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High Throughput Pretreatment and Enzyme Hydrolysis of Biomass: *Screening Recalcitrance in Large Sample Populations*

Presented by

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BioEnergy Science Center

A multi-institutional, DOE-funded, Bioenergy Research Center dedicated to understanding and modifying ***Biomass Recalcitrance***

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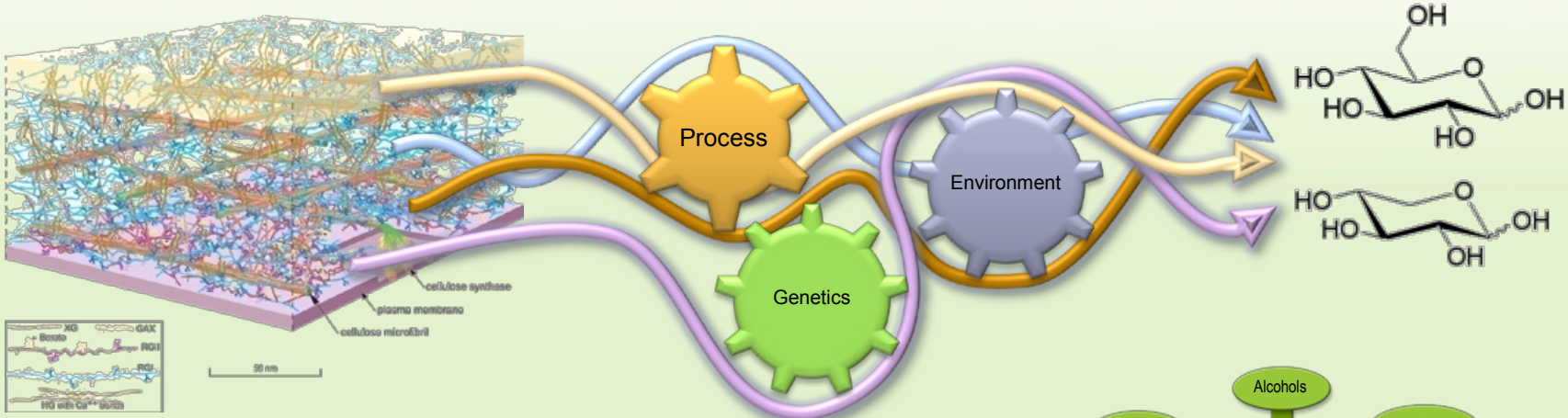
Biomass Recalcitrance



Somerville et al

- Plant cell walls are complex, heterogenous structures
  - Three major polymers
    - Cellulose, hemicellulose (multiple types), and lignin
    - Components closely associated and differentially reactive
  - Cell wall polysaccharides are *structural*
    - More recalcitrant than storage carbohydrates such as starch
  - Steps to overcome recalcitrance
    - Pretreatment- enables efficient enzymatic hydrolysis
    - Enzymatic hydrolysis is less damaging than pretreatment
- Recalcitrance- genetic and environmental factors
  - Must screen large populations to find and correlate underlying principles and effects
  - Affects pretreatability and enzyme digestibility

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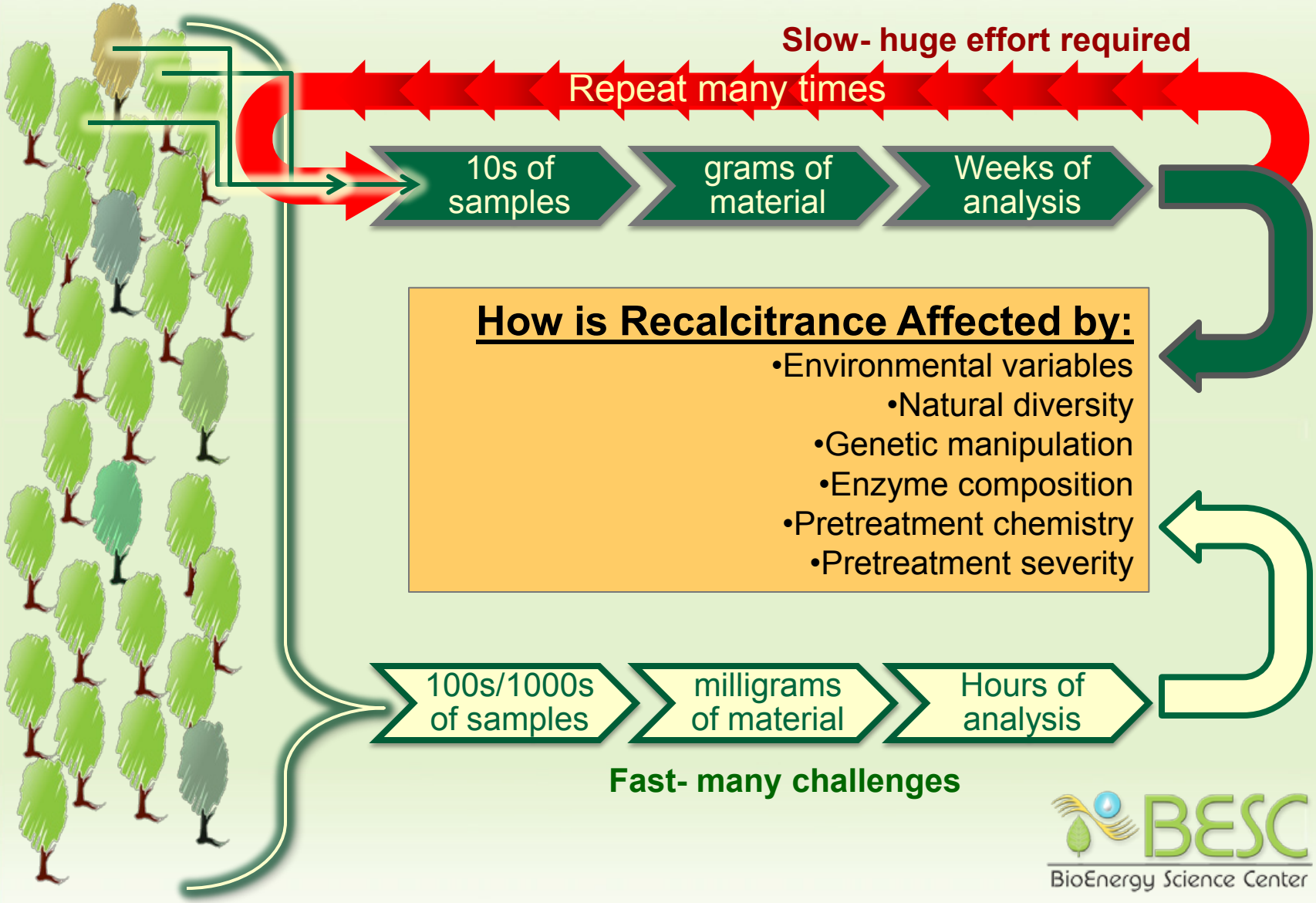
Screening Large Populations for Recalcitrance Variants



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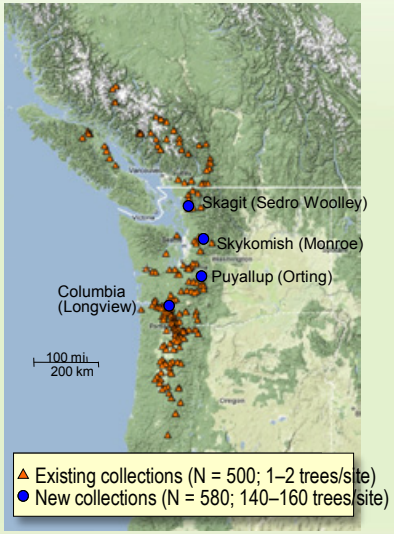
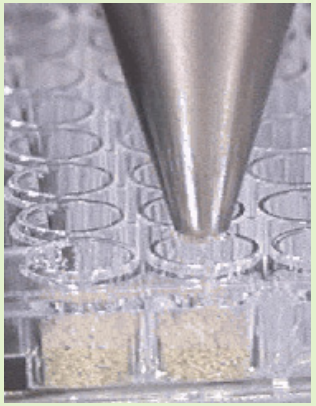
Barriers to High Throughput Screening in Biomass Conversion

- Defined by large sample sets
  - Manual labor in collecting, milling, extracting, starch removal
  - Demands small-scale, automated processing
- Biomass is insoluble, heterogeneous
  - Accurate dispensing, distribution is difficult
  - Static is major problem
- Severe pretreatment conditions
  - High temperatures (150-200°C), pressures, low pH
- Solids/Liquids separation
  - Small scale losses→ large impact on analysis
- Enzyme systems are complex, slow
  - Long conversion times (days, not minutes)
- Sugar product detection
  - HPLC is slow (one plate = 2 days of HPLC time)
- Chemical analysis/composition
  - Standard techniques too slow
- Systematically addressed/constrained the barriers





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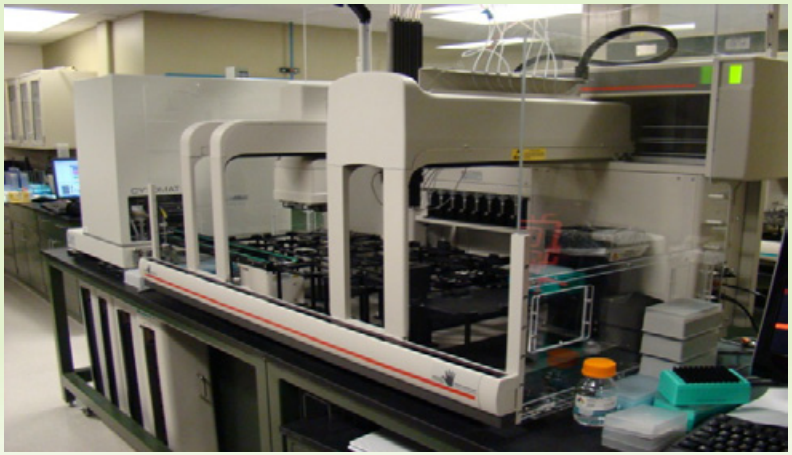
Biomass Preparation and Dispensing



- Collect and mill by hand
- De-starch/extract in “teabags”
  - Wash extensively, extract in Soxhlet

- Solids- Symyx MTM Powdernium (2)
  - Dispense/track quantities
    - Automated, 0.1 mg precision
  - Multiple substrate capacity
    - 144 or 200 deck capacity
    - 15 plate capacity
- Liquid- Beckman-Coulter Biomek FX
  - Catalyst, enzymes, sugar assays

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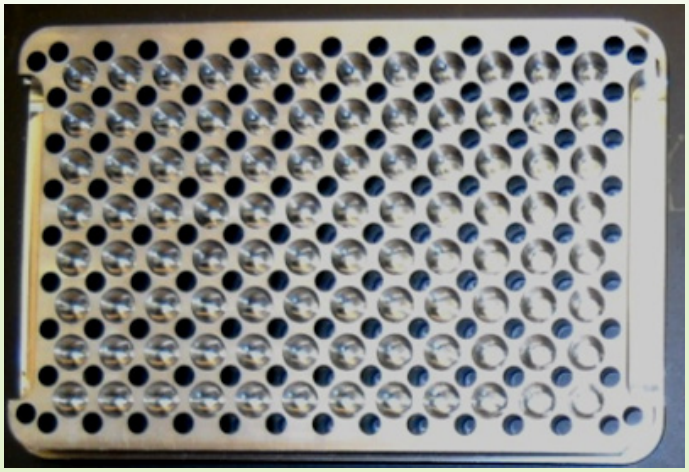
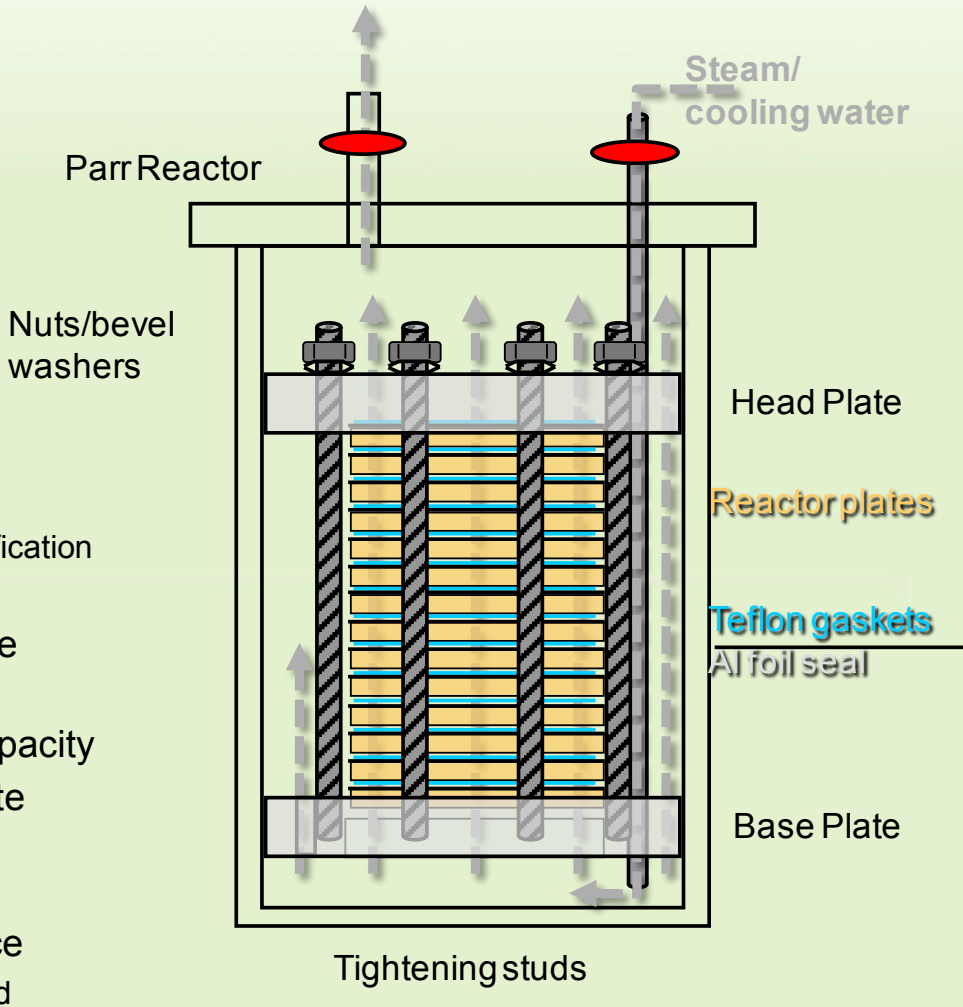
96-Well Pretreatment Reactor Plate





- Single piece reactor plate
  - Sequential pretreatment/enzymatic saccharification
  - No Solid-Liquid separation- neutralize only
- Outer dimensions of standard 96-well plate
  - Robot friendly
- Larger well diameter increases volume capacity
- 117 steam/cooling water channels per plate
  - 4 channels/well
  - even heating and cooling
- Ni/Au plated wells add corrosion resistance
  - Hastalloy, other coatings option for dilute acid
- Stackable to enable multi-plate pretreatments
- Custom designed and manufactured
  - Available from Aspen Machining, Lakewood, CO, USA

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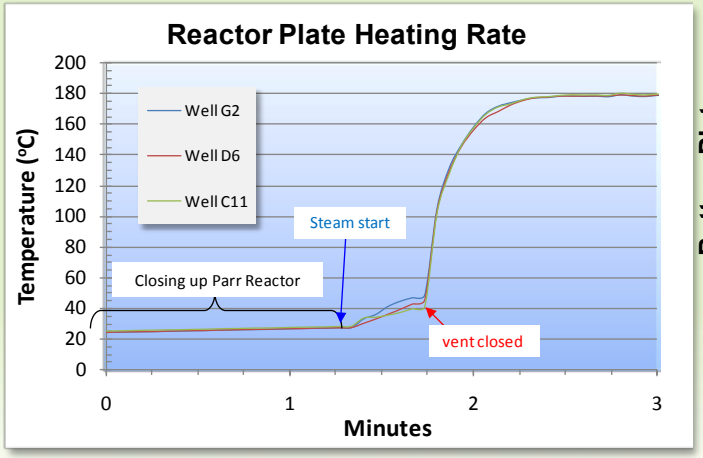
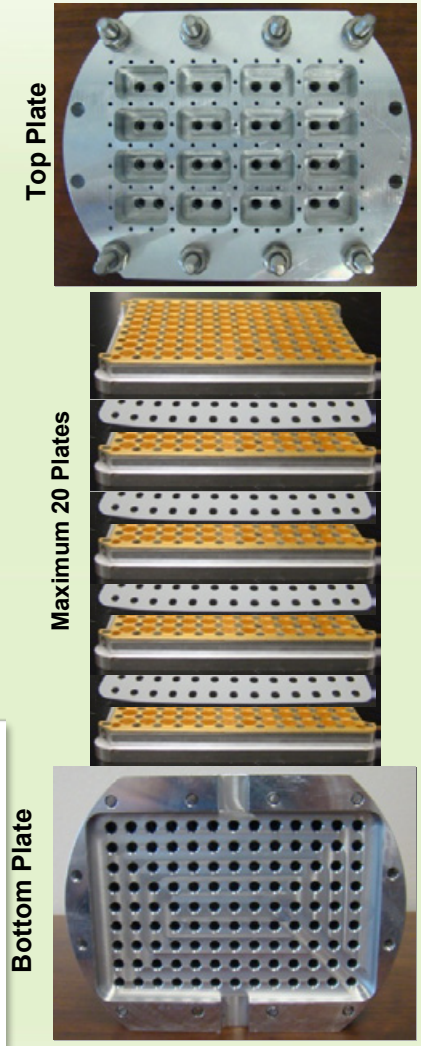
HTP Plate Clamping System Design

- Stacked system
  - 20 plates, 1920 pretreatments
- Aluminum head and base plates
  - Fit snug in Parr reactor
  - designed for rigidity
- Plate sealing
  - 0.020” glass-reinforced PFTE gaskets
  - Aluminum foil seals on plates
- Steam/cooling water enters through bottom clamping plate
- Even heating well-to-well and vertically





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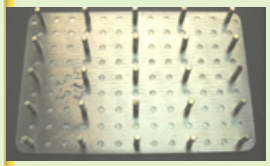
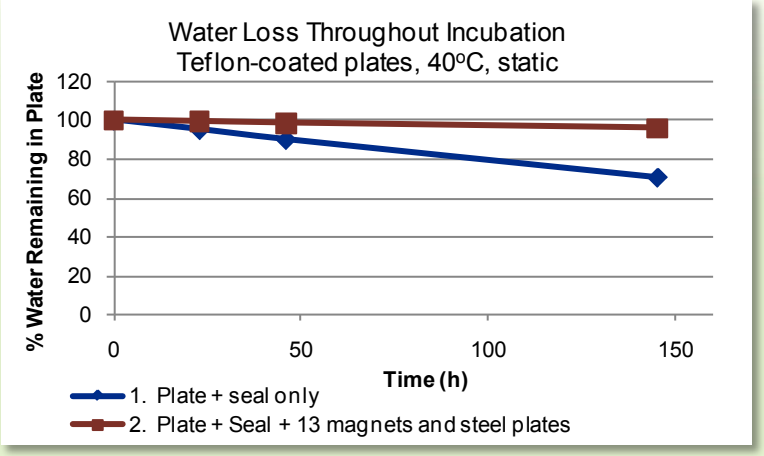
Evaporation Control-Sugar Quantitation

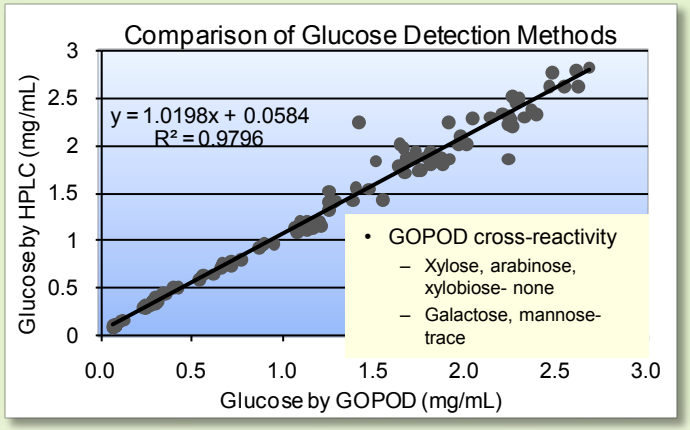
- 3 days at 40°C
- Clear adhesive seal was inconsistent
  - Edge effects
- Used magnets in steam ports
  - Press fit into aluminum base plate
  - Thin magnetic stainless steel top plate





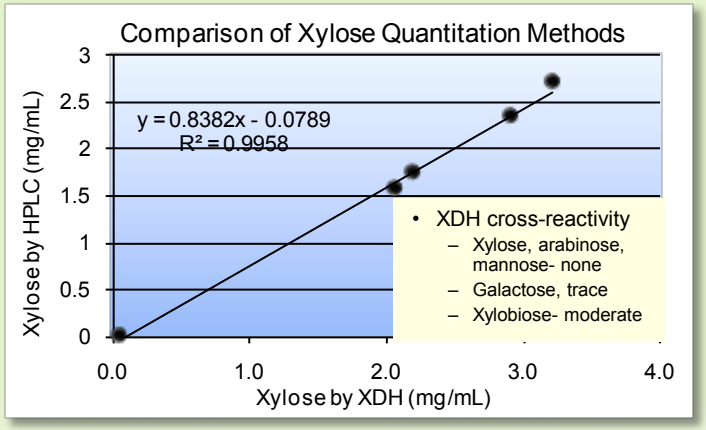
Glucose - glucose oxidase/peroxidase

D-Glucose + O_2 + H_2O → D-gluconate + H_2O_2
H_2O_2 + p-HBA + 4-aminoantipyrine → quinoneimine dye (A_{510})



Xylose - ß-D-xylose dehydrogenase

ß-D-Xylose + NAD^+ → D-xylonic acid + NADH (A_{340}) + H^+



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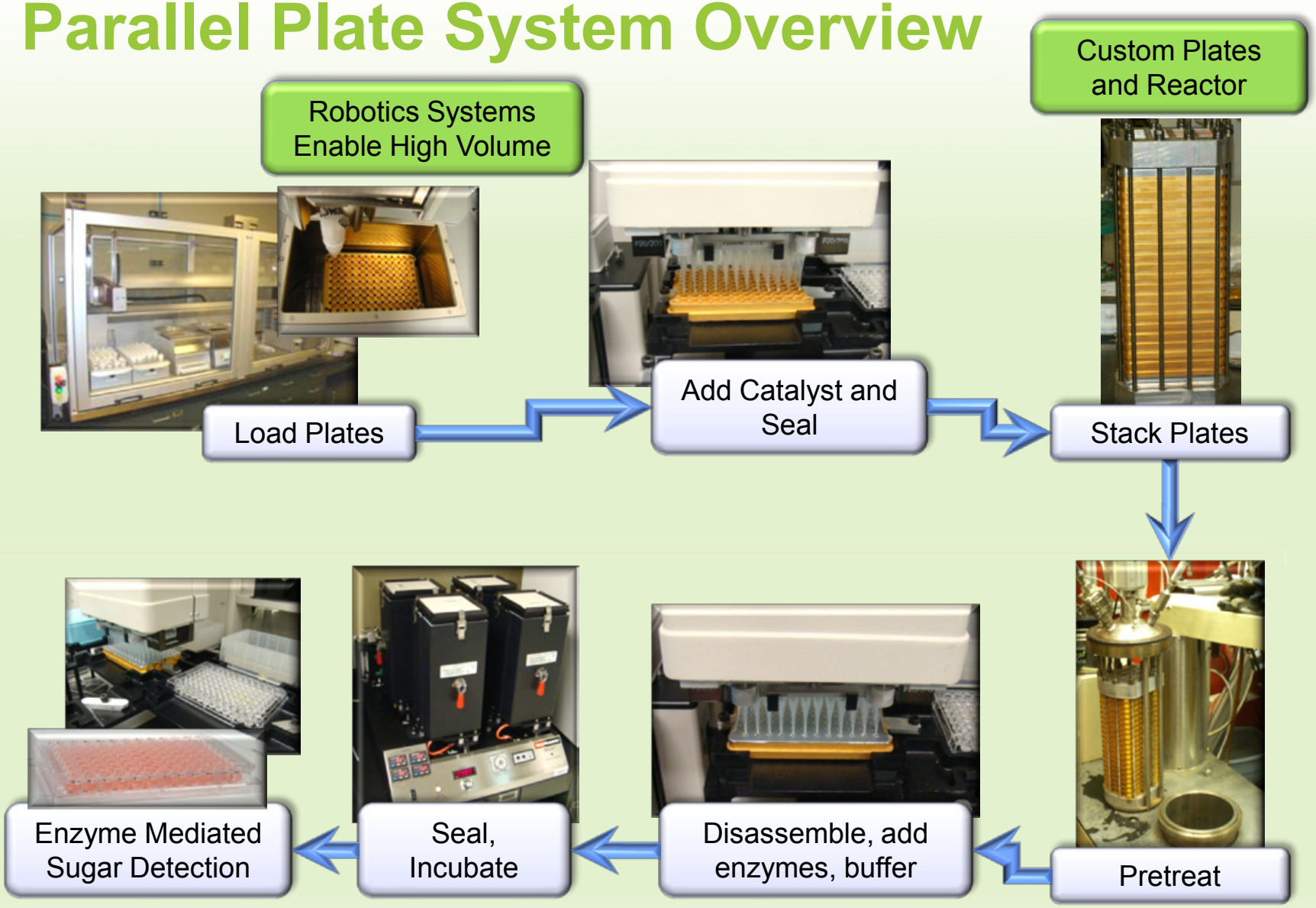
Parallel Plate System Overview



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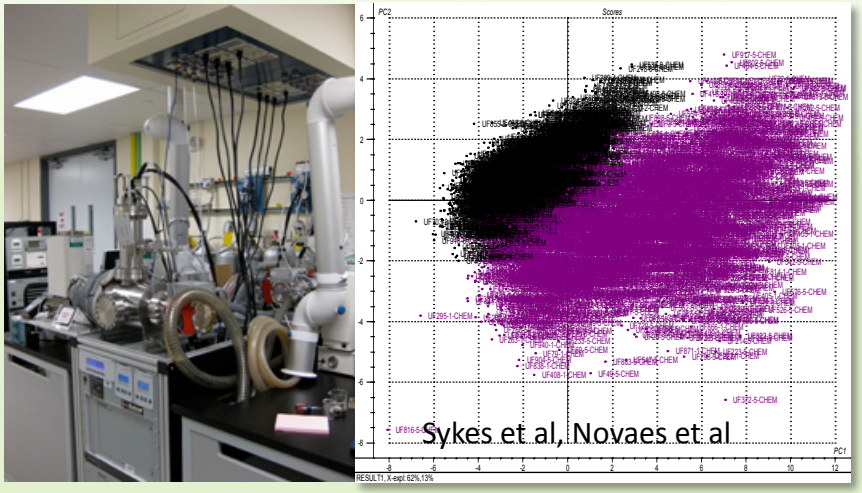
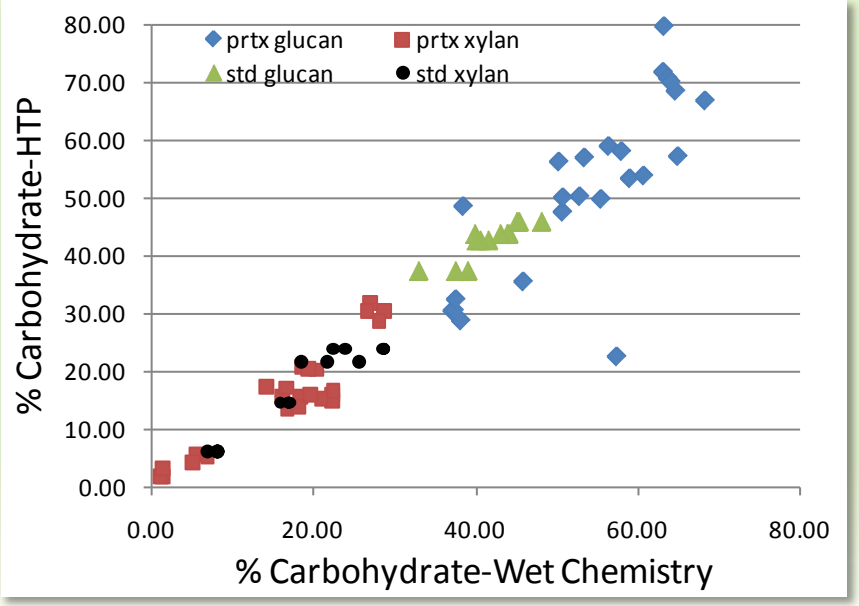
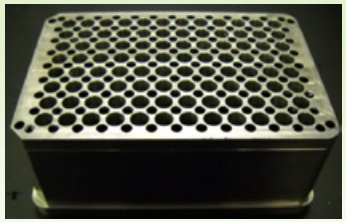
Analysis of Cell Wall Chemistry



- MBMS- rapid estimate of cell wall chemistry
  - High information content
  - Complements wet chemistry
- PCA of MS from ~1500 greenhouse poplar.
  - Black = low nitrogen
  - Purple = high nitrogen.
- The two clusters indicate differing cell wall chemistry
  - Different lignin composition



- HTP compositional analysis
  - Glucan and xylan content
  - Correlates with wet chemistry
- 24 samples in 4 hours- single reactor plate
  - 2-3 days by wet chemistry
  - Expandable to multiple plates
- Native or pretreated biomass



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Poplar Association Study-1st 2 Sets

- Error bars represent standard deviation of triplicate runs
  - reps equally distributed throughout plate; 24 samples per plate;
  - red bars are samples repeated in multiple plates
- All plate normalized to BESC poplar standard
  - 8 standard runs per plate
- Data didn't look very encouraging at first……

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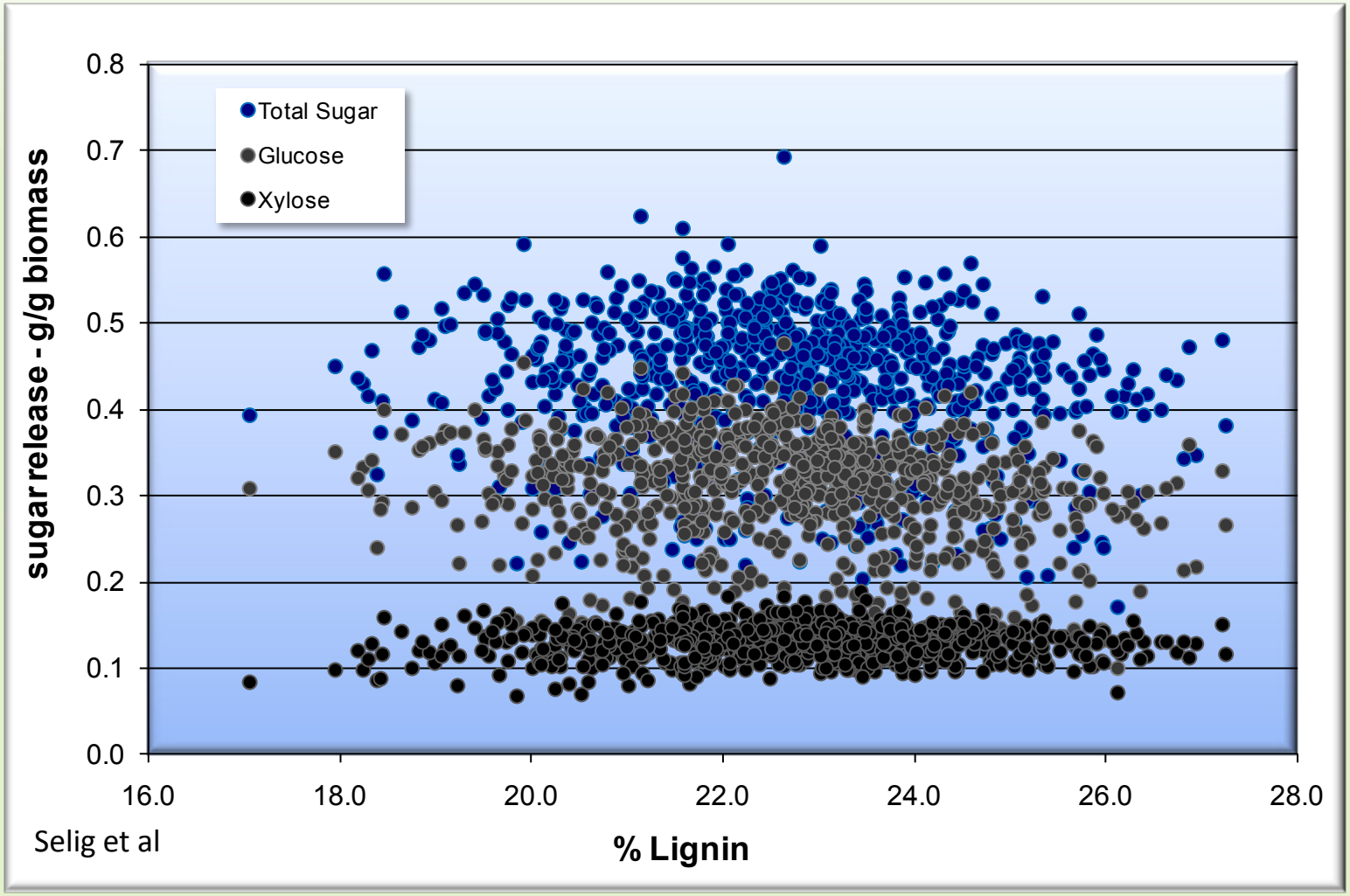
Poplar Association Study-755



- No apparent impact of lignin content on sugar release after pretreatment/enzyme hydrolysis

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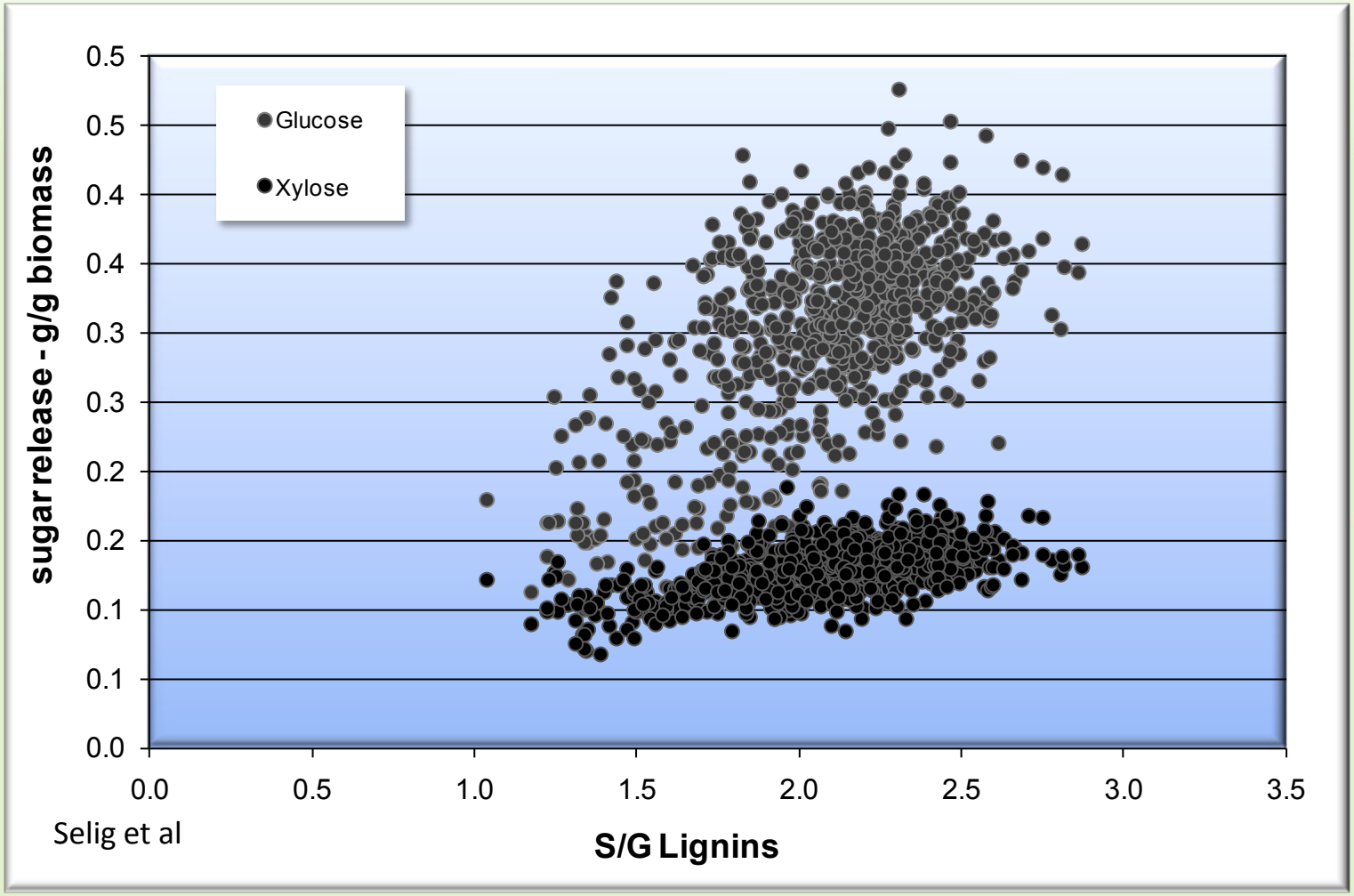
Poplar Association Study- 755



- Glucan and xylan conversions show trend with S/G
- Xylan conversion is somewhat less scattered

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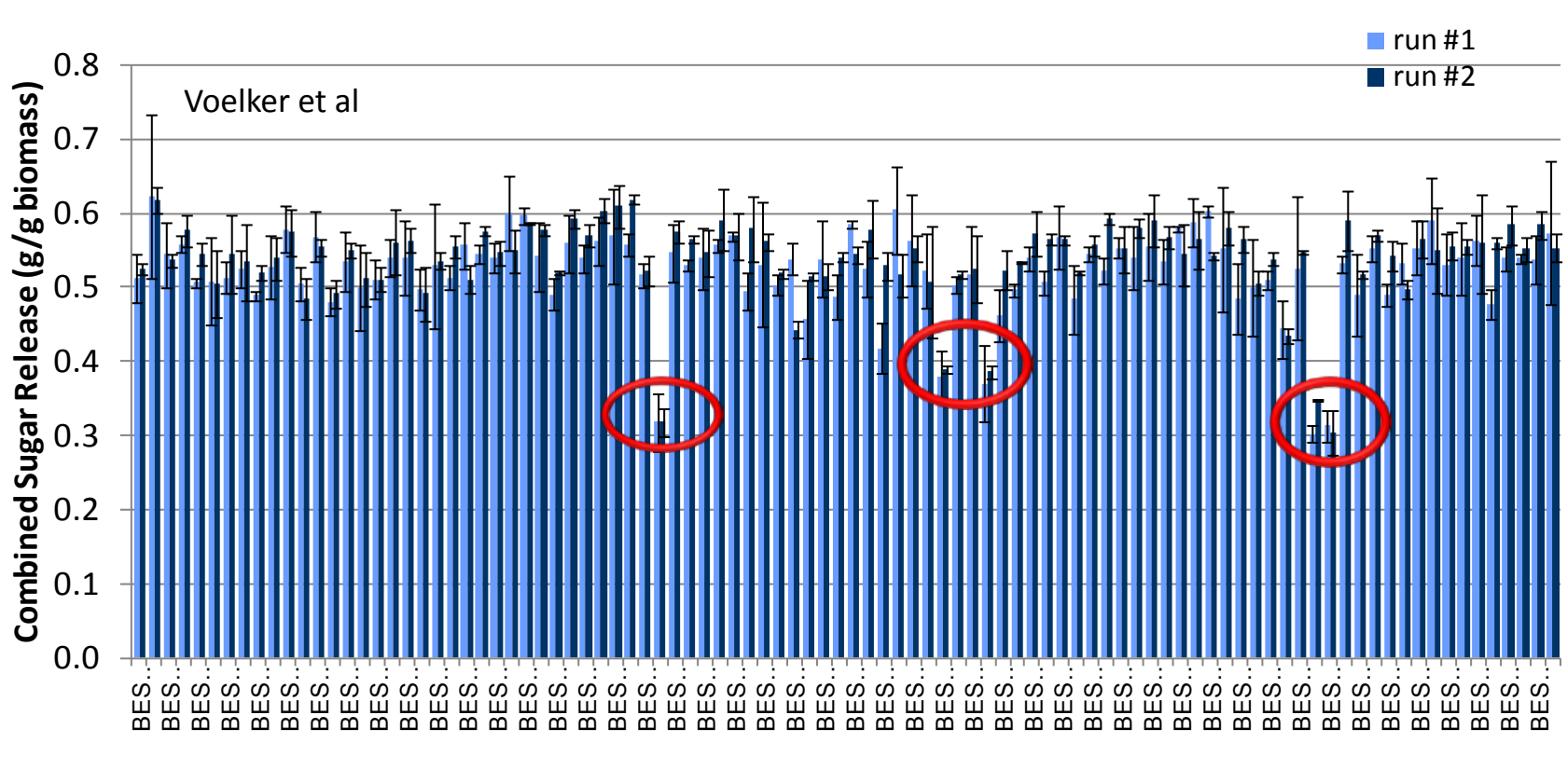
Poplar 4-CL Study- 100



- System consistently identifies outliers within small & large sample sets

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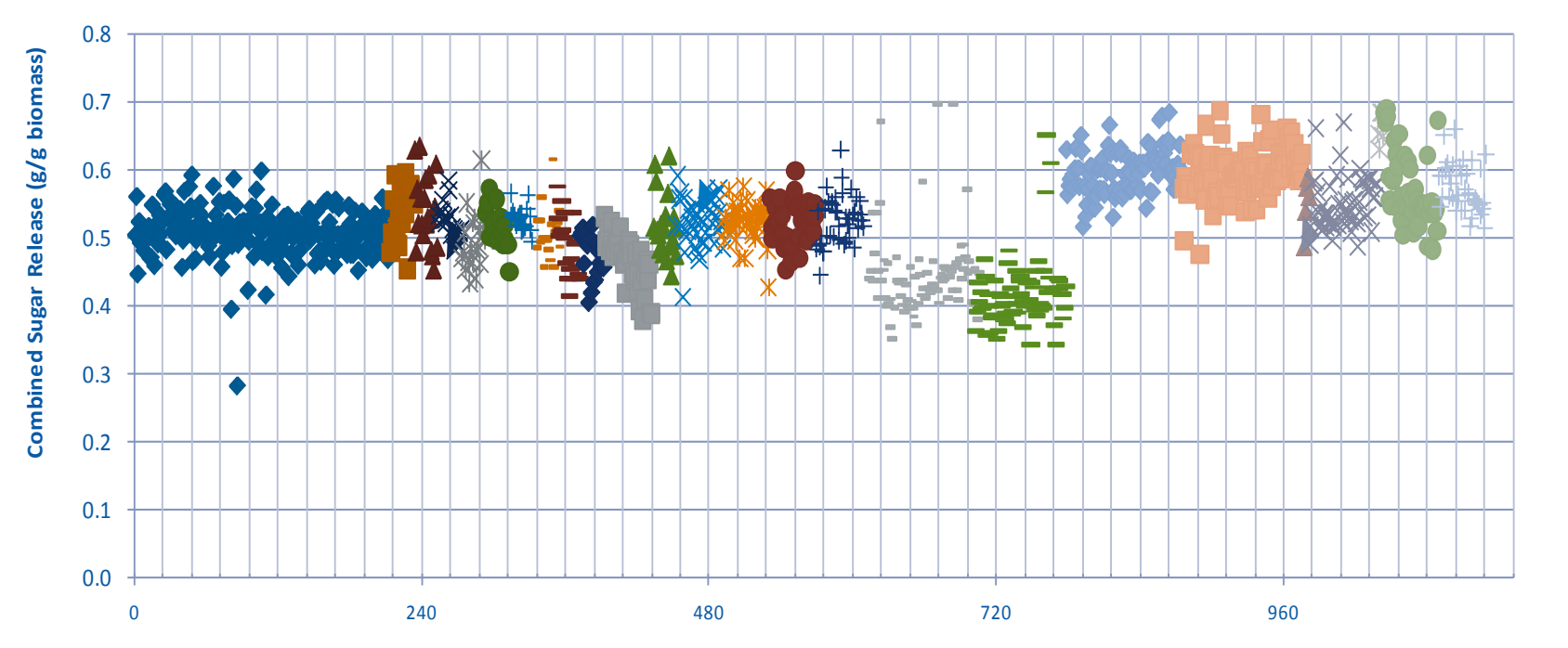
Danish Wheatstraw- 1130



- 1100 sample set – 25 separate experimental sets; strains, harvest conditions…etc
  - Collaboration with Claus Felby, U. Copenhagen
- Consistent overlap between plates
- Variation between sets and not plates

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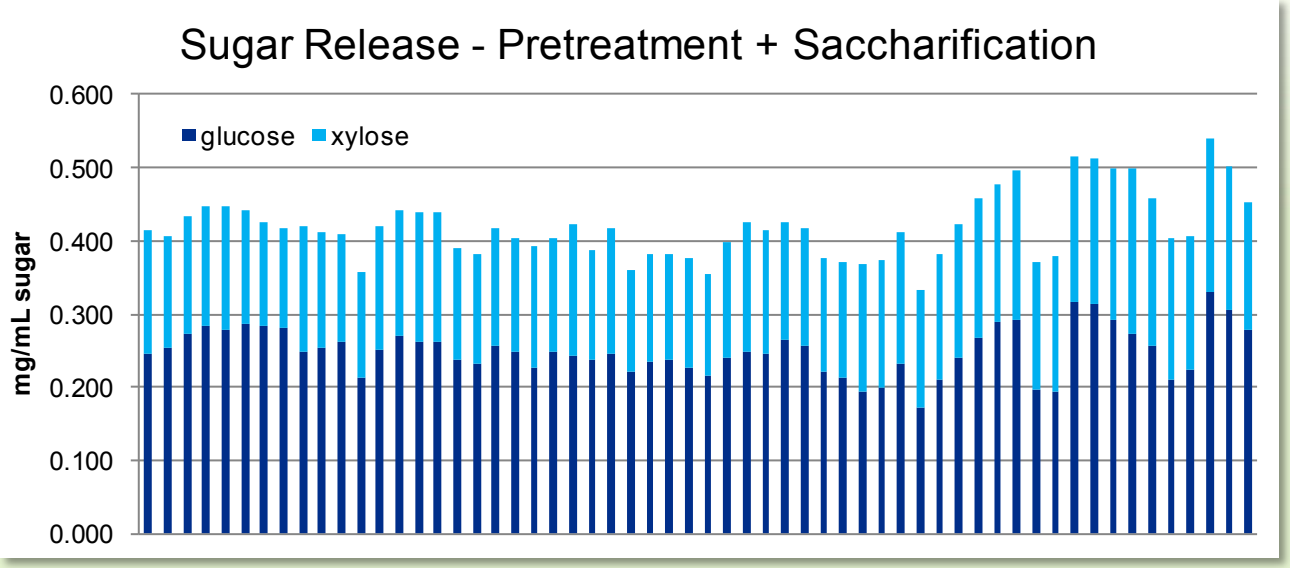
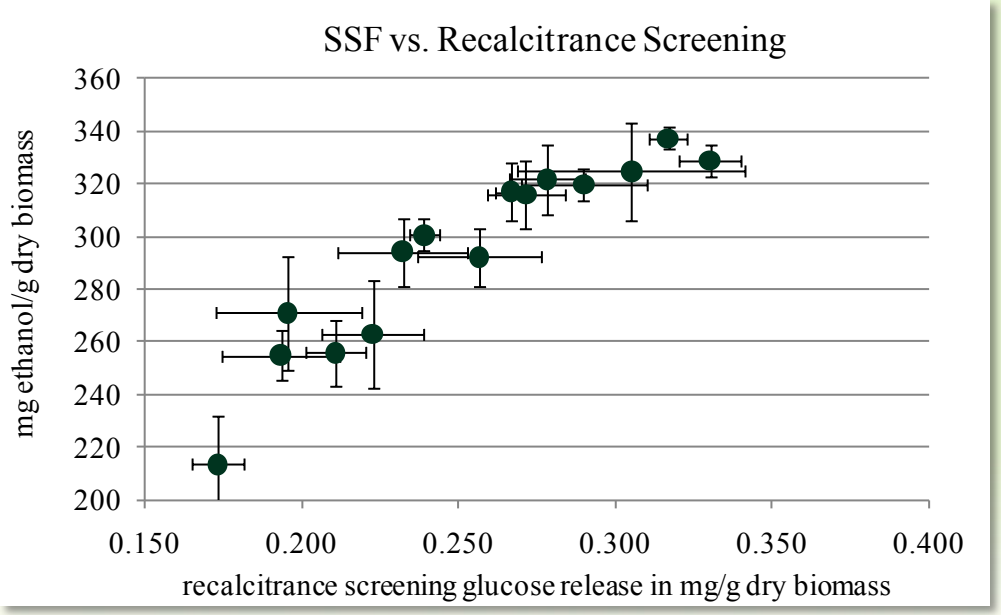
Noble Foundation Switchgrass-58





- 58 samples included controls and replicates
- SSF carried out at ORNL
  - Jonathon Mielenz lab
- First demonstration of fermentation/HTP pipeline correlation

Summary

- First high-throughput thermochemical pretreatment/enzyme digestion pipeline
  - We have run dozens of campaigns
  - ~10,000 samples analyzed
- Solved numerous obstacles
  - Biomass processing and dispensing
  - Extractives and starch variability
  - Pressure and evaporation control
  - Rapid sugar analysis
  - HTP chemical analysis
- Severe pretreatment conditions possible
  - High temperatures (150-200°C), pressures, low pH
- HTP Analytical chemistry for lignin/carbohydrate content
  - MBMS for total lignin, S/G ratios
  - HTP compositional analysis for cellulose and xylan content
  - Starch analysis is possible
- Data is comparative within sample sets
  - Determine correlations of recalcitrance with environmental factors
  - Measure effects of genetic variation
  - Find outliers in large sample populations
- Capacity of 100s of samples/day
  - Current bottleneck is sample preparation

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