

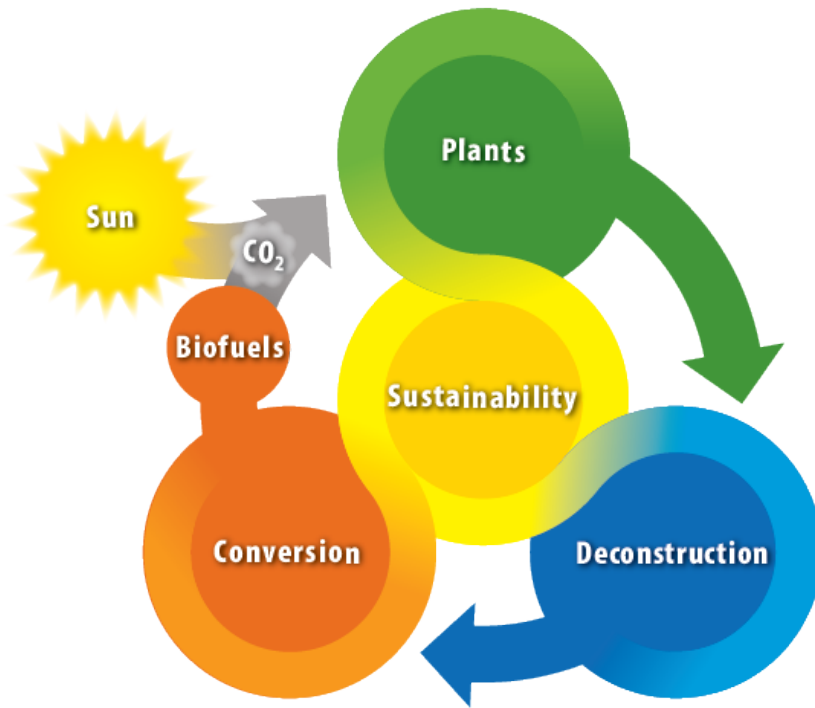


BER Advisory Committee Meeting

Timothy Donohue
Director, Great Lakes Bioenergy
Professor of Bacteriology
University of Wisconsin-Madison

October 15, 2012

Great Lakes Bioenergy



Goals

- ✘ Improve relevant plant traits & sustainable agronomic systems
- ✘ Improve energy conversion of cellulosic biofuels production

Our Members

- ✧ International team of 10 universities, two national labs & one company
- ✧ UW-Madison (lead), MSU (major partner)
- ✧ ~400 scientists, students & staff, including
 - 78 – Faculty
 - 65 – Graduate Students
 - 64 – Post Docs
 - 46 – Scientists
 - 80 – Technicians
 - 76 – Undergraduates
 - 45 – Support Staff



2012 GLBRC Retreat

Great Lakes Bioenergy Collaborators

BER (projects, workshops, scientific advisory board)



Host Institutions (farm to bench to combustion)



External (DOE, USDA)



Products of Research Integration

Biofuel synthesis from corn stover hydrolysates

Cropping Systems



Pretreated Biomass



Hydrolysate



Biofuels



Measurables

- Site/Soil Type
- Crop/Seed/Row
- Plant/Harvest Date
- Fertilizer/Herbicide
- Season/Weather

- Cellulose
- Hemicelluloses
- Lignin
- Plant Cell Residue

- Total CHO
- C-5 & C-6 Sugars
- Amino Acids
- Organic Acids/Amides
- Ammonia/Phosphate
- ~30 Metals/Inorganic Ions

- Hydrolysate Inputs
- Transcripts
- Targeted Metabolites
- Excreted Products
- Fuel
- Input/Output COD
- Microbial Growth

Data Management

Scientific Discoveries

Sustainability

Sustainability



Goals:

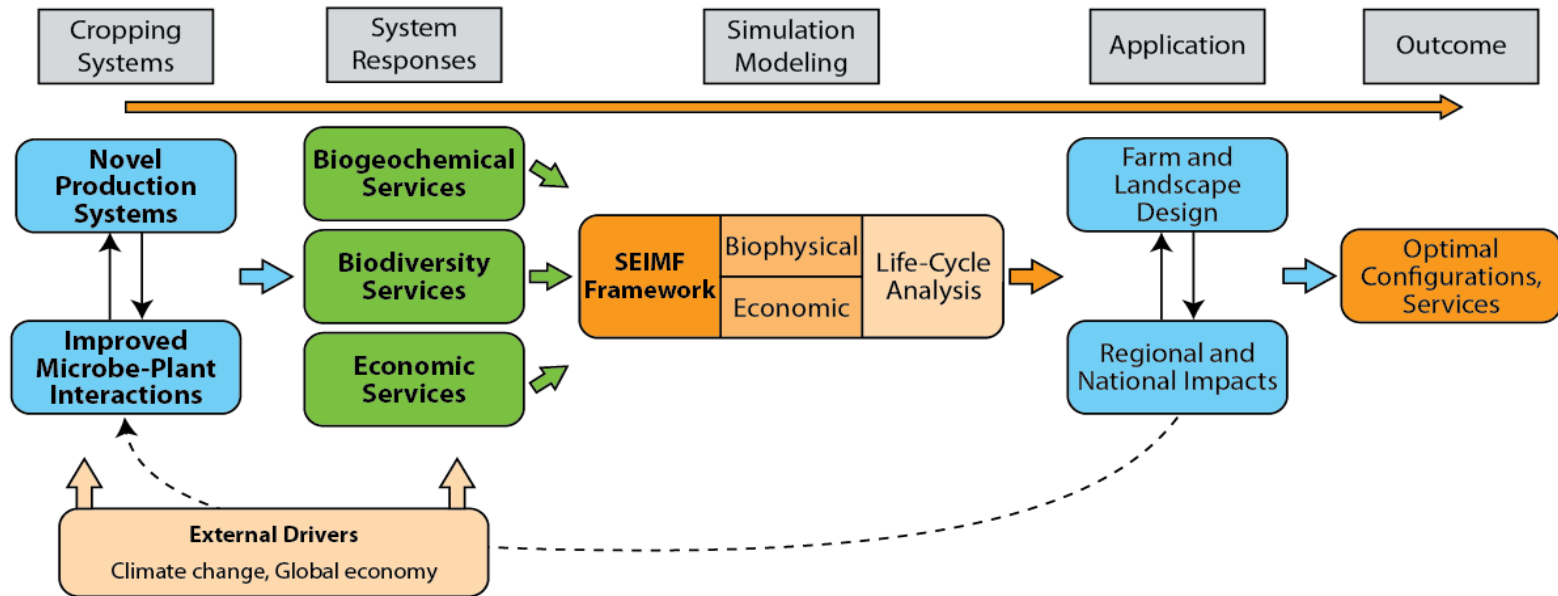
- ✘ Discoveries to design sustainable biofuel production systems
- ✘ Model alternative biofuel systems at field to regional scales

Objectives:

- ✘ **Economic** - basis for farmer, refiner & policy makers decisions about what to plant where & when
- ✘ **Environmental** - climate mitigation, water, nitrogen conservation & delivery of biodiversity services
- ✘ **Social** - energy and food security

Sustainability research roadmap

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✧ Cropping Systems

- Productivity & rhizosphere communities of a range of cropping systems

✧ System-Level Responses to Potential Crops

- C, N, water balances & impacts on pest suppression & pollination
- Predict factors that will impact farmers' acceptance

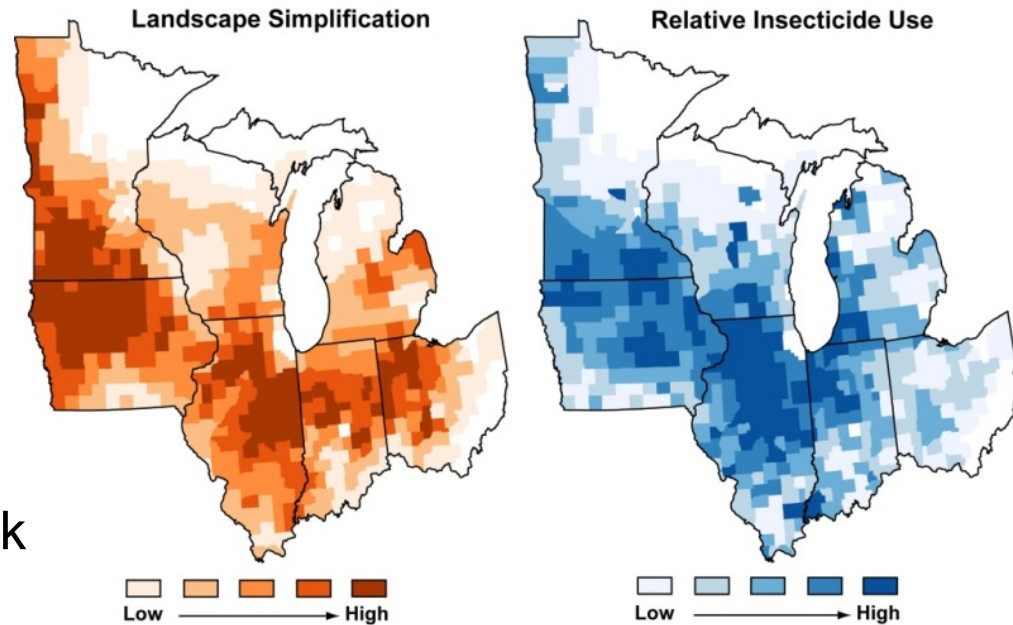
✧ LCA Simulation Modeling & Integration

- Parameterizing and testing local models
- Begin spatially explicit extrapolations to regional & national scales

Research Highlights: Sustainability

Land to support biofuel cropping systems

- ✘ System responses to land use changes

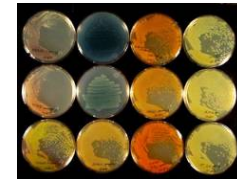


Carbon debt of converting CRP to crop land

- ✘ Cropping system impacts payback time

Value of crop perenniality & diversity

- ✘ C & N benefits of perennial cropping systems
- ✘ Beneficial insects offset ~ \$240 M/yr in chemical pest control



Plants

Plants



Goal: develop productive energy crops that can be easily processed into fuels

- ✘ Alter lignin to reduce recalcitrance
- ✘ Increase energy density of biofuel crops
- ✘ Manipulate hemicelluloses for improved processing & energy yield
- ✘ Improve crop plant properties for sustainable bioenergy production

Research Highlights: Genome-enabled improvement of plants

✧ Gene discovery in model plants

- EST deep-sequencing from tissues enriched in desired activity (JGI)

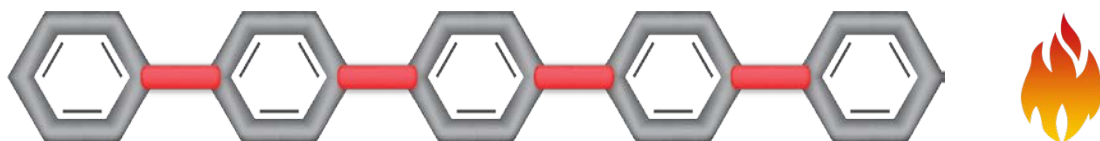


Examples:

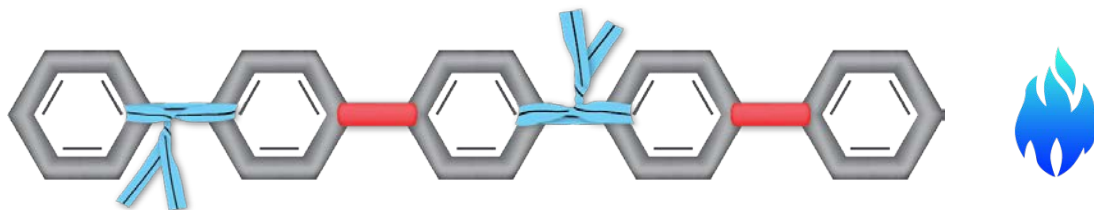
- ✧ Lignin – Alter composition & lower energy needs for release
- ✧ Oils – Increase energy density of vegetative tissue
- ✧ Hemicelluloses – Change C₅:C₆ ratio to improve conversion

Altering lignin to decrease recalcitrance

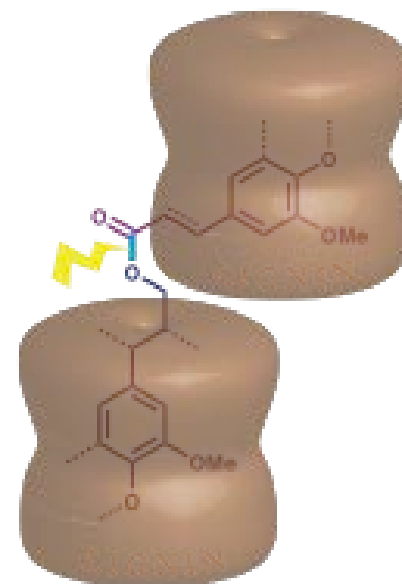
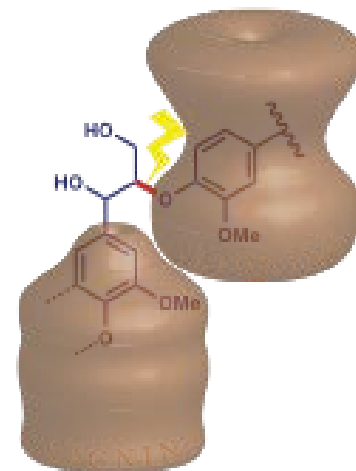
- ✦ **Lignin today:** Weakest bonds cleave at $\sim 170^\circ\text{C}/\text{alkali}$ or $>190^\circ\text{C}/\text{acid}$



- ✦ **Lignin's future:** Weakest bonds cleave at $<100^\circ\text{C}$ (replace backbone ethers with esters)



- ✦ **Result:** Less energy to depolymerize
- ✦ **Ferulate monotransferase (FMT):** one key to producing "Zip" lignins that cleave easier



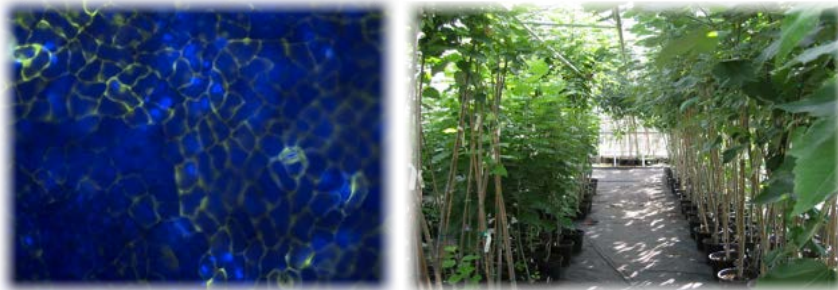
Research Highlights: Identifying an FMT Gene

Approach: EST sequencing of *Angelica sinensis* roots (~2% coniferyl ferulate)

- ✗ FMT candidate genes
- ✗ Recombinant proteins
 - Expected activity with ferulate (+), *p*-coumarate (-) & other substrates



Produce “Zip” lignin in energy crops



- ✗ FMT active *in planta*
- ✗ Poplar field trials ongoing
- ✗ Evaluating in other crops

Research Highlights: Improved Plants

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New lignin varieties

- ✦ Rapid/quantitative NMR imaging (ARRA)
- ✦ Adjust flux to other lignin precursors
- ✦ Simplify subunit composition
- ✦ Improve lignin release & valorization

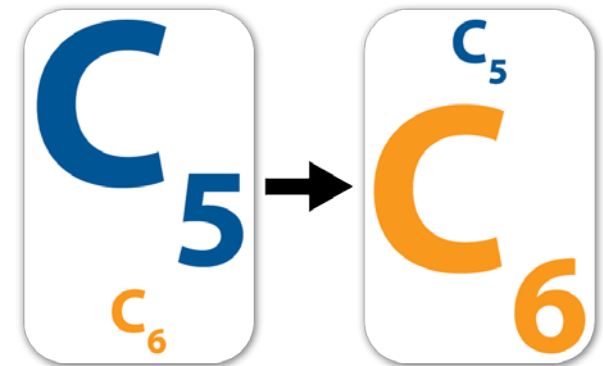
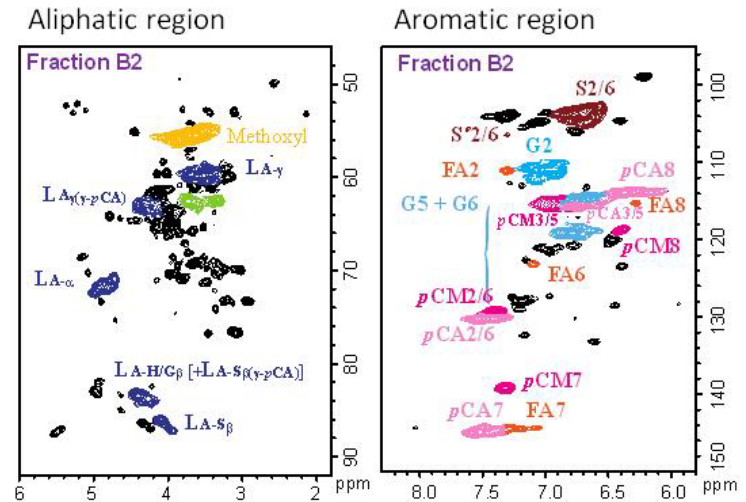


NextGen fuels in vegetative tissues

- ✦ Increase energy density of plant biomass
- ✦ Plant-derived oils & biodiesel substitutes
- ✦ Moving into energy crops & combustion trials

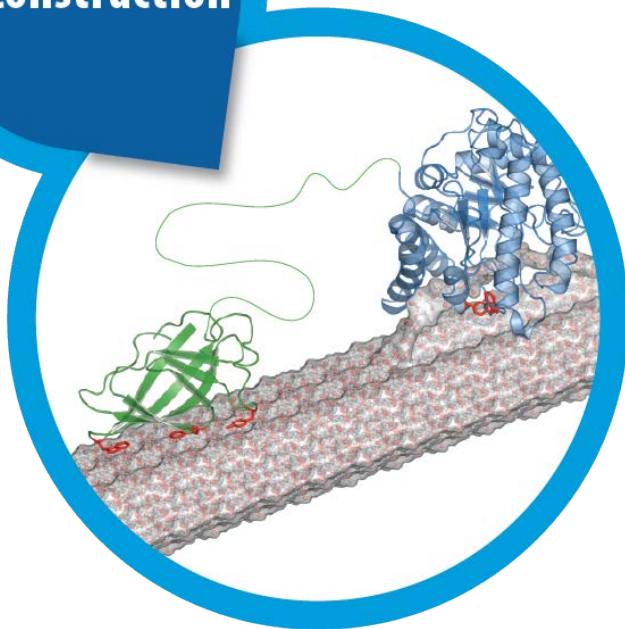
Improved hemicelluloses

- ✦ Identify genes to increase C₆ content
- ✦ Improve deconstruction/conversion efficiency



Deconstruction

Deconstruction



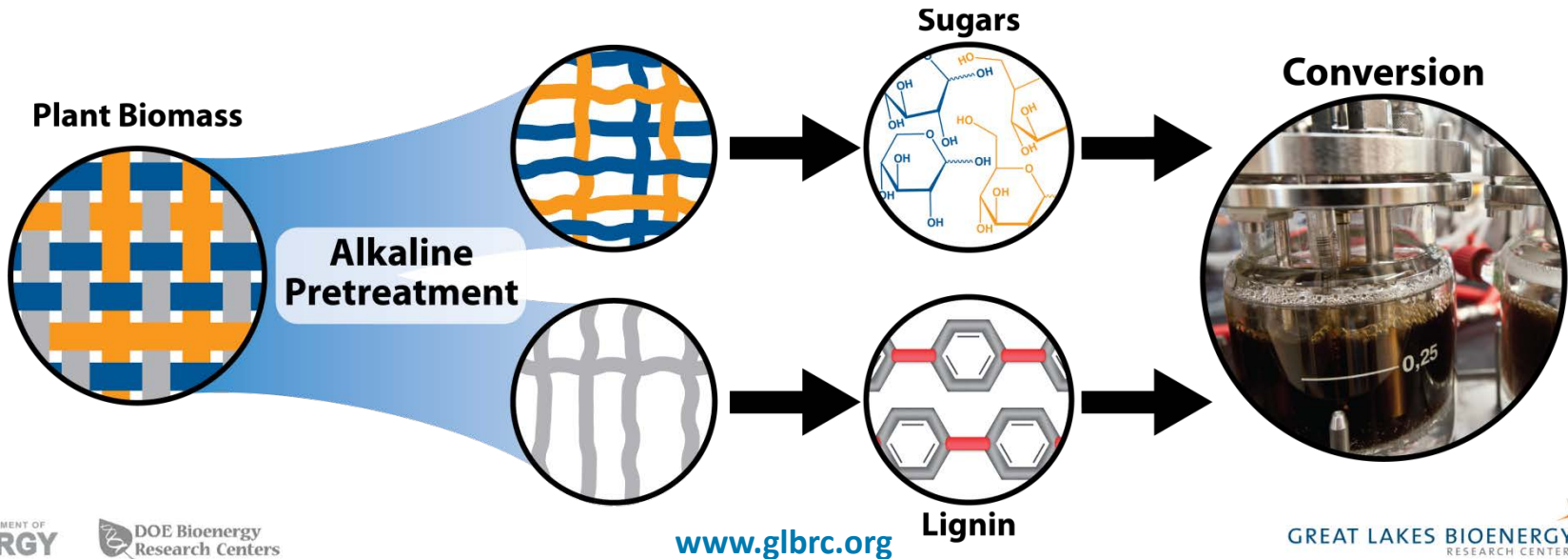
Goal: improve release of monomers or short oligomers from lignocellulosic biomass

- ✦ Improve alkaline pretreatments to open plant cell wall polymer matrix
- ✦ Improve enzyme cocktails to release useful intermediates

Research Highlights: Pretreatment advances

Alkaline pretreatments

- ✗ Preserve energy rich compounds
- ✗ **C₅** & **C₆** stream for conversion
- ✗ Cellulose III – easier to digest



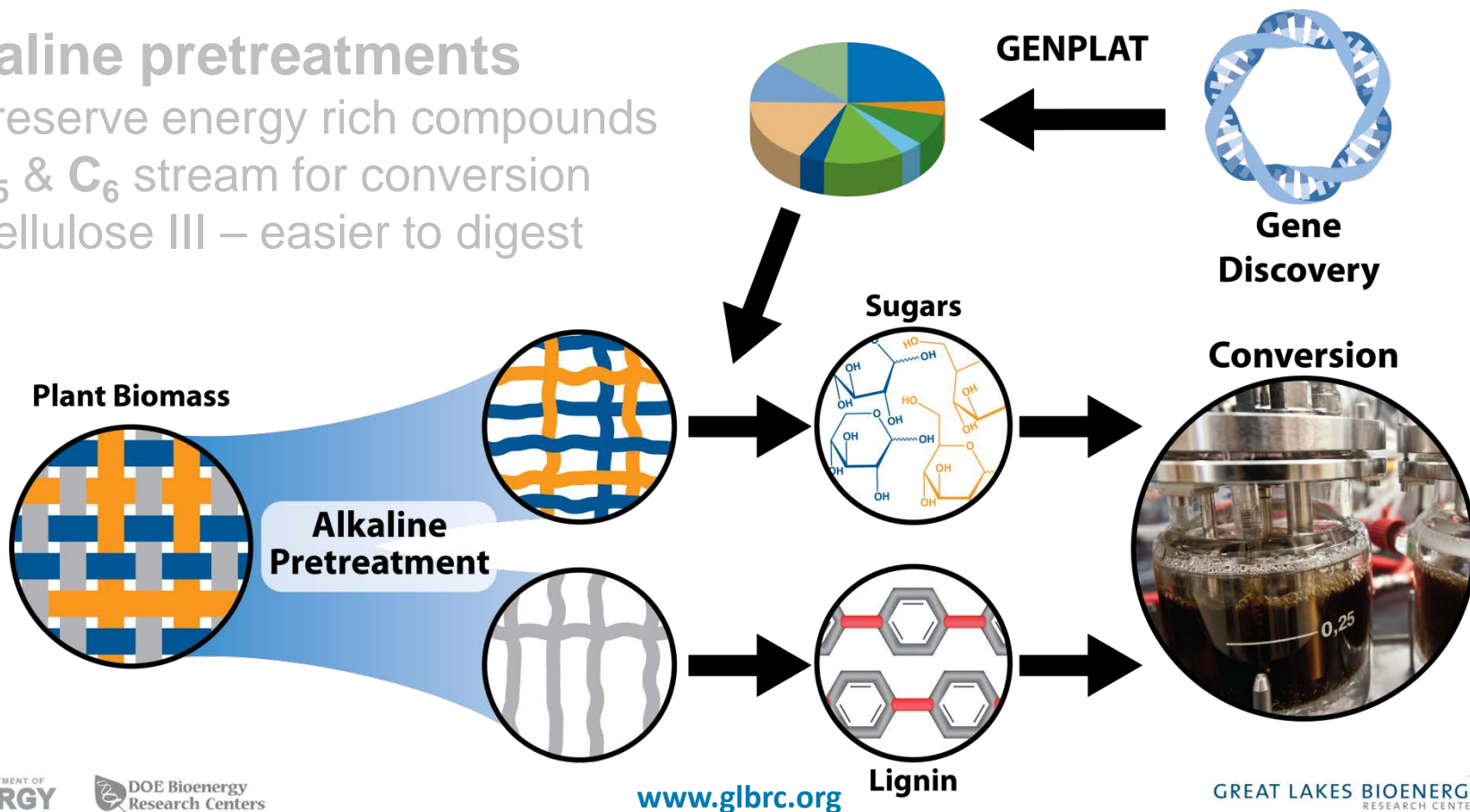
Research Highlights: Improved enzymes

Improved enzyme mixtures

- ✧ GENPLAT – HT, higher SA, enzyme sets
- ✧ Lower energy & cost of providing enzymes
- ✧ ID feedstock- or hydrolysate-limiting catalyst

Alkaline pretreatments

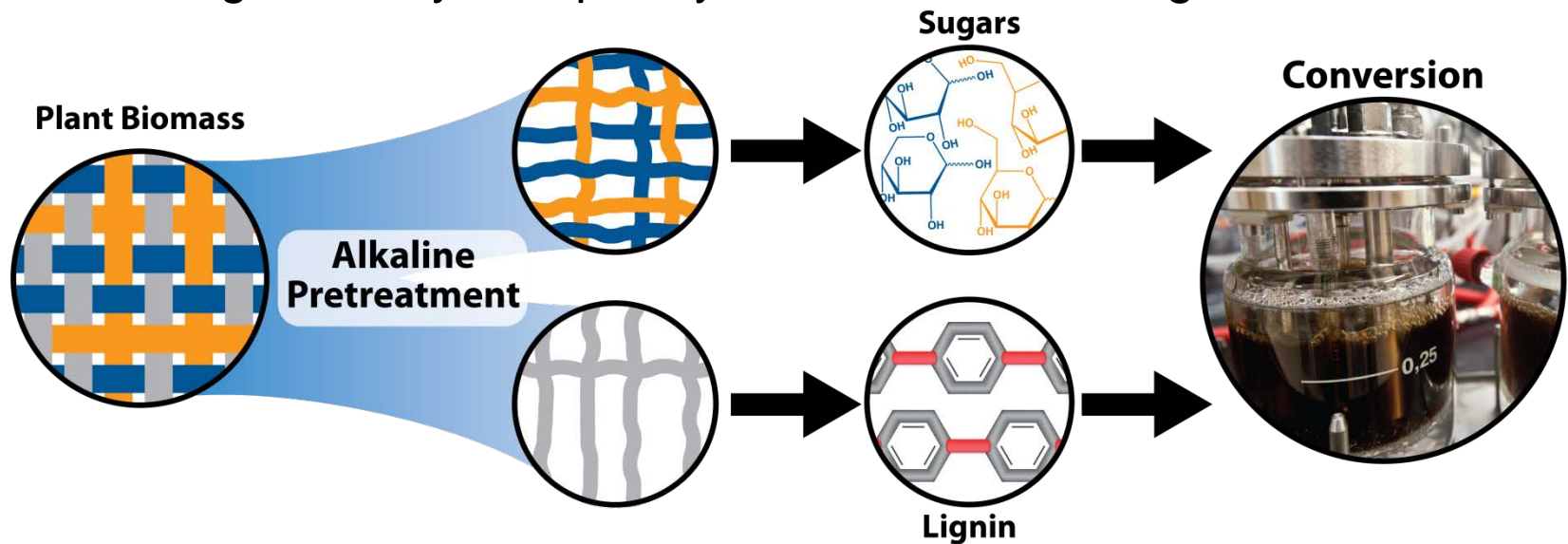
- ✧ Preserve energy rich compounds
- ✧ C₅ & C₆ stream for conversion
- ✧ Cellulose III – easier to digest



Research Highlight: Added value from lignocellulose ¹⁷

Pre-treatment advance improves energetic/economic sustainability

- ✦ Potential lignin stream for fuel & co-product synthesis
- ✦ Recover additional energy/add value to lignin fraction
- ✦ Evaluating recovery, complexity & conversion strategies



Conversion

Conversion



Goal: overcome barriers in converting lignocellulose products into biofuels & valuable co-products

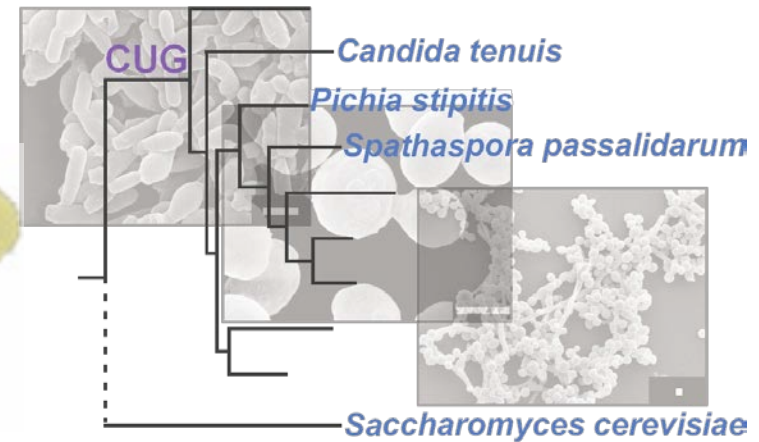
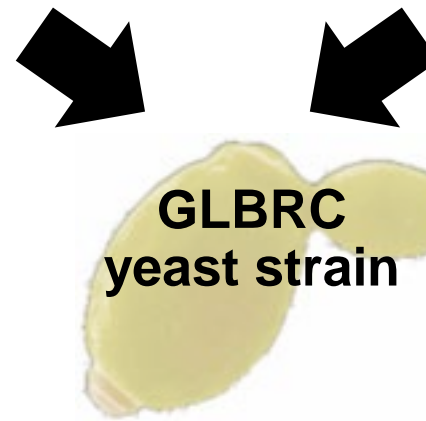
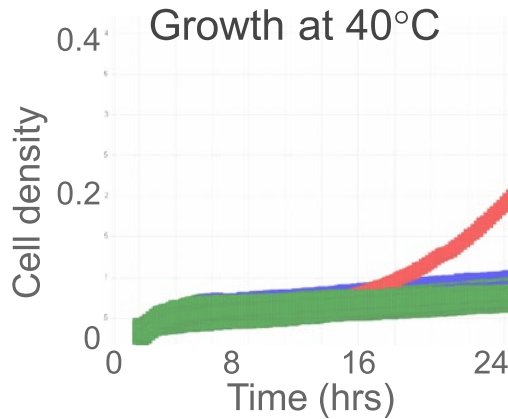
- ✘ Improve efficiency of biomass-to-ethanol conversion
- ✘ Engineer routes to NextGen biofuels

Research Highlights:

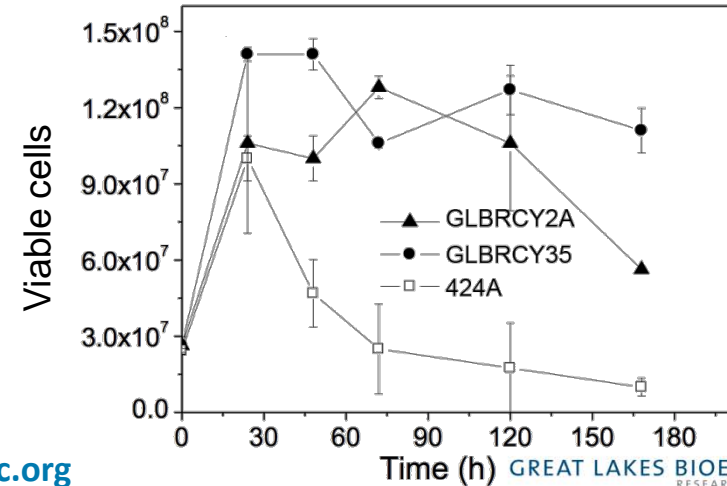
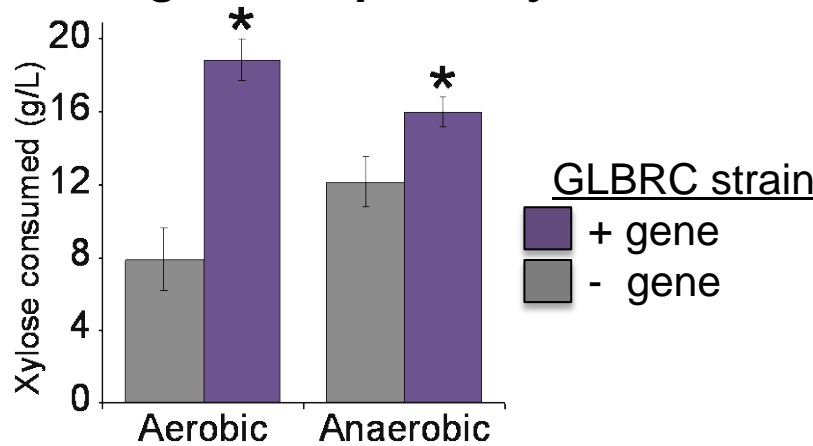
Genome-enabled yeast improvement

High-throughput screening of 587 wild strains in 6 hydrolysates

Sequencing (JGI) & expression profiling of xylose utilizers



Novel genes improve xylose metabolism Evolve stress tolerance to hydrolysate



Research Highlights: Multi-omic analyses of hydrolysate fermentation

Hydrolysate



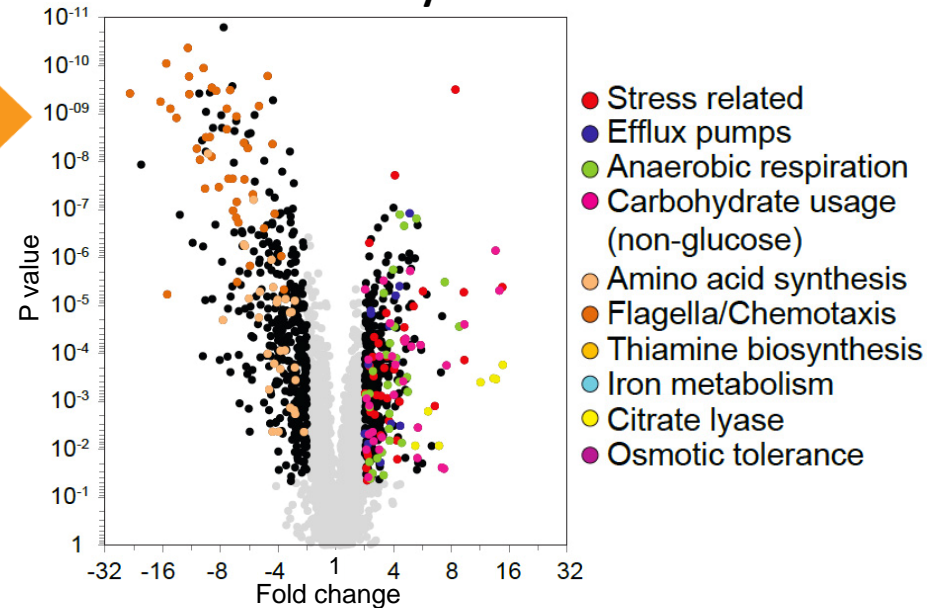
Total CHO
C-5 & C-6 Sugars
Amino Acids
Organic Acids/Amides
Ammonia/Phosphate
~30 Metals/Inorganic Ions

Biofuels



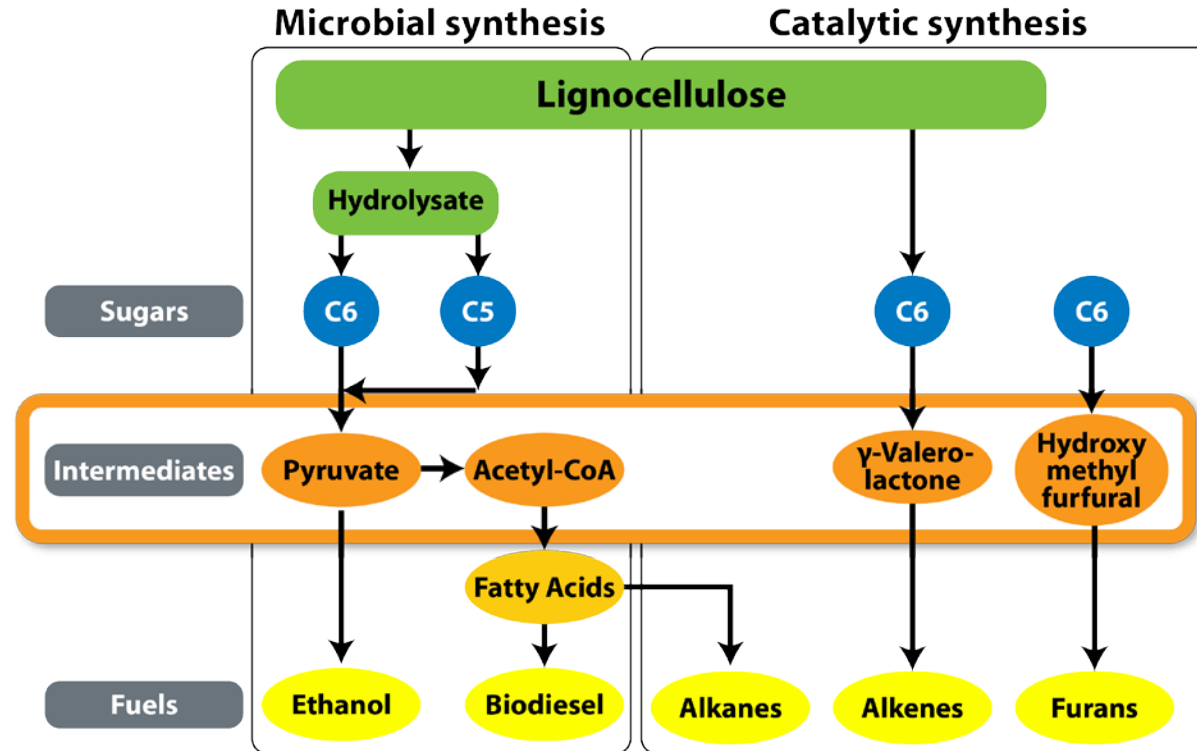
Hydrolysate Inputs
Transcripts
Targeted Metabolites
Excreted Products
Fuel
Input/Output COD
Microbial Growth

Multiomic analyses



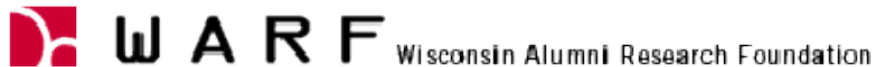
- ✘ Hydrolysate factors (osmolytes) aid growth
- ✘ Amino-acid depletion & concomitant ATP demand of multiple stresses arrests *E. coli* growth in corn stover hydrolysate
- ✘ Regulatory strategies to improve fermentation performance

Research Highlights: Key intermediates for biofuels



- ✧ Microbial fatty acids yield alkanes by catalytic decarboxylation of biodiesel by biological condensation with alcohols
- ✧ γ -valerolactone yields alkenes without exogenous H_2
- ✧ Ionic liquids produce sugars (for fermentation) or furans (for fuels)
- ✧ Producing high value co-products or fuels from lignin aromatics

Partners in transferring advances to private sector



Moving GLBRC Discoveries to Products

- ✘ Patent landscapes (Law students, WARF, MSU Technologies)
- ✘ De-risking & economics (MBI, Business & Graduate Schools)
- ✘ Sponsored research (non-DOE funds) to improve invention

Technology Transfer (WARF/MSU Technologies)

- ✘ Evaluate invention disclosure reports (IDR)
- ✘ Patent & commercialize (options, licensing, start ups)

Industry perspective on our Scientific Advisory Board



Education and Outreach

- ✘ Bioenergy STEM content
- ✘ Informing, inspiring & training future leaders
 - Undergraduates
 - Graduate students
 - Post-docs
 - K-12 student & teacher programs
- ✘ Foster development of energy literacy
- ✘ Provide science content for citizens, stakeholders & policy makers



Future Challenges & Opportunities

- ✘ Cellulosic biofuels success depends on “multiple winners”
 - ✘ Vary based on region, crop, engine or fuel

- ✘ Advances needed to integrate systems
 - ✘ Sustainable cropping practices
 - ✘ Field test new crop lines
 - ✘ Fuels from other crops
 - ✘ Valorize lignin

