer Review

LBNL PDU Support
Advanced Biofuels Process Demonstration Unit

The First Year

Date: May 22, 2013
Technology Area Review: Biochemical Conversion
WBS 2.3.1.6

Principal Investigator: Julio Baez
Organization: Lawrence Berkeley National Laboratory

Goal Statement

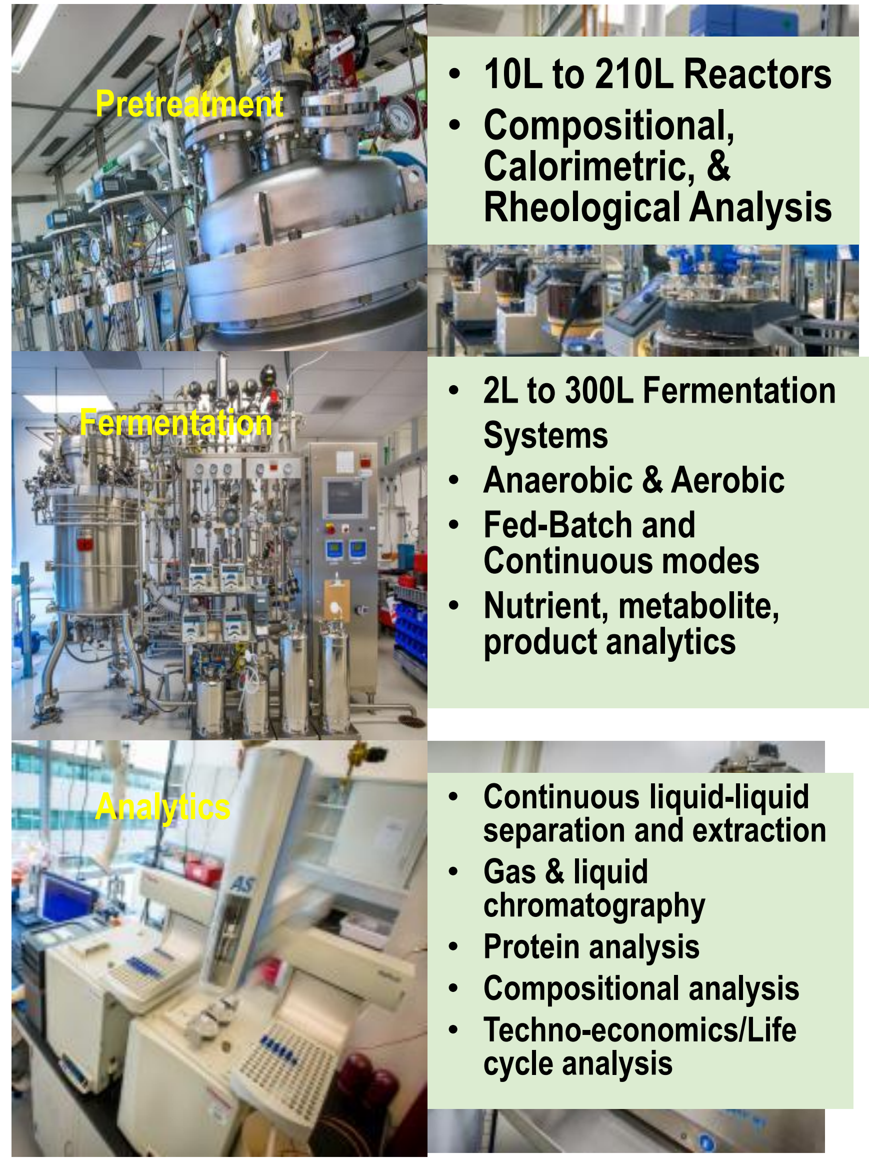
Established that the unit can enable researchers (public & private) to evaluate, adapt, develop, demonstrate, and transfer commercially viable processes for advanced biofuels and biochemical production from grasses, wood, gases, and agricultural/industrial/municipal waste leading to efficient biorefineries.

UNIT OPERATIONS

- Pretreatment
- Depolymerization
- Fermentation
- Recovery
- Analytics

ABPDU Core Competencies

- Feedstock Flexibility using lignocellulosics, cellulosics,algae, gas
- Up-to-date automated data acquisition, process control systems, and analysis of process performance
- Diverse technologies integrated for process demonstration, process validation, and Techno-economic/Life Cycle analysis
- Projects designed to generate intellectual Property and helping industrial partners commercialize technologies.
- Versatility incorporating equipment from sponsors and collaborators into end-to-end process integration



Quad Chart Overview

Timeline

- Facility funded in 2010 and functional in 2012
- Initial projects completed in Mar 2013
- Currently working in 3 DOE projects and considering 15 potential project for DOE labs and the biofuel industry

Barriers

- Facility/Equipment Start-up
- Staffing and training
- Identifying clients and projects

Budget

- Funding for FY11 = 1.2M
- Funding for FY12 = 3.0M
- Funding for FY13 = 1.0M

100% DOE funding

FY10 = $17.7M from the American Recovery and Reinvestment Act funds

Partners

- JBEI ionic liquid pretreatment
- JBEI advance biofuel production
- GLBRC cellulase production
- GLBRC alkaline pretreatment
- INL biomass processing
- Over 10 companies for biomass pretreatment and biofuel production

Project Overview

- First year projects from JBEI demonstrated capability of all the unit operations as illustrated by successful technology transfer and demonstration of:
  - Use of ionic liquids for deconstruction of lignocellulosic biomass
  - Saccharification of ionic liquid pretreated biomass
  - Production of ionic liquid tolerant thermophilic cellulases
  - Production of an advanced biofuel - bisabolene

1. Approach

- Technical discussions with clients to define the technology and objective of the program at the ABPDU
- Techno-economic/Life cycle analysis
- Proposal approval by DOE
- Technology transfer
- Technology adaptation and development as needed
- Technology demonstration
- Production of biological reagents as needed
- Use of SOPs, Batch Records and reports shared with clients
- Development of innovative equipment, such as a proprietary reactor for biomass pretreatment
- Increased pipeline for business development
- Encouraging publications

2. Technical Accomplishments/ Progress

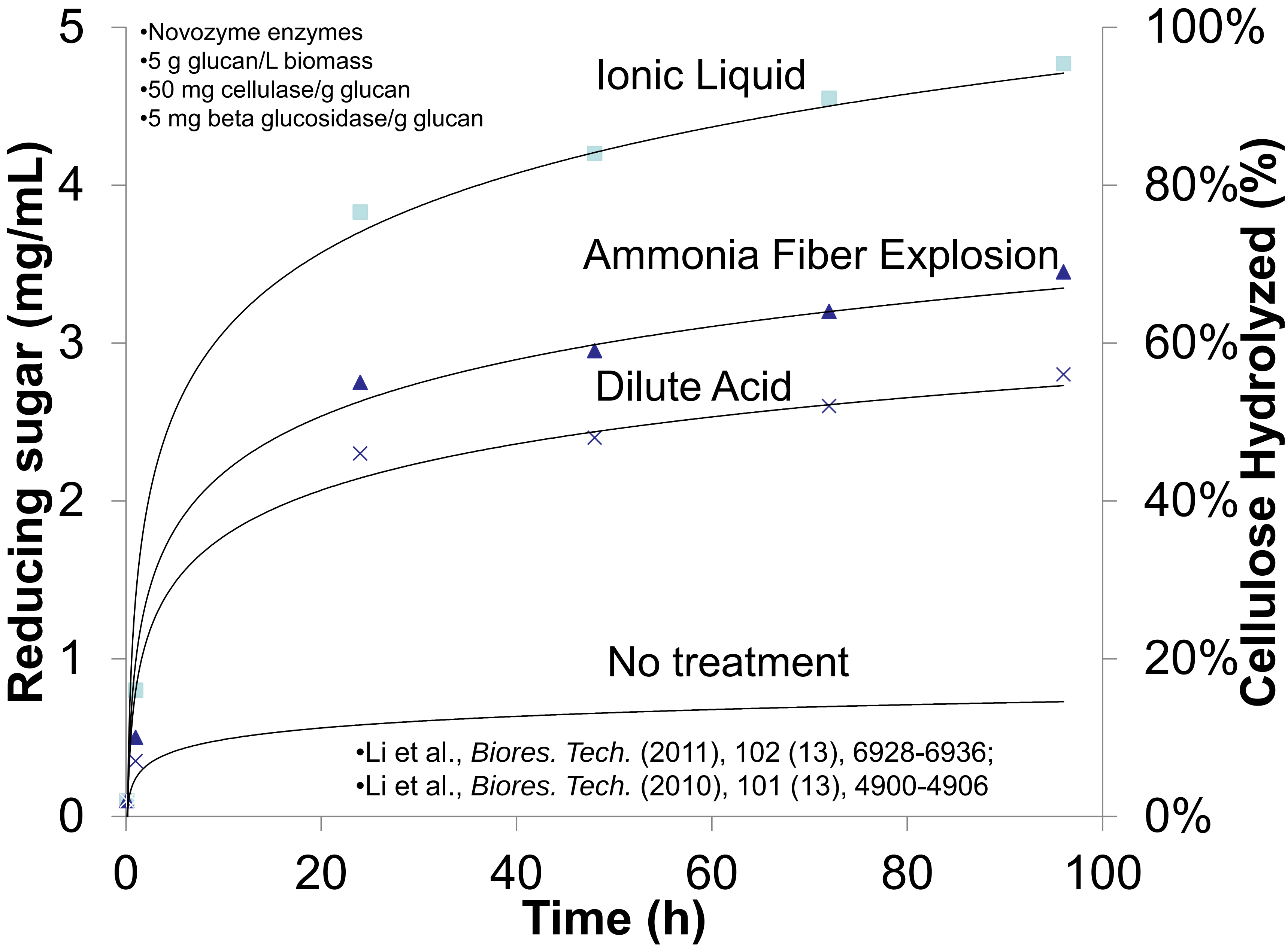
- Demonstration of ionic liquid (ionic liquid imidazolium chloride) pretreatment technology.

- Production of thermophilic cellulases tolerant to residual ionic liquids.

- Saccharification of ionic liquid treated biomass

- Technology transfer of advanced biofuel – bisabolene

Efficiency of the Ionic Liquid Treatment corn stover

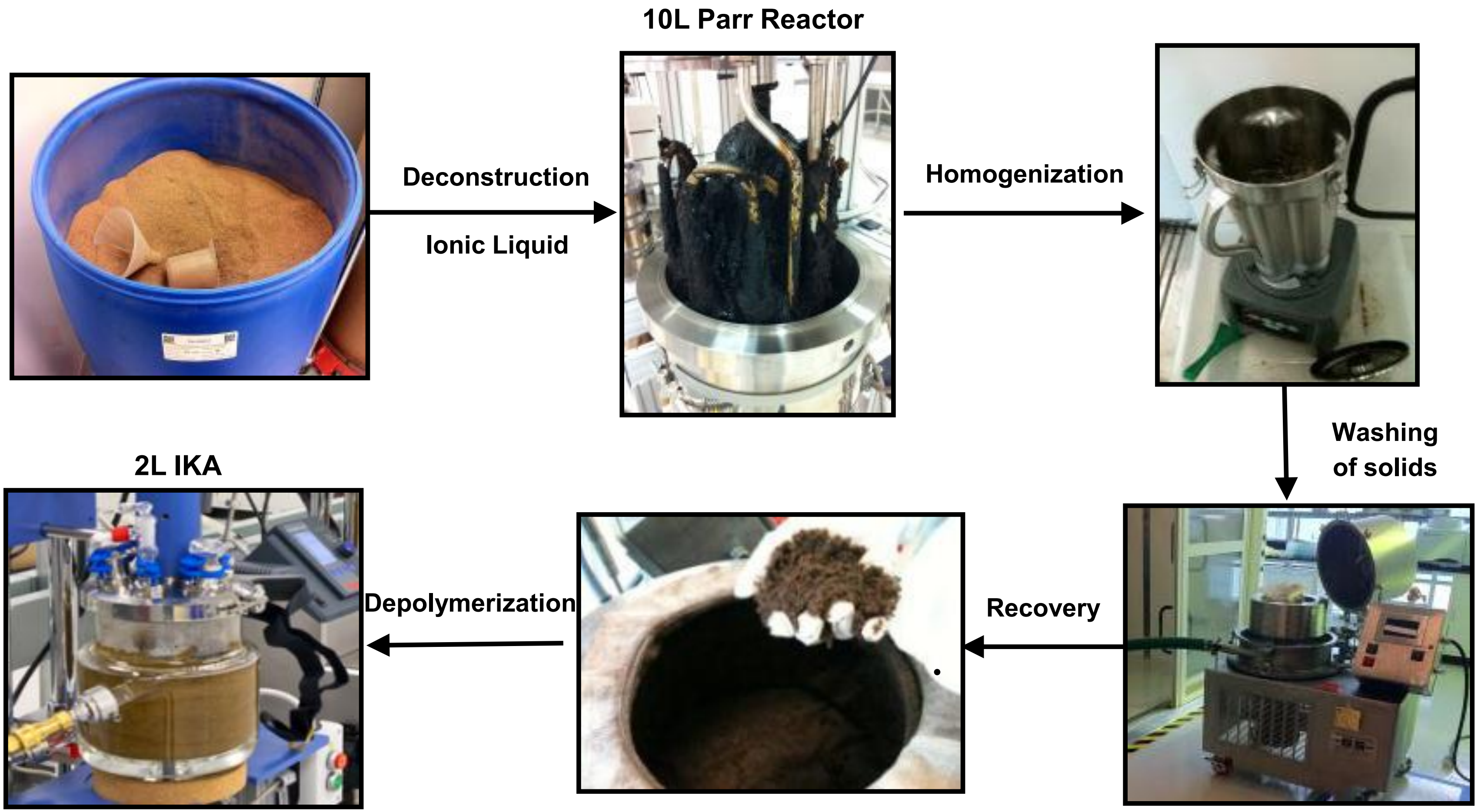


Ionic Liquid-based Biomass Pretreatment and Depolymerization

- Objective
  - ➢ Demonstrate Ionic Liquid deconstruction of various feedstocks
    - High solid loading from 10% to 15% (w/w)
    - Deconstruction 600X scale-up (0.010 to 6L *)
    - Depolymerization 25X scale-up (0.06 to 1.5L *)
- Process
  - ➢ Integration of unit operations for high titer sugar production
  - ➢ Optimization to minimize ionic liquid inhibition
  - ➢ Reduction of water usage
- Results
  - ➢ First scale up demonstration of ionic liquid-based biomass deconstruction process
  - ➢ Complete mass balance and energy flow of the process

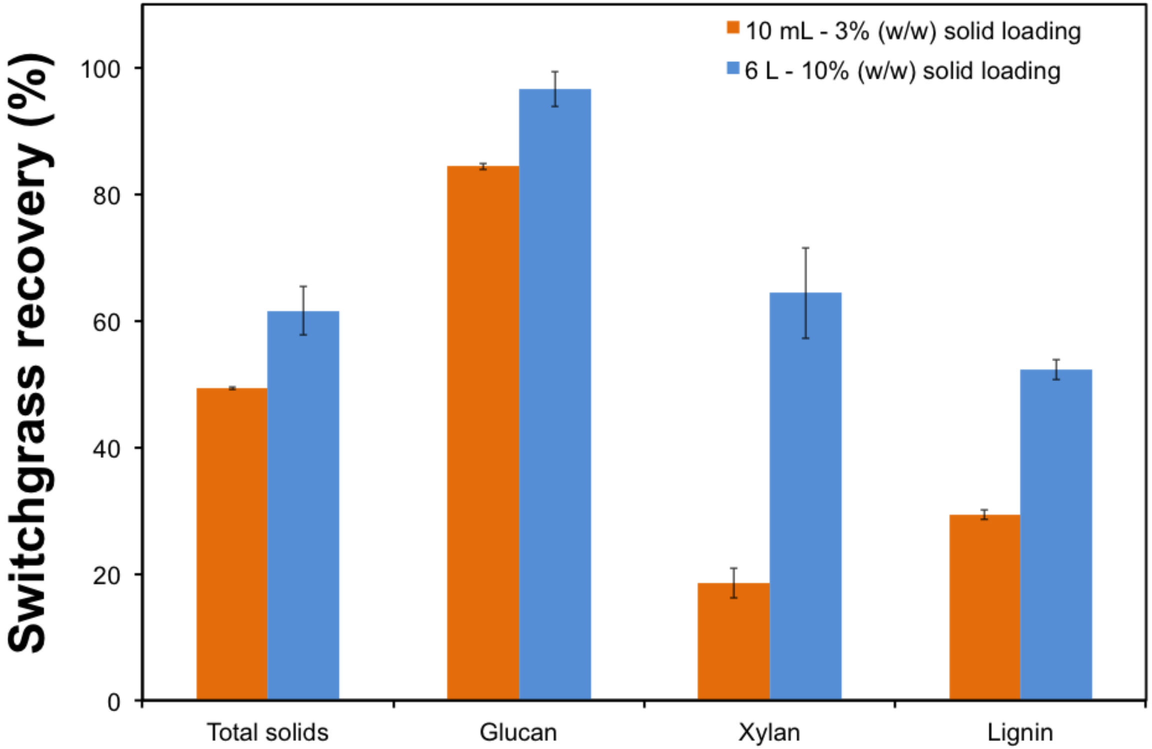
•* Working Volume

Ionic Liquid-based Deconstruction and Depolymerization



Product Recovery - Switchgrass

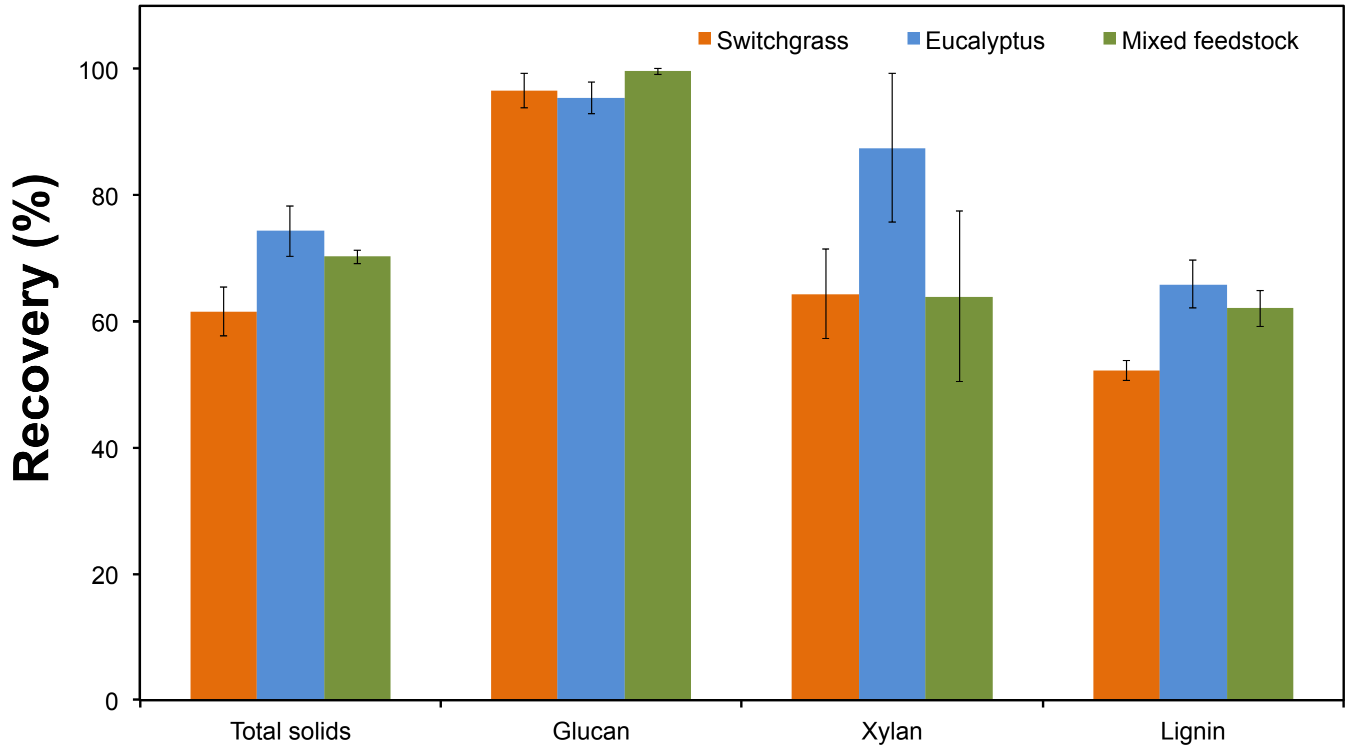
Post ionic liquid-based deconstruction



- Significant glucan enrichment upon scale up
- Higher xylan recovery

Product Recovery from Different Feedstocks

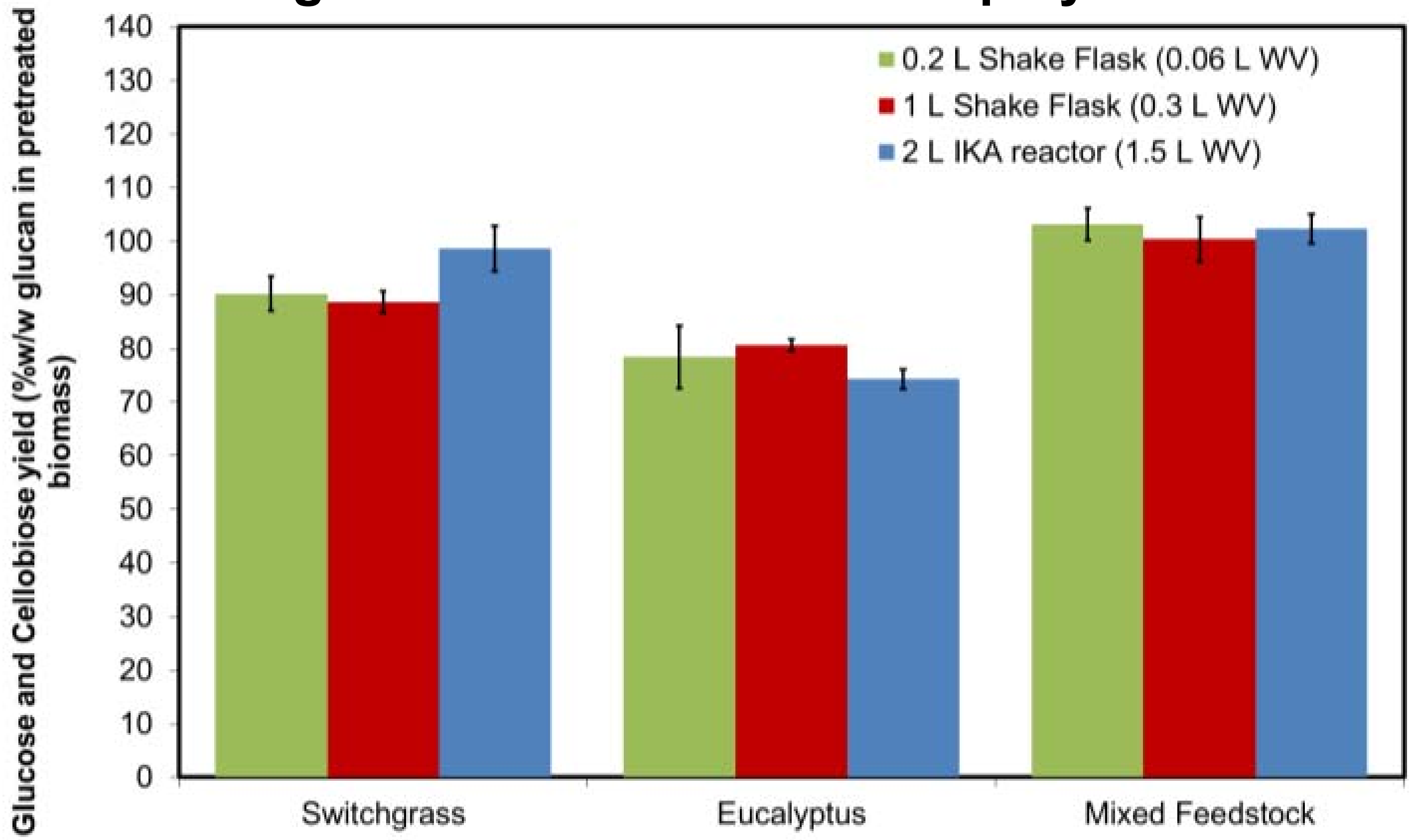
Post IL-based Deconstruction



- Significant glucan enrichment in all feedstocks
- High xylan recovery in Eucalyptus
- High lignin removal from Switchgrass

Glucan Yields from Different Feedstocks

Using IL-treated biomass for Depolymerization



*WV: Working Volume

- Glucose yields were similar or better with scale up from .06 L to 1.5L

Thermophilic Ionic Liquid-tolerant Cellulase Production

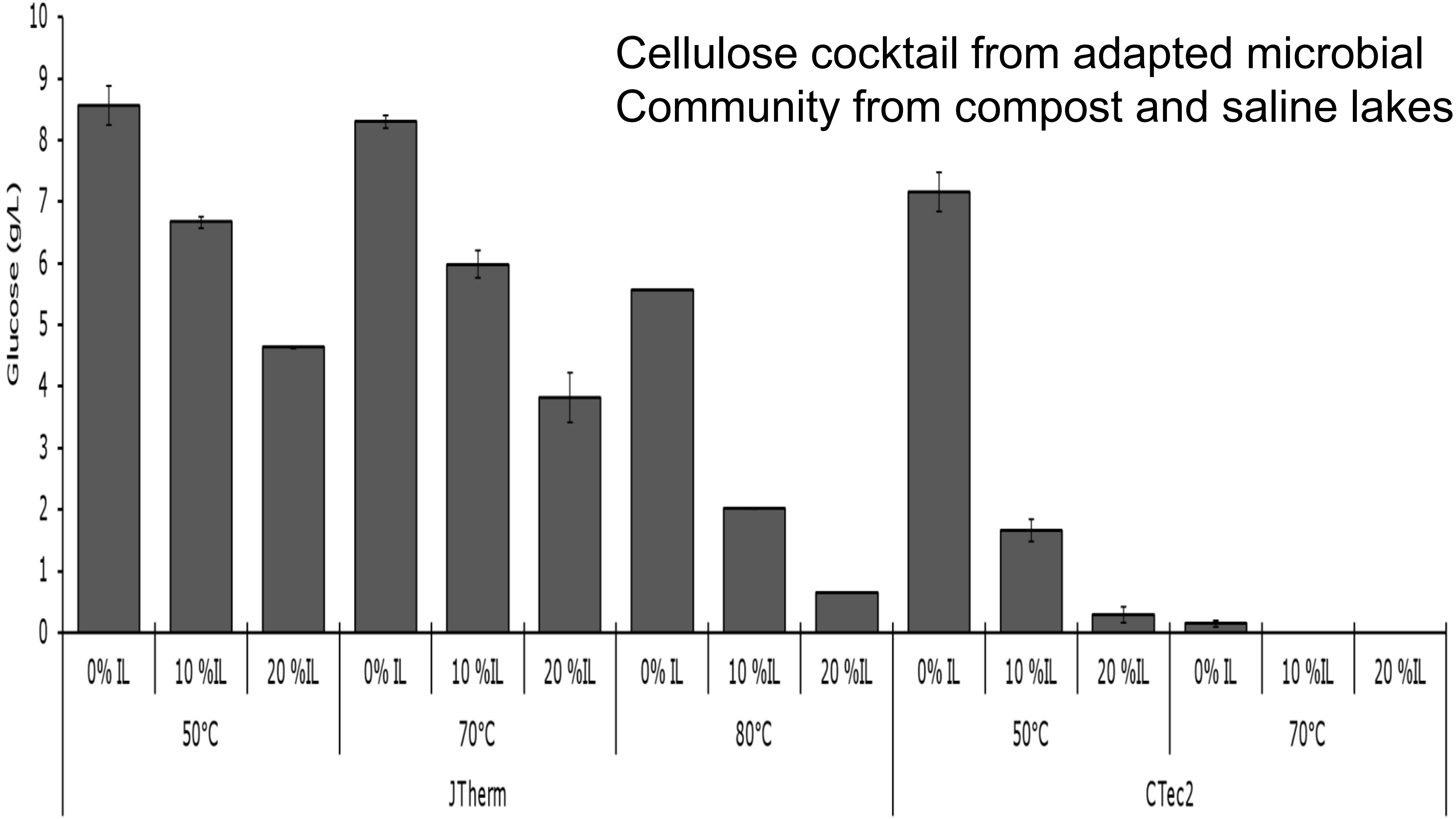
- Objective
  - ➢ Scale up of three component Jtherm Cellulase enzyme cocktail
    - Tolerates up to 20-30% ionic liquid
    - Temperatures up to 50-70° C
    - Thermophilic community 30X scale up (0.5 to 15L)
    - Exocellulase – cellobiohydrolase (CBH), Beta glucosidase 100X scale up (0.5 to 50L)

- Process
  - ➢ Fermentation
  - ➢ Continuous centrifugation
  - ➢ Tangential Flow Filtration
  - ➢ Enzyme separation and purification

- Results
  - ➢ Comparable enzyme activity upon scale up
  - ➢ Successfully recovered the necessary enzyme quantities

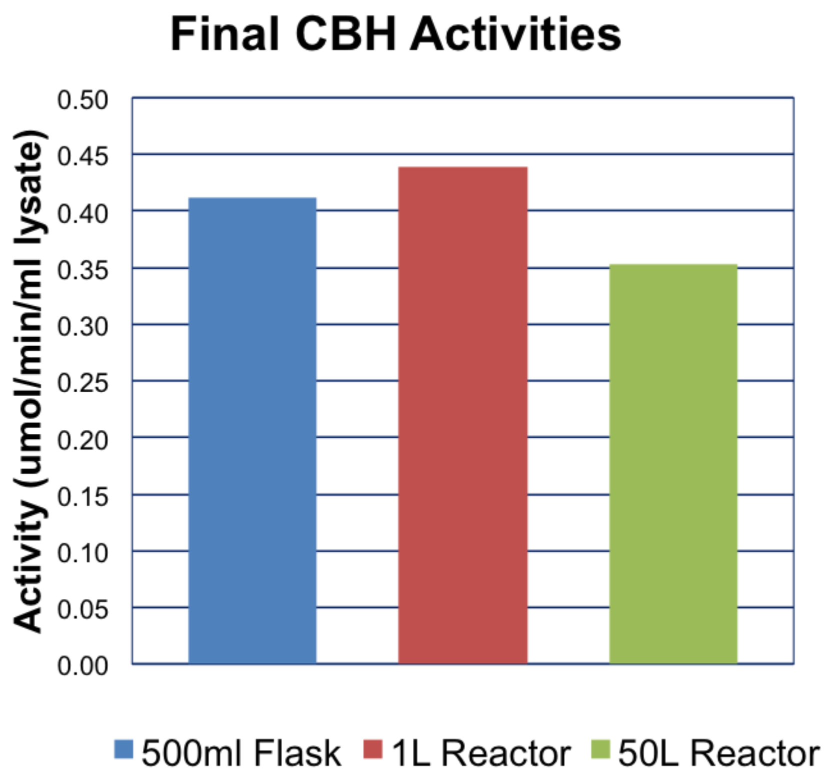
Thermophilic Ionic Liquid –Tolerant Cellulase Cocktail -JThem

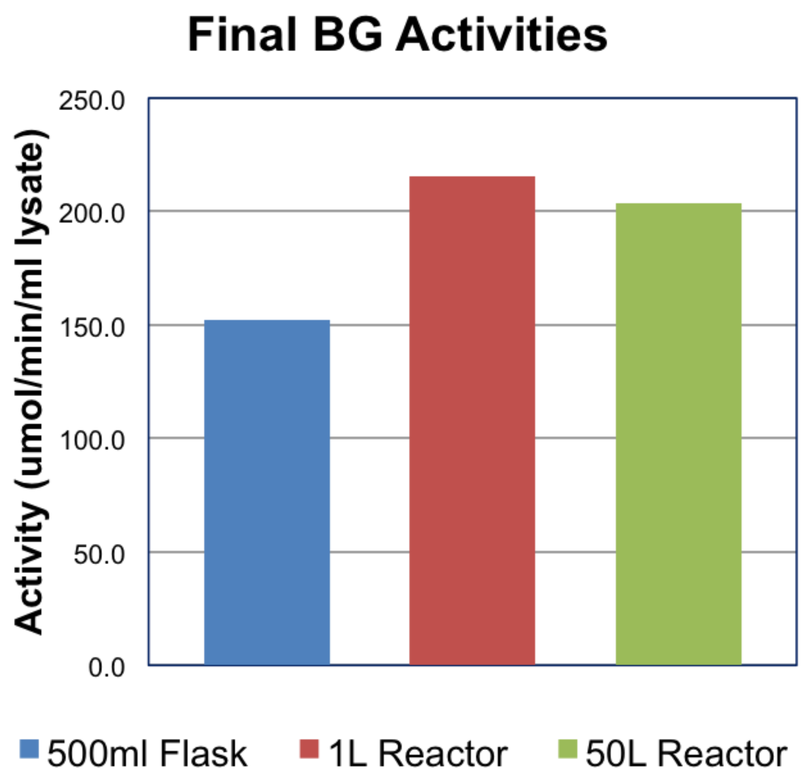
Cellulose cocktail from adapted microbial Community from compost and saline lakes



Switchgrass at 100 g/L, 120C for 3hm\, washed, added ionic liquid
Enzyme at 1 mg/g biomass for JTherm, 25 mg CTec2/g biomass

50L CBH and BG Production Results





- 100X scale up with similar enzyme activity
- Recovered sufficient quantities of all enzyme components upon scale up

Techno-Economic for Saccharification

Develop models for biofuel/biochemical process technologies

- Feedstock generation and handling
- Deconstruction and Depolymerization
- Fermentation
- Product and cell recovery
- Waste water chemical treatment, solvent recycling
- Microbial inactivation

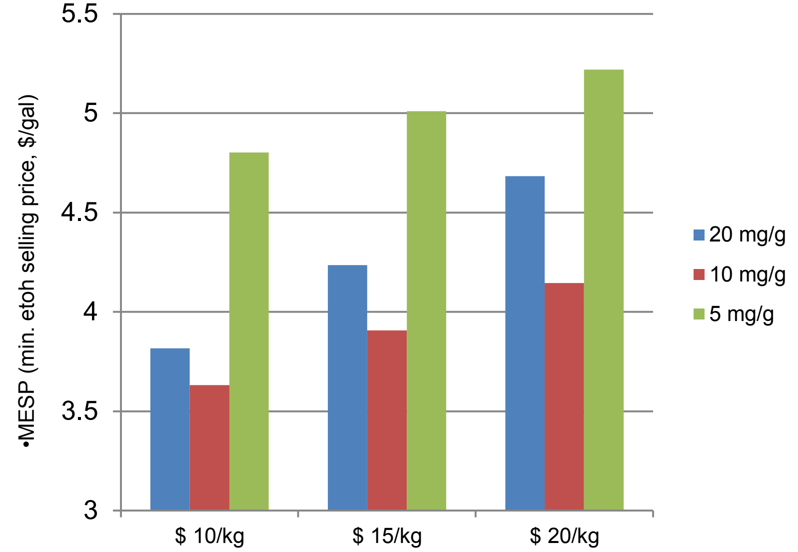
Capabilities

- Process design
- Mass and energy balance
- Cost estimations
- Sensitivity analysis
- Profitability analysis
- Risk analysis

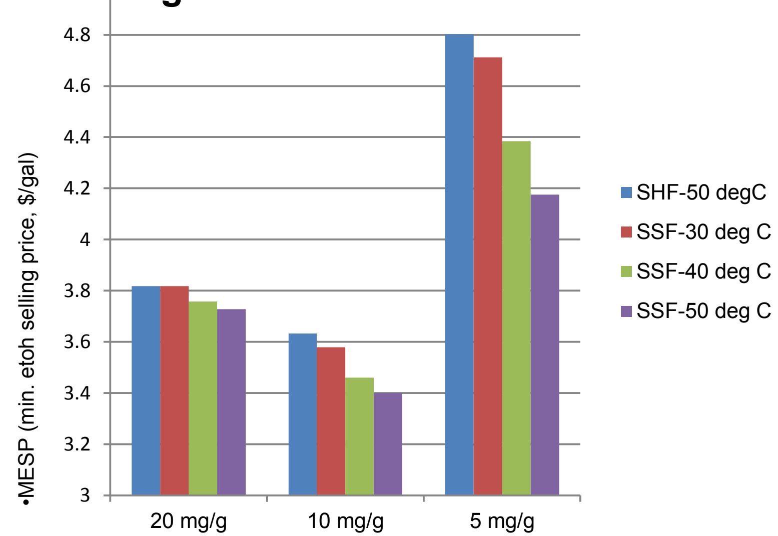
Software

- SuperPro Designer
- GREET
- MATLAB

Effect of Enzyme cost at various enzyme loadings



Effect of Fermentation Mode & Temperature at various enzyme loadings

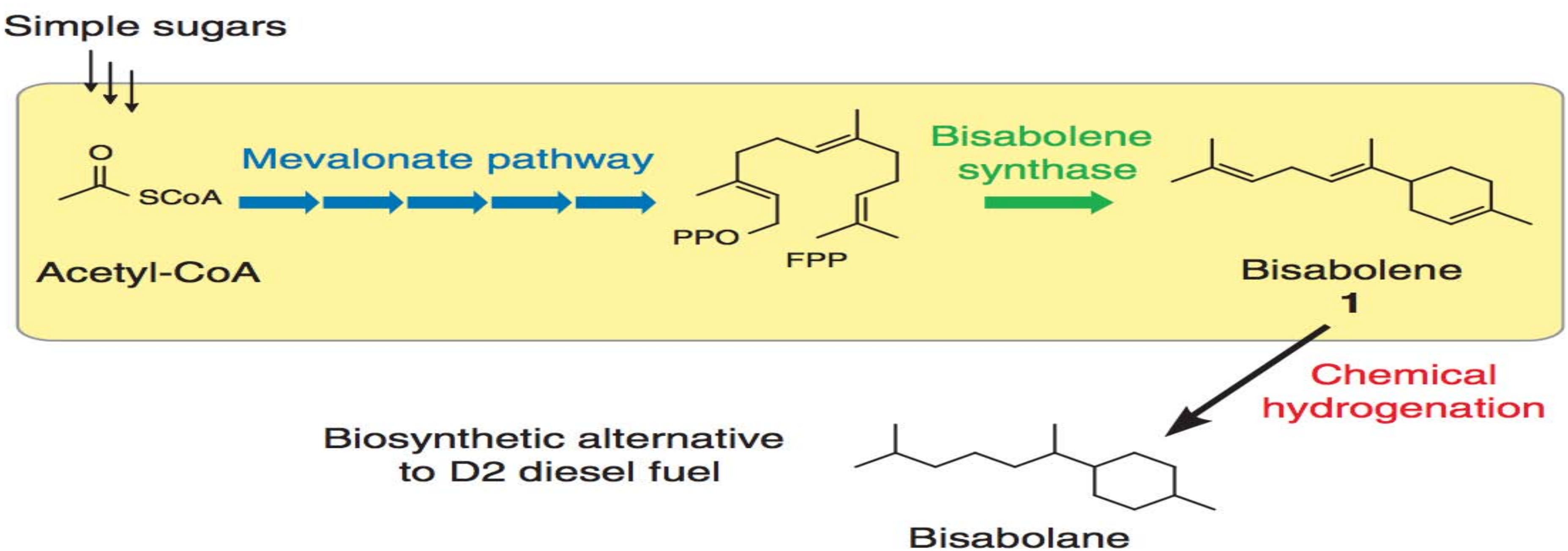


BISABOLENE FERMENTATION

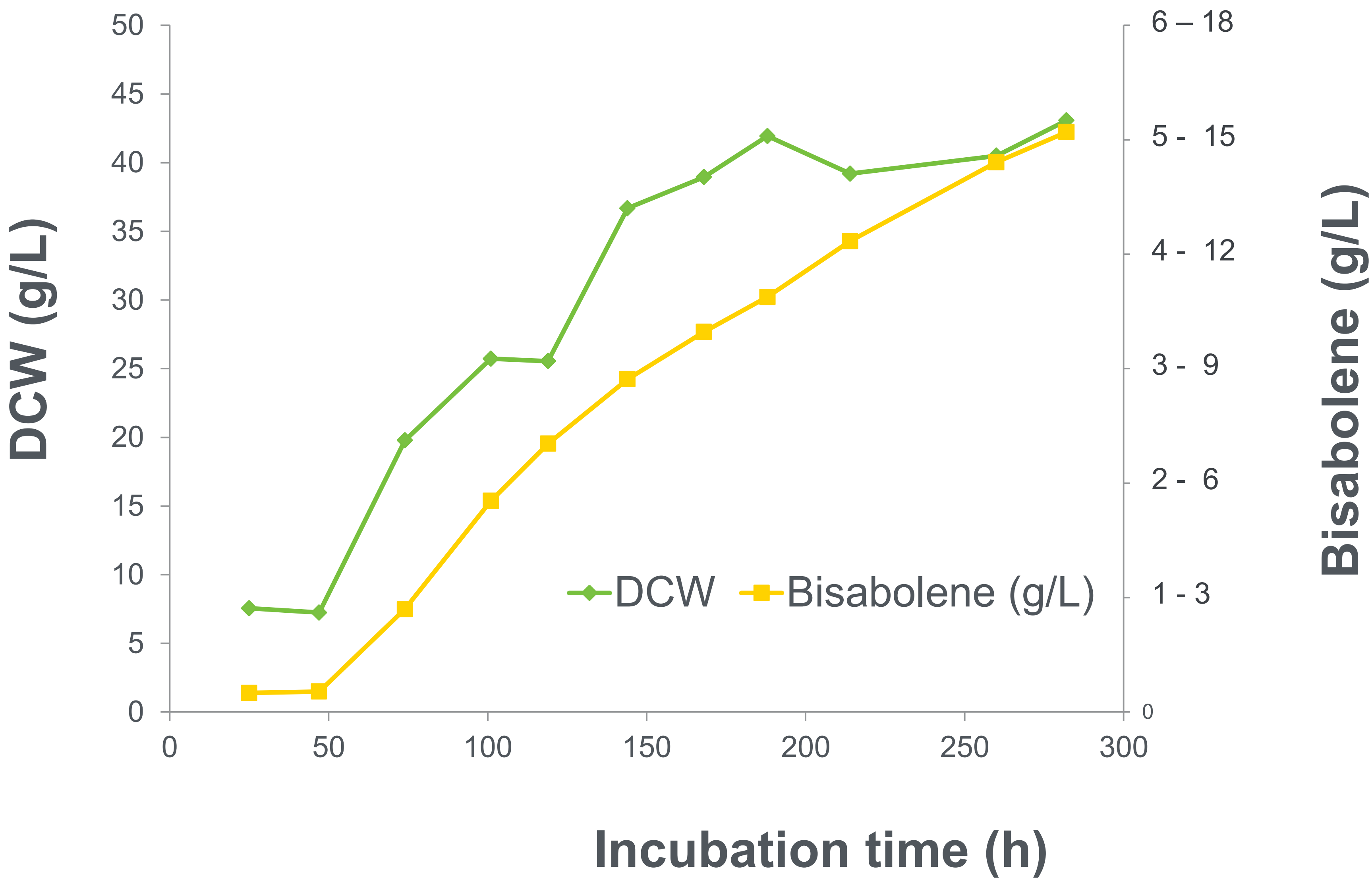
Properties

- Derived Cetane Number (DCN) comparable to No. 2 diesel
- Beneficial cold fuel properties
- Non-corrosive

- Produced in small quantities by spruce and fir trees
- Inserted a gene from the Grand Fir tree into E. coli and S. cerevisiae producing over 1 g bisabolene/L



Production of Bisabolene at 2L Scale



3 - Relevance

- Demonstration of novel, improved efficiency deconstruction technology along with production suitable tolerant enzymes critical to lower the cost of sugars used in advanced biofuel production.

- Novel biofuels like bisabolane could be cost effective alternatives to other biofuels while providing improved performance.

- Combining the development of efficient biomass pretreatment with advanced performance biofuels will benefit the bioeconomy.

4 - Critical Success Factors

- High productivity and conversion yield in fermentations for advance biofuels.
- Effective recovery of sugars/biofuels followed by recycling pretreatment additives/solvents.
- Ruggedness of pretreatment technologies to accept the diverse and variable nature of biomass.
- Integration of advances in synthetic biology to develop novel biofuels with improved performance compared to other energy sources
- Integration of novel expression systems (fungi/plants) for the production of enzymes related to biofuel production
- Understand the role of pretreatment-derived inhibitors on saccharification and fermentation and develop effective technologies to reduce their negative impact.

5. Future Work

- Improved IL pretreatment (JBEI newly developed one-pot process/IL recovery) and alkaline hydrogen peroxide (GLBRC)
- Improved productivity of microbial derived advanced performance biofuels resulting from synthetic biology.
- Integrated bacterial, yeast, fungal, algal, and plant expression technologies for the production of processing enzymes.
- Advanced biomass blending strategy (INL) and municipal/post-consumer cellulosic waste with robust pretreatment and novel enzyme cocktails to produce low cost sugars.
- Extended pretreatment expertise to algae (green, brown).
- Adapted gas (H_2/CO_2,syngas, methane) feedstocks for biofuel production

Summary

1. Established that the ABPDU is capable to work with research organizations to demonstrate technology for:
    - Effective pretreatment of diverse feedstocks
    - Enzymes adapted to to be used in novel pretreatment technologies
    - Advanced biofuel production through the use of synthetic biology

2. Developed effective collaborations with DOE research centers (JBEI, GLBRC, INL) and with over 10 companies working in the development of improved technologies for biomass/post-consumer product pretreatment and biofuel/biochemical production.

3. Integrated Techno-economic analysis in all programs.

4. Considering novel technologies to better serve clients (gases as feedstock, plant expression systems)

Questions?

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Publications & Presentations

List of Publications (6)

1.One-pot Ionic Liquid Pretreatment and Saccharification of Switchgrass. 2013. Jian Shi, John M. Gladden, Noppadon Sathitsuksanoh, Lucas Sandoval, Pavan Kambam, Debjani, Mitra, Sonny Zhang, Steve W. Singer, Anthe George, Blake A. Simmons, and Seema Singh. *Submitted to **Green Chemistry***.

2.Scale-up and Evaluation of High Solid Ionic Liquid Pretreatment and Enzymatic Hydrolysis of Switchgrass. Chenlin Li, Deepti Tanjore, Wei He, Jessica Wong, James L. Gardner , Kenneth Sale, Blake A. Simmons, Seema Singh. *Ready for submission to **Biotechnology for Biofuels**.*

3.Mapping Energy Flow in Advanced Biofuel Production: A Case Study of Large Scale Ionic Liquid Pretreatment and Enzymatic Hydrolysis of Lignocellulosic Biomass. Wei He, Jessica Wong, Deepti Tanjore, Chenlin Li, James L. Gardner, Kenneth Sale, Blake A. Simmons, Seema Singh. *Under Internal Review for **Applied Energy**.*

4.Scale-up of Ionic Liquid Based Sugar Production from Mixed Feedstocks - Part I: Pretreatment. Chenlin Li, Deepti Tanjore, Wei He, Jessica Wong, James L. Gardner , Kenneth Sale, Blake A. Simmons, Seema Singh. *Under Preparation for **Biotechnology for Biofuels**.*

5.Scale-up of Ionic Liquid Based Sugar Production from Mixed Feedstocks - Part II: Enzymatic Hydrolysis. Deepti Tanjore, Chenlin Li, Wei He, Jessica Wong, James L. Gardner, Seema Singh, Blake A. Simmons, Kenneth Sale. *Under Preparation for **Biotechnology for Biofuels**.*

6.Rheological Properties of Ionic Liquid Pretreated Lignocellulosic Biomass. Deepti Tanjore, Chenlin Li, Wei He, Jessica Wong, James L. Gardner, Seema Singh, Blake A. Simmons, Kenneth Sale. *Under Preparation for **Rheologica Acta**.*

List of Presentations (9)

1.Resolving Process Scale-Up Issues of Ionic Liquid Pretreatment and Saccharification of Biomass to Monomeric Sugars. **Deepti Tanjore**, Chenlin Li, Wei He, Jessica Wong, James Gardner, Ken Sale, Seema Singh and Blake Simmons. **Poster Presentation** for Symposium on Biotechnology for Biofuels and Chemicals, Apr 2013, Portland, OR.

2.A Calorimetry Study of the Process Energy Flow in Ionic Liquid Pretreatment of Lignocellulosic Biomass. **Wei He,** Jessica Wong, Deepti Tanjore, Chenlin Li, James L. Gardner, Kenneth Sale, Blake A. Simmons, Seema Singh. **Oral Presentation** for 244th ACS National Meeting, Aug 19-23, 2012. Philadelphia, PA.

3.Scaling and Validating Energy Innovation - the Advanced Biofuels Process Demonstration Unit. **Chenlin Li**. **Oral Presentation** for Joint BioEnergy Institute Retreat, Aug 20-22, 2012. Monterey, CA.

4.Properties of Biomass Pretreated with Ionic Liquid at 10L Scale. **Deepti Tanjore, Chenlin Li**, Wei He, Jessica Wong, James Gardner, Ken Sale, Seema Singh and Blake Simmons. **Poster Presentation** for Joint BioEnergy Institute Retreat, Aug 20-22, 2012. Monterey, CA.

5.Online Monitoring Using FT-NIR to Facilitate Dynamic Fed-Batch Fermentations. **Lucas Sandoval**, **Debjani Mitra**, Wei He, Jessica Wong, James Gardner, and Pavan Kambam.

6.Poster Presentation for Joint BioEnergy Institute Retreat, Aug 20-22, 2012. Monterey, CA.

7.Proving Energy Innovation - ABPDU. **James Gardner**. **Oral Presentation** for Great Lake BioEnergy Research Center Retreat, May 21-23, 2012, Naperville, IL.

8.Scale-up of Ionic Liquid Pretreatment and Enzymatic Hydrolysis of Lignocellulosic Biomass for Biofuel Production. **Chenlin Li**, Deepti Tanjore, Wei He, Jessica Wong, James L. Gardner , Kenneth Sale, Blake A. Simmons, Seema Singh. **Poster Presentation** for Great Lake BioEnergy Research Center Retreat, May 21-23, 2012. Naperville, IL.

9.Scale-up of Thermophilic Ionic Liquid-tolerant Cellulase Cocktail for Lignocellulosic Biofuel Production. **Lucas Sandoval**, Debjani Mitra, John Gladden, Pavan Kambam. **Poster Presentation** for Seventh Annual DOE JGI User Meeting, March 20-22, 2012, Walnut Creek, CA.

Additional Slides

CORE COMPETENCIES

•Pretreatment

- Deconstruction: 3x10 & 210 Liters
- Depolymerization 4x2,50 & 210 Liters
- Basket & decanter centrifuges
- Convection & vacuum dryers
- Mill, sieve, & storage
- Composition/rheology/calorimetry
- 2 Staff Engineers

•Fermentation

- 4x4, 10, 20, 70 & 2x400 Liters
- Anaerobic or Aerobic
- Bacteria, Fungi, Yeast, Algae
- Lignocellulosic, sugar, gas feedstocks
- Batch, fed-batch, continuous
- pH, DO, redox, CO_2, weight, air/nutrient feed control, off-gas MS
- Online OD_{600}, HGA, FTNIR
- 1 Scientist, 1 RA, 1 temp

•Recovery

- Continuous solid-liquid-liquid separation/sonication
- Tangential flow filtration
- Prep liquid chromatography
- Continuous liquid-liquid extraction/solvent extraction
- Lyophilization
- 1 RA (to be hired)

•Analytical

- HPAEC-ECD
- HPLC-UV/RI/CAD
- GC-FID
- YSI, moisture, ash
- Rheometry
- Calorimetry
- Mass & Energy balance
- Techno-Economics, Carbon Footprint
- 2 RAs

Program Manager, Operations Manager, Business Development Manager, Administrator, Facility TA

Growth Initiatives

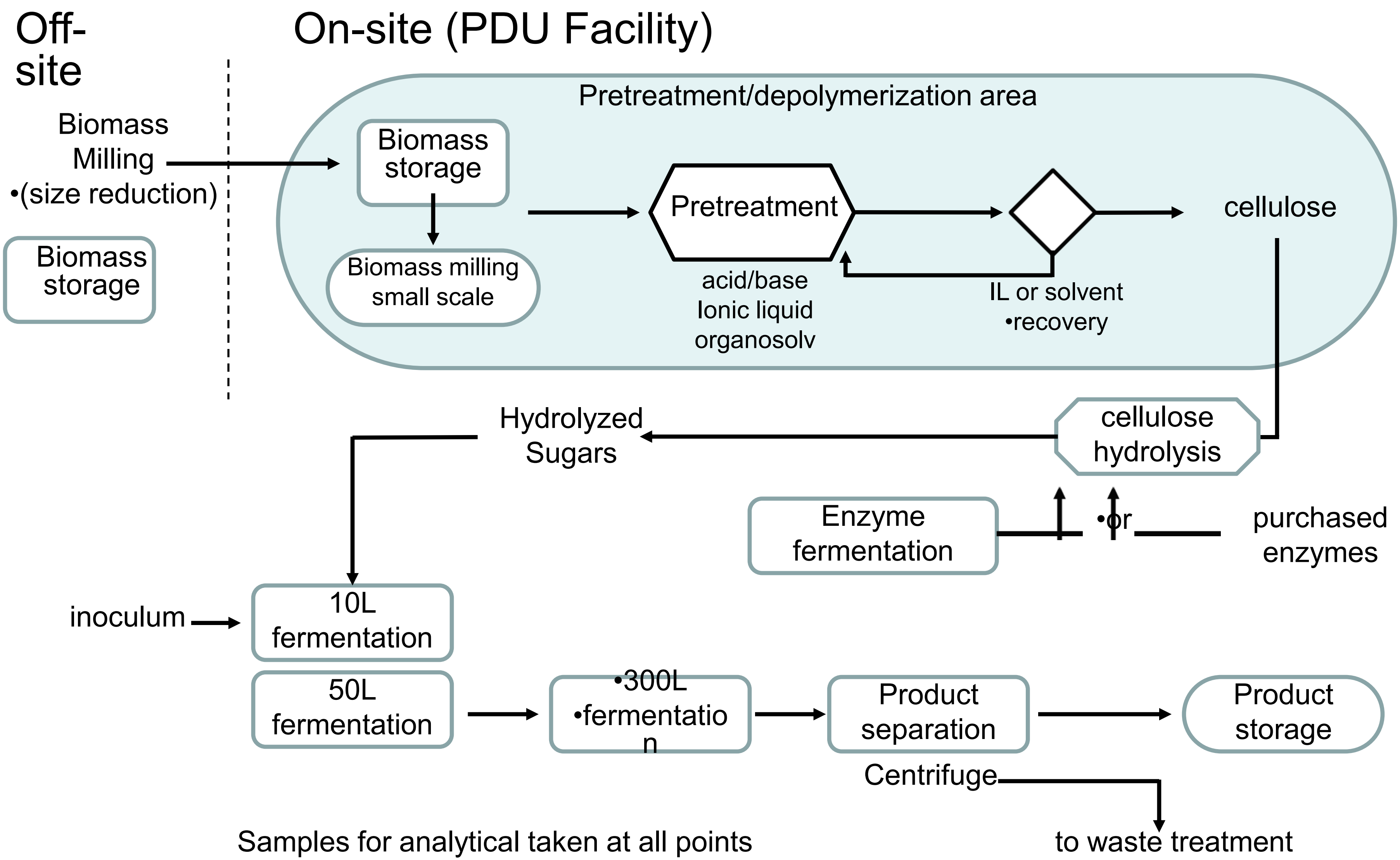
•Technologies and infrastructure to serve our clients with:

- Diverse pretreatment methods:
  - Acid/alkaline treatment
  - Ionic liquids
  - Hydrogen Peroxide
- Multiple feedstocks
  - Tropical plant material
  - Recycled post-consumer cellulosic
  - High quality and content oil seeds
  - Brown and green algae
- Fermentation/recovery process demonstration
  - Multiple sugar/protein based feeds
  - H_2/CO_2, Syngas, methane fermentations

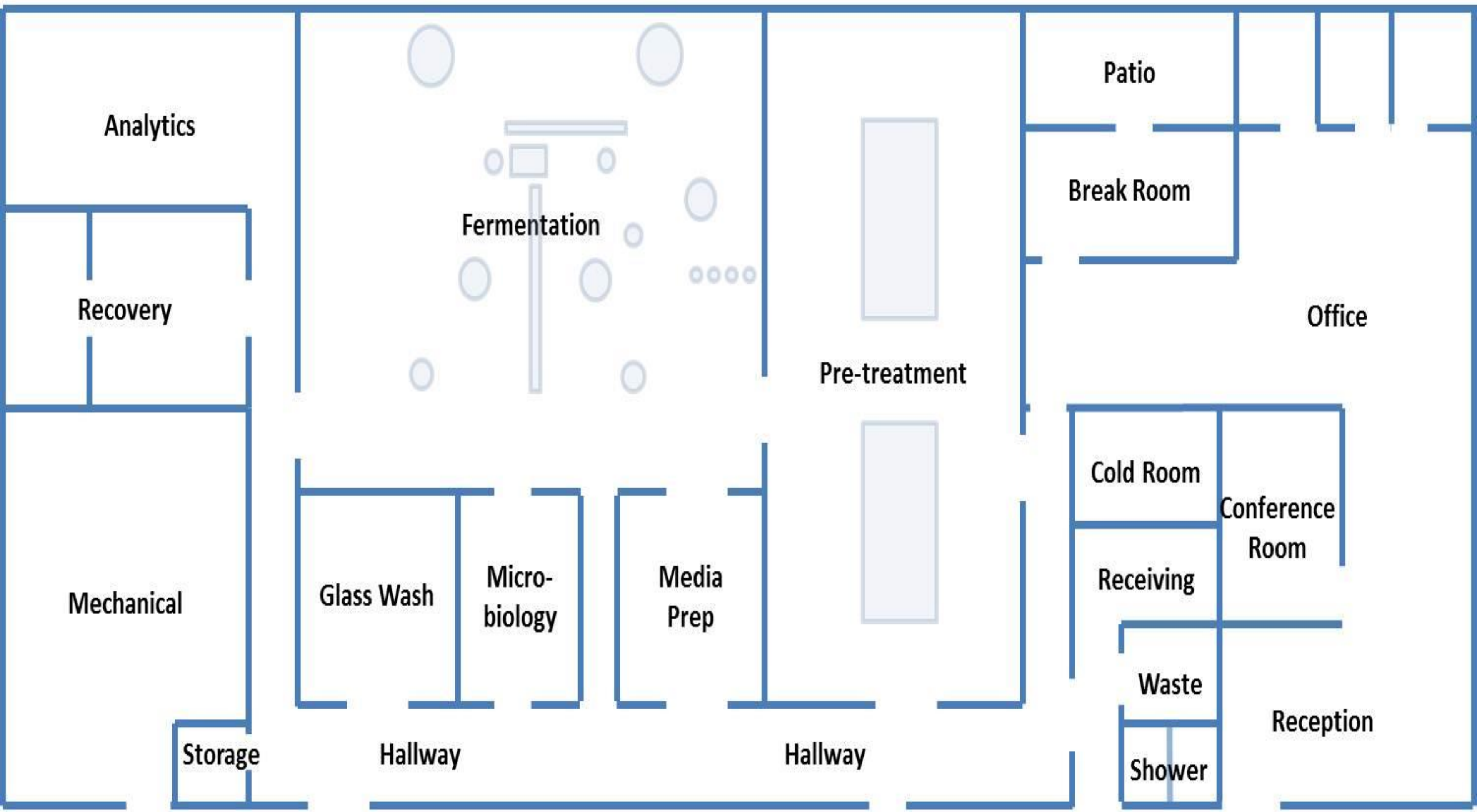
•Expanding Collaborations:

- Partner with other DOE facilities to:
  - Share test materials and methods
  - Discuss best practices & lessons learned
  - Help extend & broker technological collaborations
  - Offer collaborators opportunities for rapid follow-on testing
  - Third party technology validation
  - Biofuel use evaluation
- Partnering with QB3 to build relationships with start-up companies
- Collaborating with UC Berkeley to establish the ABPDU internship program providing hands-on biofuels development experience

ABPDU Process Flow



ABPDU Laboratory Schematic



Pretreatment

•Capabilities

- 3X 10L, 1X 210L
- Biomass size reduction and pelletization
- Dilute acid, hydrothermal, alkaline, ionic liquid, and organosolvent processing up to 30% w/w
- Compositional and rheological analysis
- Solid/liquid separation

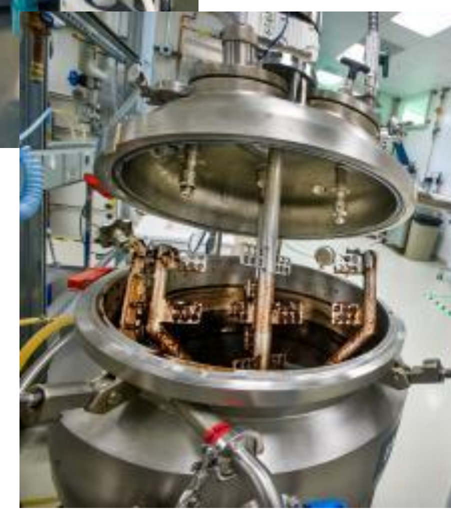
•Types of Biomass

- Herbaceous
- Softwoods and hardwoods
- Agricultural bi-products and residues
- Municipal waste
- Algae

Depolymerization

•Capabilities

- 4X 2L, 1X 50L
- Process optimization with commercial and research enzymes
- High solids enzymatic hydrolysis (up to 30% w/w)
- Solid/liquid separation
- Compositional and real-time rheological analysis



Fermentation

•Capabilities

- 4X 2L, 1X 10L, 1X 15L, 1X 50L, 2X 300L
- Anaerobic and Aerobic processing
- Microbial and Algal batch, fed-batch and continuous processing
- Gaseous (syngas, methane) fermentations with controlled sparging and head pressure
- Rushton and Pitch-blade impeller

•Monitoring and Control

- pH, temperature, dissolved oxygen, redox, cell density, weight, substrate feeding
- Mass spectrometric off-gas analysis
- On-line FT-NIR monitoring nutrients and metabolites
- Off-line nutrient and metabolite
- Microscopic analysis

Gas Fermentation

•Retrofit

- Liquefied or compressed gas in large dewars will be used as source tanks
- Connection between dewars to the sparge and overlay lines equipped with pressure regulators and mass flow controllers
- Gas detectors to address safety

•Capabilities

- Controlled sparging of a gas or mixture (0-1vvm) at desired flow rate both through sparger and overlay
- Head pressure control (0-2bar) with desired gas to improve mass transfer

•Timeline

•Available May-June 2013

Recovery

•**Capabilities**

•Continuous centrifugation
•Batch and continuous cell lysis
•Continuous liquid-liquid extraction
•Tangential flow filtration
•Freeze drying
•Enzyme separation and purification using Fast Protein Liquid Chromatography (FPLC)
•Conventional solvent extraction

•**Equipment**

•Alfa Laval Centrifuge up to 150 liters/hour
•Qsonica- Q700 sonicator, batch and continuous up to 0.5 liters/min
•KARR® Reciprocating Plate Extraction Column, 2L
•Cogent M1 Tangential Flow Filtration (TFF) systems
•6-L LABCONCO Freeze dryer and Shell freezer
•Column Pilot-scale enzyme separation and purification using (FPLC) with the ÄKTA Avant 150

Analytical

•**Capabilities**

•Optical microscopy
•Compositional analysis
•Enzyme activity
•Gel electrophoresis
•Gas and liquid chromatography

•**Equipment**

•Gas Chromatograph with Flame Ionization Detection (GC-FID)
•UV-Vis Spectrometer
•Precision bomb calorimeter for solids and liquids
•Microplate reader
•Rotational rheometer
•Column Pilot-scale enzyme separation and purification using (FPLC) with the ÄKTA Avant 150
•HPAEC for sugar analysis
•UPLC-UV/RI/CAD for sugar, organic acids, alcohols, and protein analysis