2017 Project Peer Review
WBS# 2.5.1.101
Analytical Development and Support

Technology Area Session: Biochemical Conversion

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March 8, 2017

NREL is a national laboratory of the U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, operated by the Alliance for Sustainable Energy, LLC.

Goal Statement

Goal: The ADS Project supports and enables biofuel and bioproducts R&D by ensuring high quality analytical data and advancement of the tools available to the wider community through method development and globally adopted procedures

Highlight	Outcome	Relevance
• Development of tools that enable industry and R&D	• Published and adopted procedures	• Broad community enablement with validated accepted methods to measure and analyze data
• Providing reliable actionable data	• Measurement and evaluation of new technologies and processes	• Advances in technology from cross team collaboration and data-technology assessments

Role in the Overall Platform

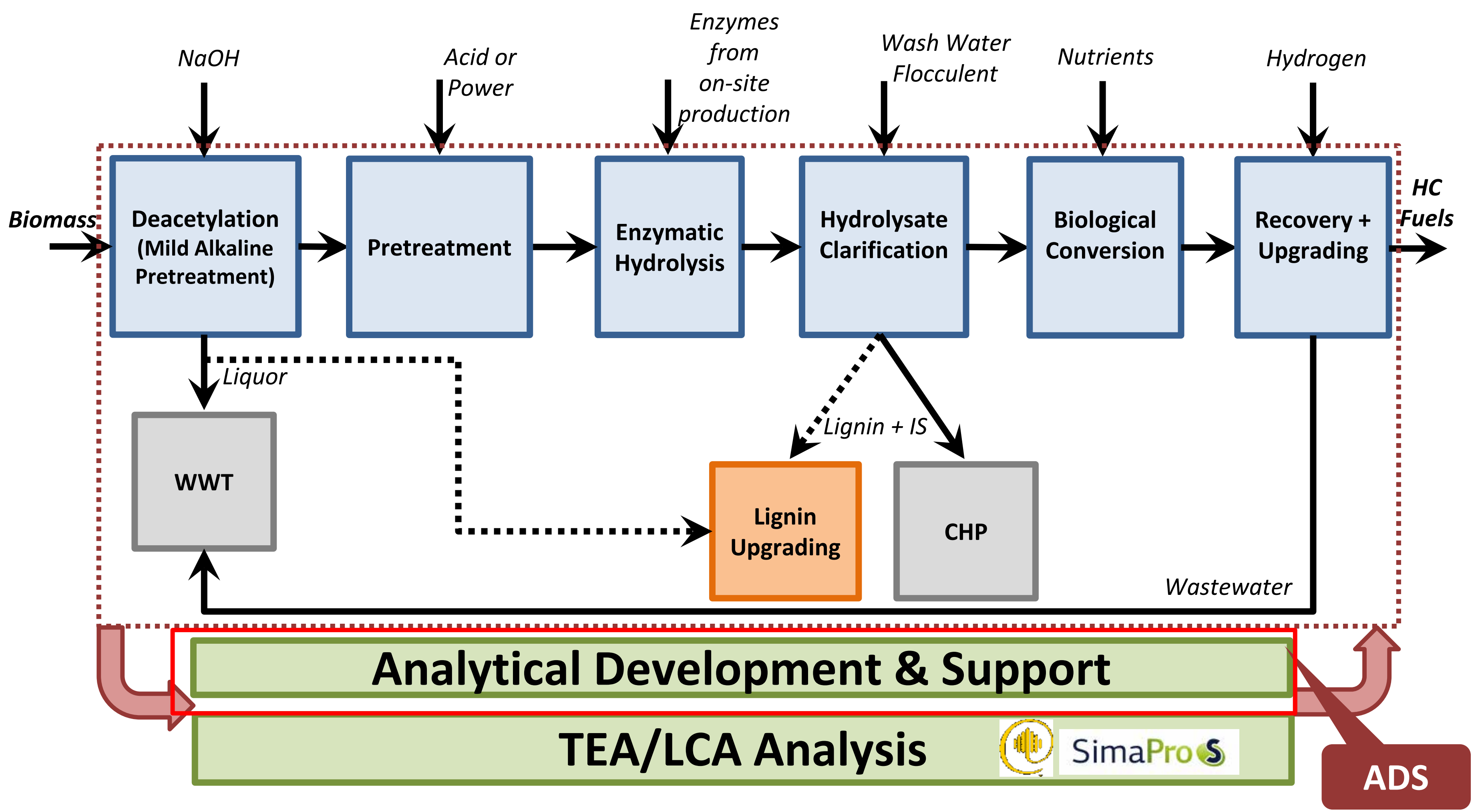
Enabling/Fundamental Technologies	Synthesis and Upgrading Technol.	Process Development	Process integration, Scale-up, Verification

- Biochem. Process Modeling and Simulation
- Targeted Microbial Development
- Enzyme Engineering and Optimization
- Biological Lignin Depolymerization
- Lignin Utilization
- Biological Upgrading of Sugars
- Catalytic Upgrading of Biochem. Intermediates
- Feedstock-Process Interface
- Pretreatment and Process Hydrolysis
- Bench-Scale Process Integration
- Separations Development and Application
- Pilot-Scale Process Integration

Analytical Development and Support

Biochemical Platform Analysis

Role in the Overall Process



Quad Chart Overview

Timeline

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

Barriers

Bt-B. Biomass Variability

- Develop methods to assess variability

Bt-C. Biomass Recalcitrance

- Measure reactivity and intermediates

Bt-D. Pretreatment Processing

- Track mass balance and constituents

Budget

	Total Costs FY12 –14	FY15 Costs	FY16 Costs	Total Planned Funding (FY17-18)
DOE Funds	$4.2M*	$1.2M	$1.2M	$2.4M

*** This project was part of former BPI Task**

Partners

Analytical chemistry work and rapid prediction models facilitated partnerships with:

- INL
- Shell
- Toyota
- Grain Millers
- **LONG LIST**

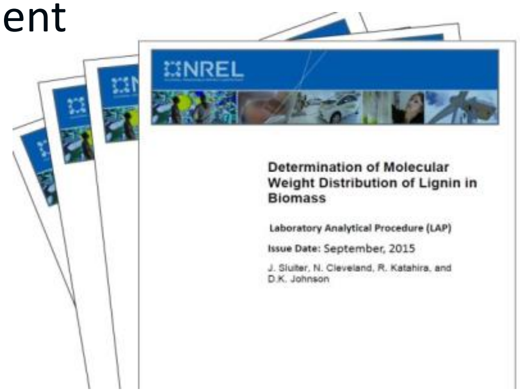
External Collaborators for previous slide

Abengoa
ASTM International
Ciris Energy
Chromatin
Cobalt
Cornell
CSU
DONG Energy
Dupont
Earth Energy
EcoPetrol
EdenIQ
Environmental Protection Agency
GrainMillers
Kellogg
Leaf Resources
KST
KiOR
Metabolix
Nexsteppe
Petrobras
Purdue
Shell
Toyota
UIUC
UT-Austin
Virent
Waste Empowered
WSU
ZeaChem

Project Overview

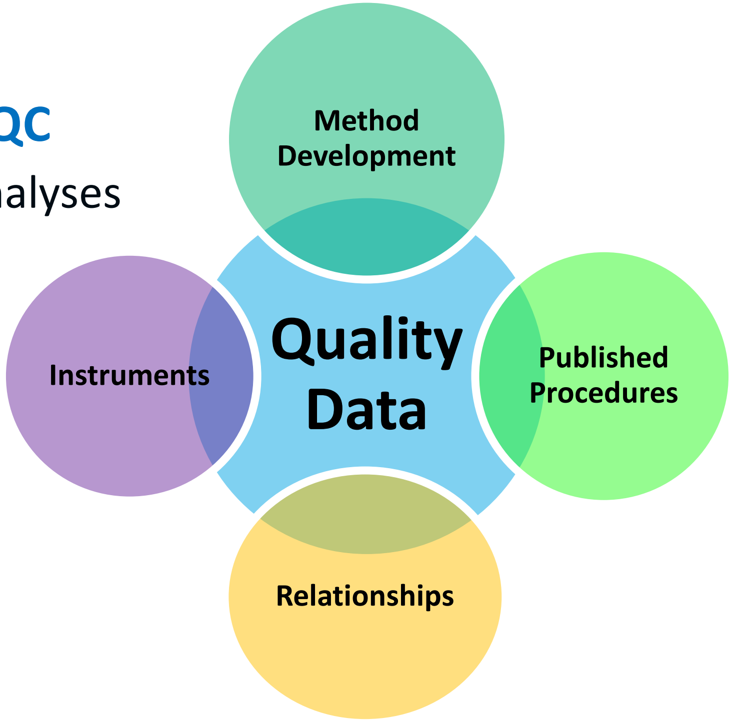
Method Development

Work with colleagues to develop and implement new analytical methods



Sample Coordination and QC

More than 14,600 platform analyses



Laboratory Support

Maintain four analytical laboratories

Maintain existing instrumentation and identify and validate new instruments

Relationships

Cultivate relationships with industry, academia, and other laboratories

Maintain NIR calibration models for licensing to partners

Approach

Analytical Development and Support (ADS) Project

Approach – Management

We conduct relevant research across the conversion value chain and work jointly with other projects across the platform to provide quality data to evaluate and meet all technical goals

Task 1: Analytical Development

Perform cross cutting research to enable technologies by providing high quality data, and recommend and develop methods

Task 2: Analytical Support

Ensure consistent data, provide functional labs, and handle sample management

Scientists

- Communicate and coordinate with PIs from program tasks
- Anticipate analytical needs based on work plans
- Recommend new methods for development
- Carry out milestones and publish work

Research technicians

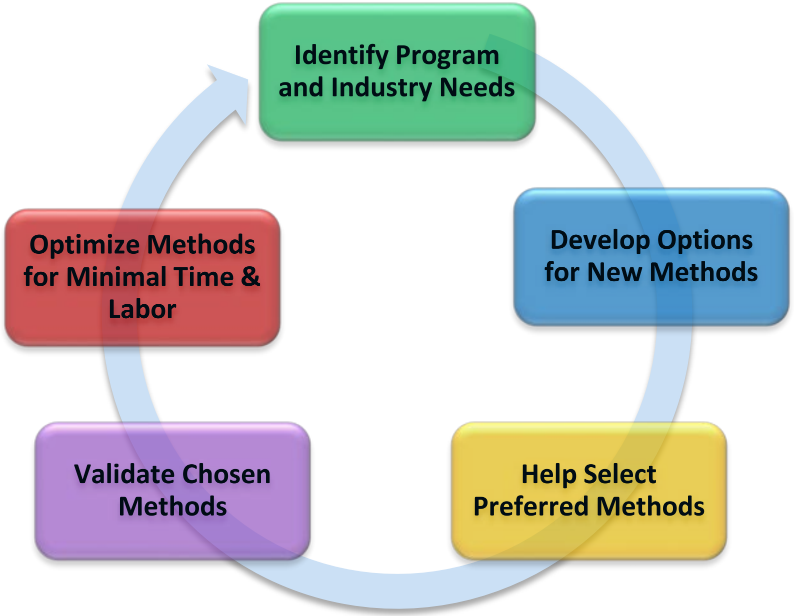
- Produce consistent quality data
- Maintain laboratory equipment
- Cross train on methods to have staff available to all projects

Approach – Technical

We focus on the evolving needs of our colleague's research, always a moving target. We are responsive to new analytical needs across multiple projects.

Critical Success Factors

- Provide quality data in a timely manner to allow research to proceed
- Ensure data is reliable and actionable
- Provide methods to the broad biomass conversion research, development, and commercialization community
- Go/no-go decision provides all analytical methods necessary for reliable data analysis for advanced biofuels/bioproducts processes

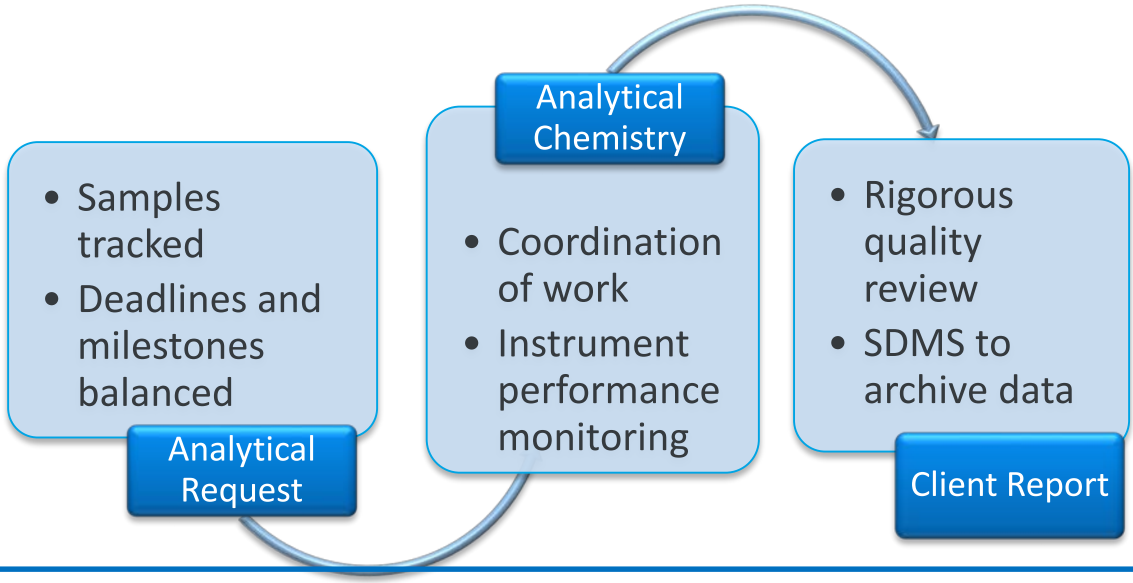


Challenges

- Keeping abreast of changing program needs
- Developing robust methods quickly
- Managing analysis for multiple platform projects
- Maintaining four laboratories and a large number of analytical instruments

Approach – Sample Management & Data Handling

Organization of thousands of samples to ensure that highest quality data is provided on time to projects to make decisions and meet deadlines



Procedures	FY16 Samples
Acids	4400
HPLC	2000
NIR	2000
Protein	1250
FAME	900
Intermediate solids	750
AFDW	550
Liquor	550
FIS	400
Extractions	300
Feedstock Solids	300
Milling	300
ASE/APL Pretreatment	200
Starch	200
Total Solids	200
Ashing	150
Washing	100
Enzymatic Hydrolysis	50
Gravimetric	15
Neutralization Capacity	5
TOTAL	**14,600**

74 tasks

> 60,000 discrete analyses

Ensure Quality Data

- Quality data across platform = **CONSISTENCY**
- QA/QC on **ALL** data
- High data quality maintains NREL's reputation as a leader in biofuel conversion
- Satisfied clients and successful projects
- No wasted research time
- No missed deadlines

Technical Accomplishments (TA)

Analytical Development and Support (ADS) Project

TA – Extending NIR Models to Predict Reactivity

- Feedstock characterization and the identification of superior feedstock candidates is a primary step in these goals.
- NREL developed a laboratory-scale pretreatment and enzymatic hydrolysis (PT/EH) assay to screen feedstock samples (FPI Project, FY14-15)
- We developed a NIR calibration model based on this assay to predict the PT/EH results from spectral data
- Predicts <u>composition</u> & <u>sugar release and yield</u>
- Contains over 200 samples, including corn stover, switchgrass, sorghum, miscanthus, and perennial cool season grasses

Using the Model

- Used to predict over 3000 samples from ***Regional Feedstock Partnership (RFP)*** **Studies –** data transferred to **INL Biomass Feedstock Library** – saved 1000s of hours of wet chemistry time
- Also used for rapid screening of samples for multiple internal & external clients

"Rapid analysis of composition and reactivity in cellulosic biomass feedstocks with near-infrared spectroscopy", Payne et al, Biotechnology for Biofuels, 8, 43 (2015).

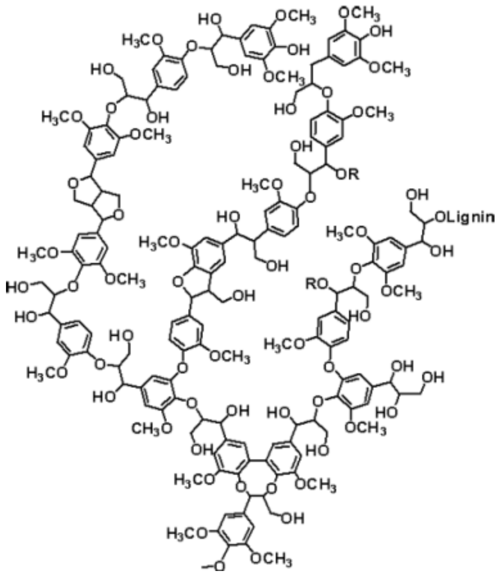
TA – Improving Analysis for Industry, New Processes, and Speed

Non-structural carbohydrates and starch

- Generation 1.5 biofuels and RIN credits require differentiation of carbohydrates
- Developing standardized methods for community use

Lignin

- Lignin utilization is critical to overall process economics, but characterization is still problematic
- Developing new methods required for alkaline pretreated samples



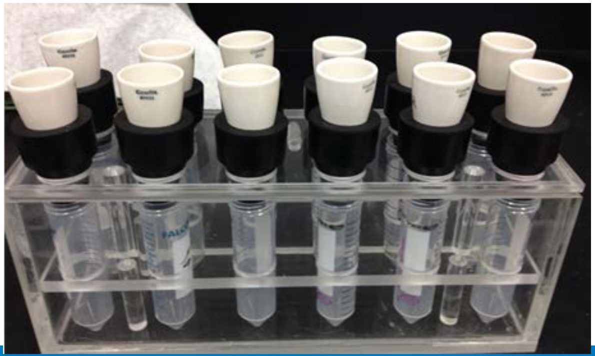
TA – Improving Analysis for Industry, New Processes, and Speed

High Throughput

- 3X increase in analytical hydrolysis throughput
- New lignin measurements 8 times faster
- Increased throughput extractions

Investing in new analytical technology

- LCMS (Q-ToF) for identification of compounds (lignin, lipids, carbohydrates) based on molecular structure
- IC-PAD for improved carbohydrate measurement



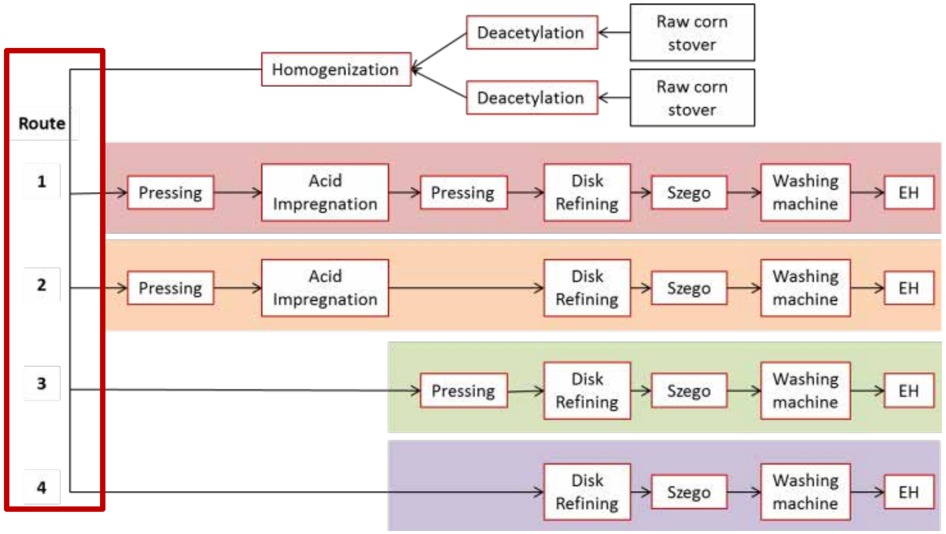
TA – Carbohydrate Tracking across DMR to Enable Process Evaluation

DMR (deacetylation and mechanical refining)

DDMR (deacetylation, dilute acid impregnation, MR)

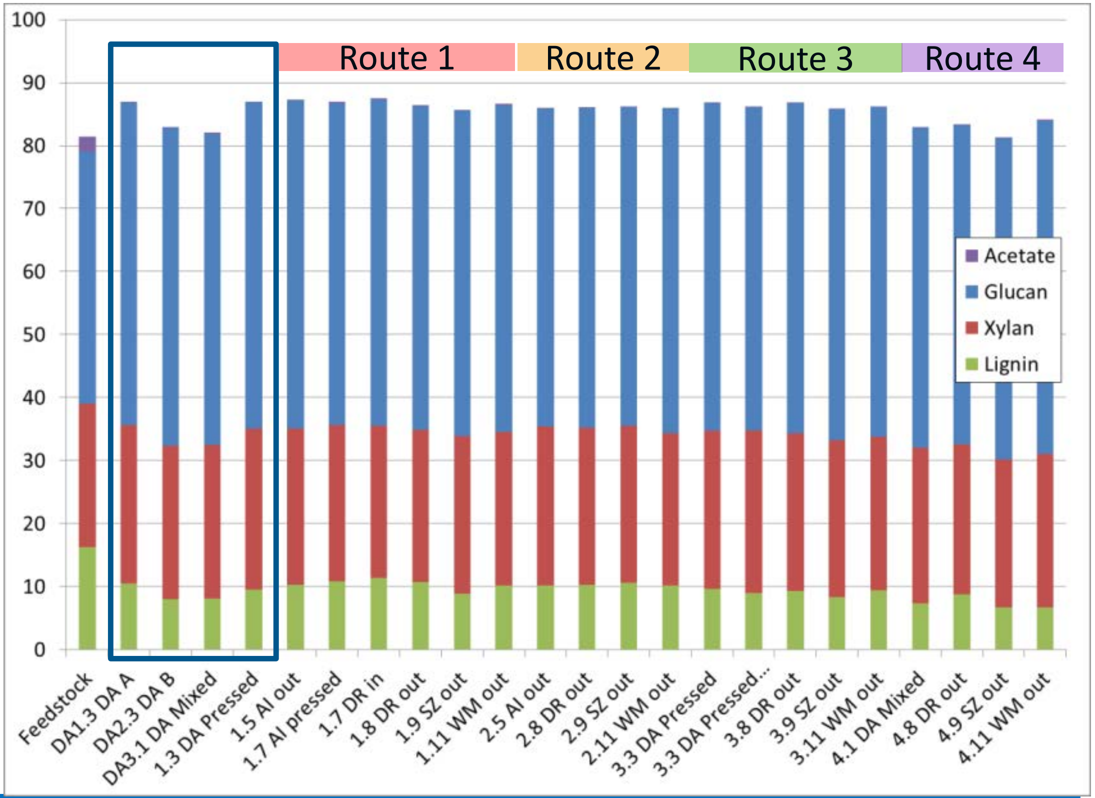
- Previous results indicated an increase in carbohydrate content after the deacetylation step in mechanical refining, which is contrary to our understanding of how DMR functions
- Hypothesis- the only change in the chemical composition of feedstock should take place at the deacetylation step

- **This work was critical to interpreting and understanding the results of the process teams**
- **This project is an excellent example of close collaboration between analytical and process development tasks to design and execute experiments**



TA – Carbohydrate tracking across DMR to Enable Process Evaluation

- We saw no practical differences in carbohydrate content across mechanical refining
- Materials were very difficult to sample representatively
- Lignin and acetate measurements under these conditions were problematic – working on this in FY17



This work demonstrates a collaboration between analytical and process development teams to deconvolute processes and data.

Additionally, work done in the pilot plant is very different than work done on the bench; the pilot plant is more representative of industrial environments.

TA – Developed Improved Fraction Insoluble Solids (FIS) Method for Throughput and At-line Capability

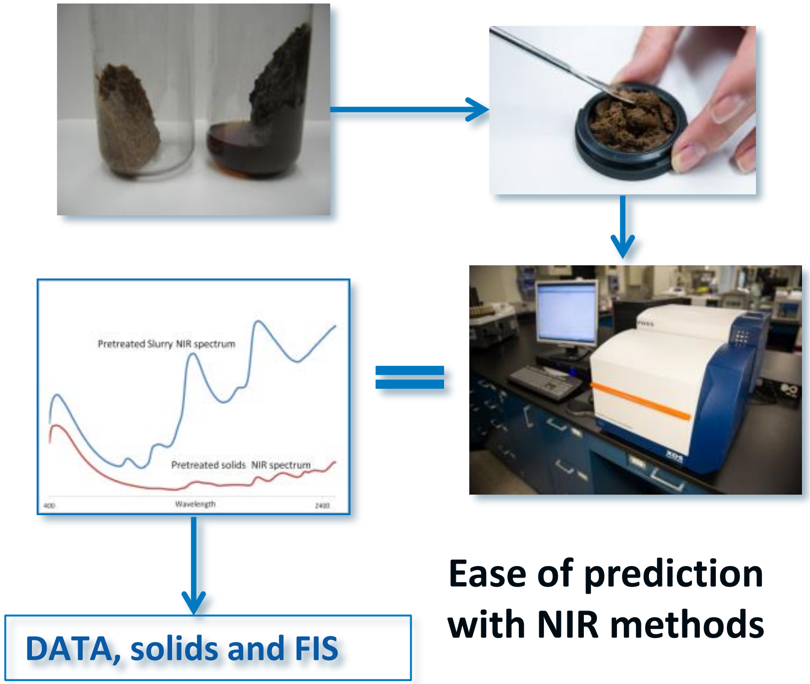
FIS is the major analytical contributor in <u>process uncertainty</u> for cellulose enzymatic hydrolysis. Traditional methods are not sufficient, so we improved them in several ways.

Improved benchtop methods

- Building on previous work on this project
- 6x throughput with increased precision
- Optimized methods based on sample type

New NIR method

- Whole slurry used, no separation hassle
- Multiple data from one scan
  - FIS
  - Solids composition
- <u>Fast and inexpensive</u>
- <u>No waiting for traditional analysis to begin enzymatic hydrolysis</u>
- Relevant to bench and process scale experiments
- Manuscript in preparation



"Improved methods for the determination of drying conditions and fraction insoluble solids (FIS) in biomass pretreatment slurry", Sluiter et al, *Biomass and Bioenergy*, 91 (2016).

Relevance

Analytical Development and Support (ADS) Project

Goal: The ADS Project supports and enables biofuel and bioproducts R&D by ensuring high quality analytical data and advancement of the tools available to the wider community through method development and globally adopted procedures

Relevance – Technology Transfer

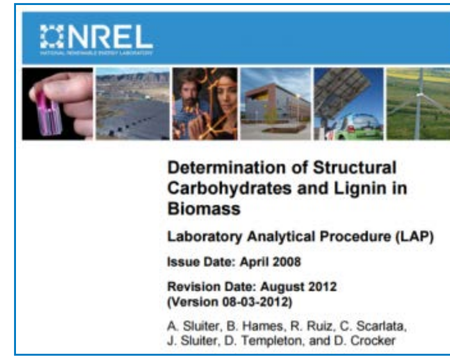
We regularly work with external partners; these relationships are based on our reputation for excellence in Biomass Compositional Analysis

- Quality data is essential for TEA, and an extremely effective tool for technology transfer
- Many partnerships begin with analytical requests
- We maintain NIR calibration sets and predictive models
- NREL licenses underlying NIR and chemical data to companies, enabling them to develop their own models

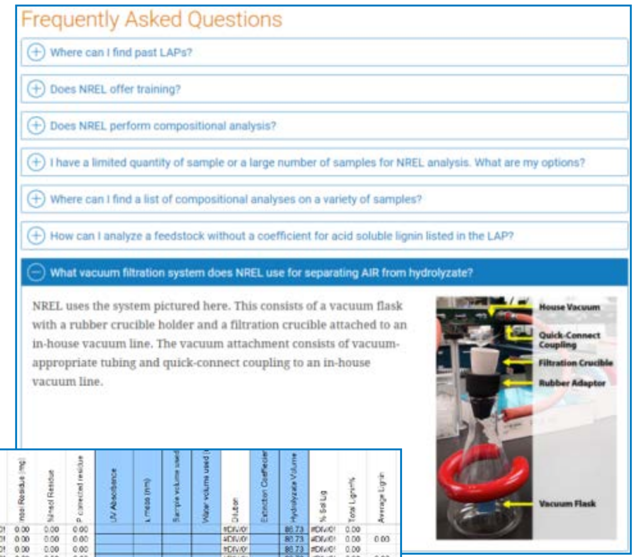
Abengoa	Dupont	Leaf Resources	Toyota
ASTM International	Earth Energy	KST	UIUC
Ciris Energy	EcoPetrol	KiOR	UT-Austin
Chromatin	EdenIQ	Metabolix	Virent
Cobalt	Environmental	Nexsteppe	Waste Empowered
Cornell	Protection Agency	Petrobras	WSU
CSU	GrainMillers	Purdue	ZeaChem
DONG Energy	Kellogg	Shell	

Relevance – Laboratory Analytical Procedures (LAPs)

NREL is a leader in biofuels analysis and our methods let the world-wide community "speak the same language"



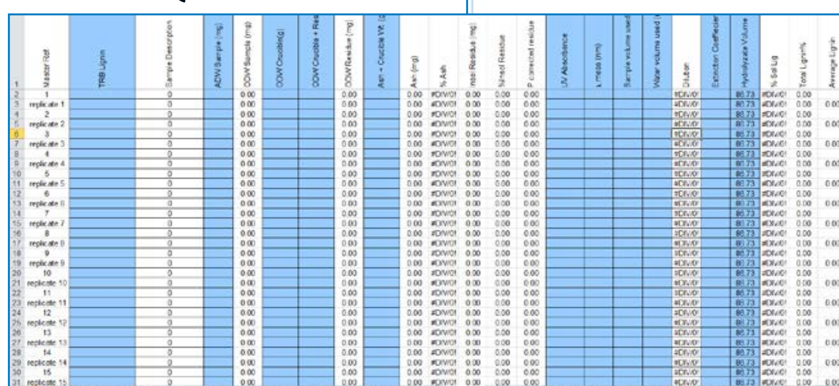
- Regularly evaluated and updated to meet the changing needs of program and industry
- Used world-wide as *de facto* standards for analysis of biomass
- Several methods have been adopted by ASTM
- Recently used for recertification of NIST's Biomass Reference Materials



www.nrel.gov/bioenergy/biomass-compositional-analysis

Includes procedures, calculations, and FAQ

- Page Views (unique)
  - FY14 25,000
  - FY15 9,800
  - FY16 10,500
- Procedure Downloads
  - FY14 6,100
  - FY15 8,700
  - FY16 3,200



Relevance – Leading Collaborative Analysis

- NREL led recertification of NIST Biomass Reference Materials
- Involved 11 academic, industrial, and government labs
- NREL coordinated analysis, contributed data, compiled results, and helped with statistical analysis
- RMs were recertified with new reference values

NIST National Institute of Standards and Technology U.S. Department of Commerce

"Compositional Analysis of Biomass Reference Materials: Results from an Interlaboratory Study", Templeton et al., *BioEnergy Research,* 9,1 (2016).

- Large international effort with NREL and seven Brazilian laboratories
- NREL contributed analytical data, compiled results, and performed statistical analysis to determine interlaboratory uncertainties
- Large variability in performance across labs

"Evaluation of Brazilian Sugarcane Bagasse Characterization: An Interlaboratory Comparison Study", Sluiter et al., *Journal of AOAC International,* 99,3 (2016).

Relevance – Outreach to External Stakeholders

- Working with EPA on the Renewable Fuels Standard (RFS) guidelines for qualifying pathways to cellulosic ethanol
- This work is developing a better understanding on suitable analytical methods for quantification of cellulose converted to ethanol in “Gen 1.5” processes
- The ADS Project is uniquely suited to this role with its history of analytical methods and relationship with the ASTM methods

ASTM INTERNATIONAL

- NREL is a member of Subcommittee E48.05 on Biomass Conversion
- NREL Laboratory Analytical Procedures (LAPs) were adopted by ASTM almost 10 years ago
- NREL is leading ongoing discussions to improve lignin analysis

Relevance – Laboratory Management and Support

Safe well-run laboratories and instruments that work every time are essential to research. This takes time, diligence, training, and communication.

Laboratory Management

- This is not the most visible work we do, but it is very important to the success of the platform
- Project members are in the lab, able to provide training and oversight for shared instrumentation

Training

- Scientists well-trained on procedures and safety to support platform tasks with analysis
- Long-term training and guidance are necessary to produce fully competent analysts

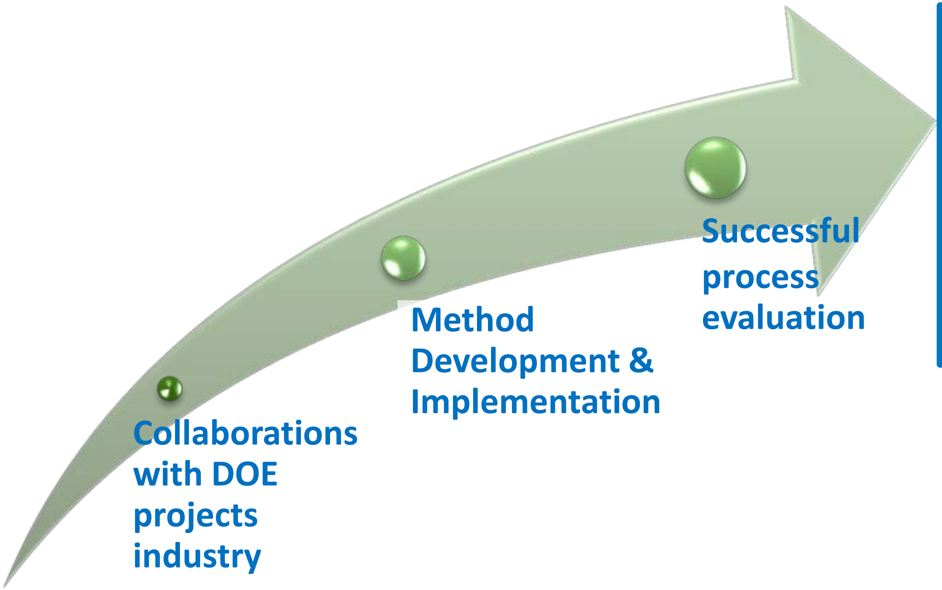
Instrumentation

- This project has primary responsibility for instrument upkeep
- Troubleshooting, repairs, and routine maintenance
- We place and monitor subcontracts for instrumentation that supports most program tasks

Future Work

Analytical Development and Support (ADS) Project

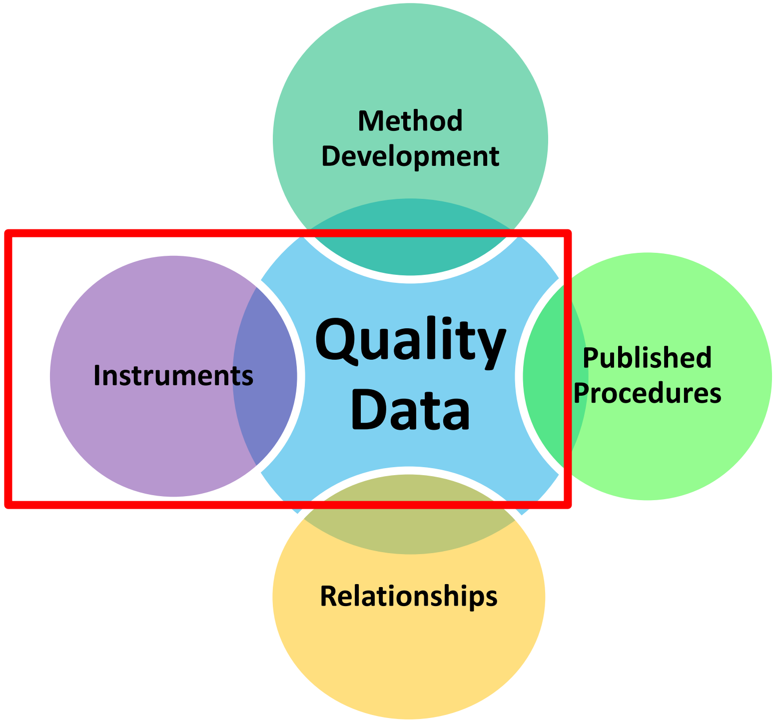
Future Work – Quality Data and Maintained Labs



Continue to provide quality data

- **Allows us to understand processes**
- **Understanding lets us optimize**
- **Moves research forward and provides industry with the best technology**

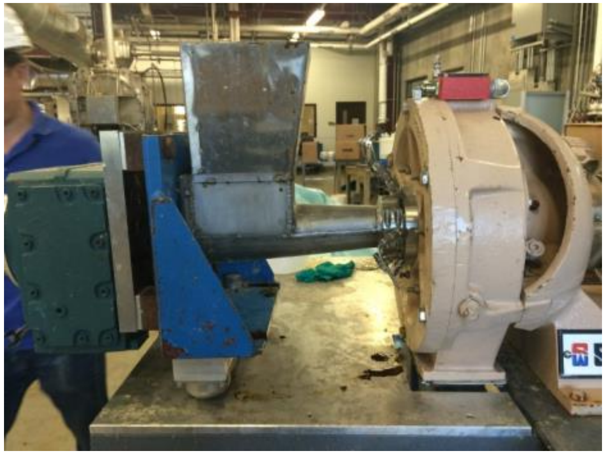
- A hallmark of this project is ensuring quality data
- Continuing to provide data with proper QA/QC ensures milestones are met
- Well maintained labs are essential to platform function
- Organizing samples and data, and keeping labs running smoothly is a function that will always be critical to program success

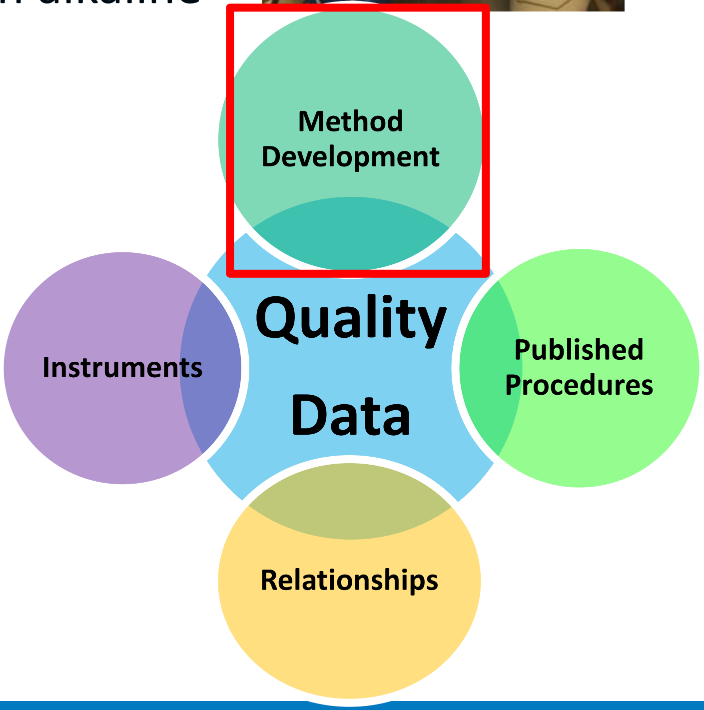


Future Work – Continue Pretreatment Tracking

DMR/Alkaline Biomass Samples

- FY17 Q4 milestone to address lignin and acetate tracking; we need improved measurement capabilities to understand these changes
- Lignin tracking and characterization is key for lignin upgrading
- Biomass appears to absorb NaOH which complicates analysis
- Lack of standardized methods for analysis of lignin in alkaline solution

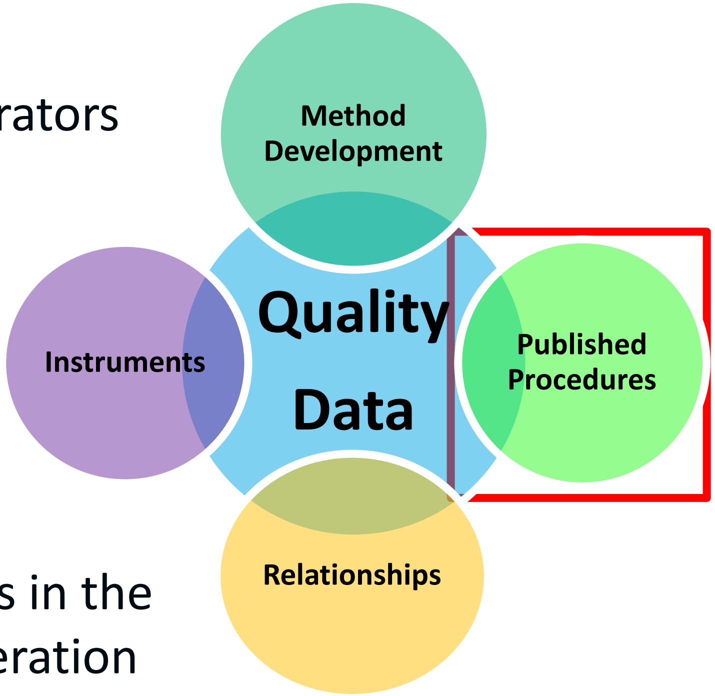




Future Work – External Engagement

Support generation 1.5 biofuels

- Engaging EPA to determine appropriate cellulosic RIN conversion
- Establishing starch standards with external collaborators
- Publishing standard starch and free carbohydrate procedures



ASTM

- Revising current ASTM methods to reflect advances in the field and changing industry needs, specifically generation 1.5 cellulosic ethanol
- Leading discussions on appropriate methods for analysis of biomass and biofuels

Future Work – Moving to more mature technologies

We will develop more effective online tools and methods for process optimization, deploying these for industrial and R&D applications

NIR- online monitoring

- At-line and on-line fermentation monitoring
- Very low-cost NIR spectroscopy approaches
- 2018 milestone to demonstrate in-line saccharification and fermentation monitoring at the pilot-scale using low-cost near-infrared spectroscopy

Characterize fermentation coproducts

- Carbon and nitrogen mass balance around fermentation including coproduct characterization
- Develop standardized procedures for use with current and future organisms
- Provide information that will minimize EPS interference in fermentations

Summary

Goal: The ADS Project supports and enables biofuel and bioproducts R&D by ensuring high quality analytical data and advancement of the tools available to the wider community through method development and globally adopted procedures

Approach

- Method development
- Quality data
- Community support
- Published procedures
- Collaborations
- Laboratory management

Accomplishments

- Developed reactivity NIR models
- Continued improvements in industrially relevant methods
- Tracked DMR carbohydrates for process evaluation
- Developed faster fraction insoluble solids method and at-line NIR capability

Relevance

- Industry support
  - Guide standards and references
  - NIR models available for license
- LAPs
- Quality reliable data is crucial for processes
- Outreach to key external collaborators and the larger community
- Laboratory Management

Future

- Maintain quality data and labs
- DMR process characterization
- Alkaline sample methods
- Support EPA and ASTM standards and regulations
- Maintain and cultivate external relationships
- Fermentation coproduct quantification
- NIR monitoring

Acknowledgements

- Ian Rowe (BETO)
- Amie Sluiter
- Justin Sluiter
- Courtney Payne
- Deborah Hyman
- David Templeton
- Bill Michener
- Michelle Reed
- Darren Peterson
- Jeff Wolfe
- Kailee Potter
- Ryan Ness
- Brenna Black
- Kelsey Ramirez

Publications & Presentations 2015-2016

Payne CE, Wolfrum EJ. Rapid analysis of composition and reactivity in cellulosic biomass feedstocks with near-infrared spectroscopy. Biotechnol Biofuels 2015;8:1–14.

Templeton DW, Wolfrum EJ, Yen JH, Sharpless KE. Compositional Analysis of Biomass Reference Materials: Results from an Interlaboratory Study. BioEnergy Res 2015.

Sluiter JS, et al. Evaluation of Brazilian Sugarcane Bagasse Characterization: An Interlaboratory Comparison Study. Journal of AOAC International 2016;99:3.

Sluiter A, Sluiter J, Wolfrum E, Reed M, Ness R, Scarlata C, et al. Improved methods for the determination of drying conditions and fraction insoluble solids (FIS) in biomass pretreatment slurry. Biomass and Bioenergy 2016;91:234–42

Sievers DA, Kuhn EM, Stickel JJ, Tucker MP, Wolfrum EJ. Online residence time distribution measurement of thermochemical biomass pretreatment reactors. Chem Eng Sci 2016;140:330–6.

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Wang DR, Wolfrum EJ, Virk P, Ismail A, Greenberg AJ, McCouch SR. Robust phenotyping strategies for evaluation of stem non-structural carbohydrates (NSC) in rice. J Exp Bot 2016;67:6125–38.

Lischeske JJ, Crawford NC, Kuhn E, Nagle NJ, Schell DJ, Tucker MP, et al. Assessing pretreatment reactor scaling through empirical analysis. Biotechnol Biofuels 2016;9:213.

Turner MF, Heuberger AL, Kirkwood JS, Collins CC, Wolfrum EJ, Broeckling CD, et al. Non-targeted Metabolomics in Diverse Sorghum Breeding Lines Indicates Primary and Secondary Metabolite Profiles Are Associated with Plant Biomass Accumulation and Photosynthesis. Front Plant Sci 2016;7:953.

"Pretreatment Reactor Scaling: Comparing Conversion Performance Across Different Pretreatment Reactors " (poster), Edward Wolfrum, Daniel Schell, James D. McMillan, Nick Nagle, Melvin Tucker, Erik M. Kuhn, Nathan Crawford and James Lischeske, 38th Symposium on Biotechnology for Fuels and Chemicals, Baltimore MD, April 2016

"Effect of drought on composition and bioconversion for Miscanthus, mixed perennial grasses, and switchgrass as bioenergy feedstocks," (poster) A. Hoover, R. Emerson*, D. Stevens, A. Ray, J. Lacey, M. Cortez, C. Payne, R. Kallenbach, M. Sousek, and R. Farris. 38th Symposium on Biotechnology for Fuels and Chemicals, Baltimore MD, April 2016

"NIR/PLS Models to Predict Pretreated Corn Stover Slurry Solids, Isolated and In-situ", Sluiter, et al, oral presentation., 37th Symposium on Biotechnology for Fuels and Chemicals, May 2015

Responses to Previous Reviewers' Comments

OVERVIEW

- Excellent overview, with clear indication of where the project fits in the overall platform.
- Quality data is key.

APPROACH

- Good technical and management approach. The collaboration management approach will support many projects and have a greater impact; good to see. Technical approach of focusing on providing what the client is and will be looking for is excellent.
- Good approach, balancing supplying services when they are needed with maintenance, developing

PROGRESS

- Outstanding performance to date. Over 6000 analyses done FY14! Development of tools and methods that have direct impact on industry is terrific. Great project.
- Vast number of analysis complete. Plus QA/QC of those.
- QA/QC support and coordinate analyses of data
- Develop of software tools to make life easier is excellent. Data management is a huge undertaking.
- Consistency of data across projects/ researchers is vital
- Enhanced mass balance closure with enhanced sample measurements is outstanding and important.
- Pro active in developing analytical procedures to keep up with the focus of the researchers is good.
- Technology transfer of models is excellent.
- Speed and quality seems to be a theme.
- Publishing the analytical procedures is really beneficial to the private sector.
- Up keep of analytical instruments and training.

RELEVANCE

- This is probably one of the most relevant projects in the portfolio, supporting so many of the others and providing meaningful analytical data. Technology transfer to industry is a major plus.
- This is so relevant that most of the other work would stop without it.
- Key enabling technology for many BETO projects and contributing to overall goals. Publication of methods has a high impact on the scientific community, enabling more effective R&D.

FUTURE WORK

- "Keep doing what you're doing" seems odd for a development project, but works perfectly here for future work. It seems that it means not just continuing to provide analytical services, but to remain agile and ahead of the industry, providing methodologies and models as needed to advance many projects.
- Directional plan in place. Much is dependent on demands from other projects.
- Keep doing what you are doing

Overall Impressions (Not Scored)

- This is one of the most important activities in the program. Consistent and reliable data is of utmost importance.
- Great project that has focused its effort on allowing other projects to progress and/or complete successfully. It sounds like the method development effort is proactive and stays ahead of industry, this is great news. Integration with paying customers has allows the project to progress with nominal funding that should continue in order to support this terrific resource.
- Absolutely the most important support task, system will come to a halt without this being done correctly.
- This activity has stepped up over the years to enable all of the NREL success as well as made pioneering inroads into the fundamental analysis and support to the industry.
- Key activity that seems to run well, responding to the needs of customers, and providing critical data and methods to the community.
- The importance of the analytical methods and procedures and the ability to deliver quality data in a timely manner is huge.

Acronyms

- DDMR - deacetylation, dilute acid pretreatment, and mechanical refining
- DMR - deacetylation and mechanical refining
- FIS - fraction insoluble solids
- NIR - near infrared
- PDU – Process Development Unit

Additional Slides

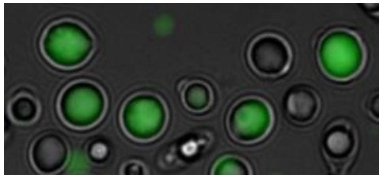
Analytical Development & Support (ADS) Project

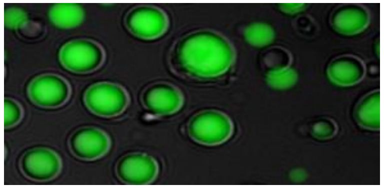
TA – EPS Characterization to Remove Fermentation Barriers

Exopolysaccharides (EPS)

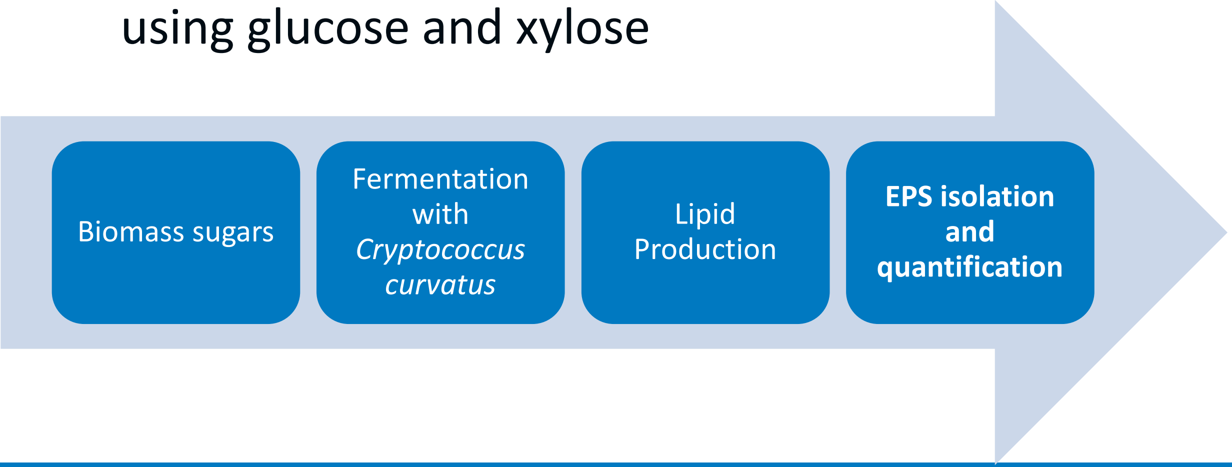
- **EPS were halting fermentation research**
- EPS is a slime coat produced in lipid fermentation
- EPS clogs HPLC columns, preventing carbohydrate & acid analysis
- Filtering samples through molecular weight cutoff filters was successful, but expensive and time-consuming
- Developed analysis with IC-PAD , requiring no extra filtering
- Characterized EPS using analytical hydrolysis approach
- EPS carbohydrate composition the same for fermentations using glucose and xylose

C. curvatus grown on glucose





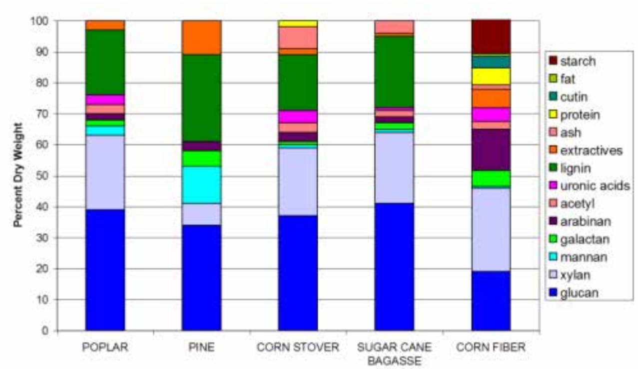
C. curvatus grown on xylose

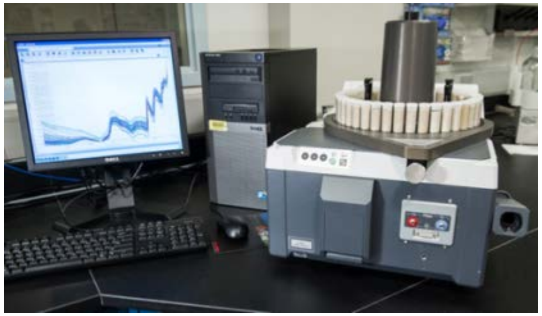


	Glucose Fermentation	Xylose Fermentation
	Lipid Production %	
Lipid Yield	72	63
	EPS Production g/L	
Glucose	3.9	3.4
Xylose	0.2	0.4
Mannose	4.3	4.1
Total EPS	**9.5**	**8.9**

TA - Resource and Data Management

- **We are past the point where a single person can keep track of everything in their head or on paper**
- We use SharePoint, which can be modified and used by our team
- Essentially shared spreadsheets on a backed up server accessible anywhere at NREL
- Some of these tools have been adapted for use in other DOE-funded work (SABC, ATP3)





Near Infrared (NIR) Spectroscopy for Rapid Analysis

Why NIR

- Minimal sample preparation
- Adaptable instruments
- Multiple scanning options
- Bulk sample analysis

Wet Chemistry

+

Calibration NIR Spectra

+

Chemometrics (multivariate statistical methods applied to chemical data)

=

Calibration Model

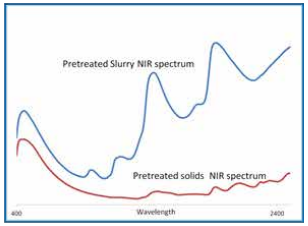
Calibration Model

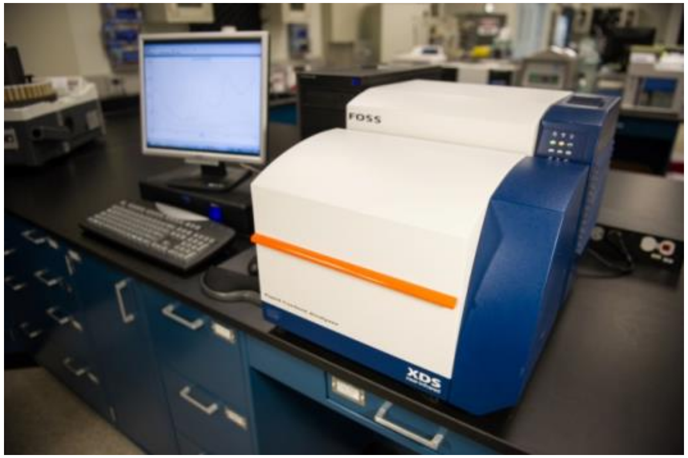
+

Unknown NIR Spectra

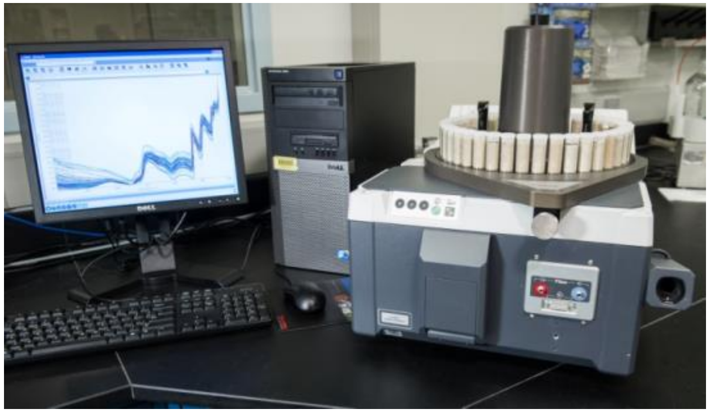
=

Rapid Predictions





Foss Dispersive NIR

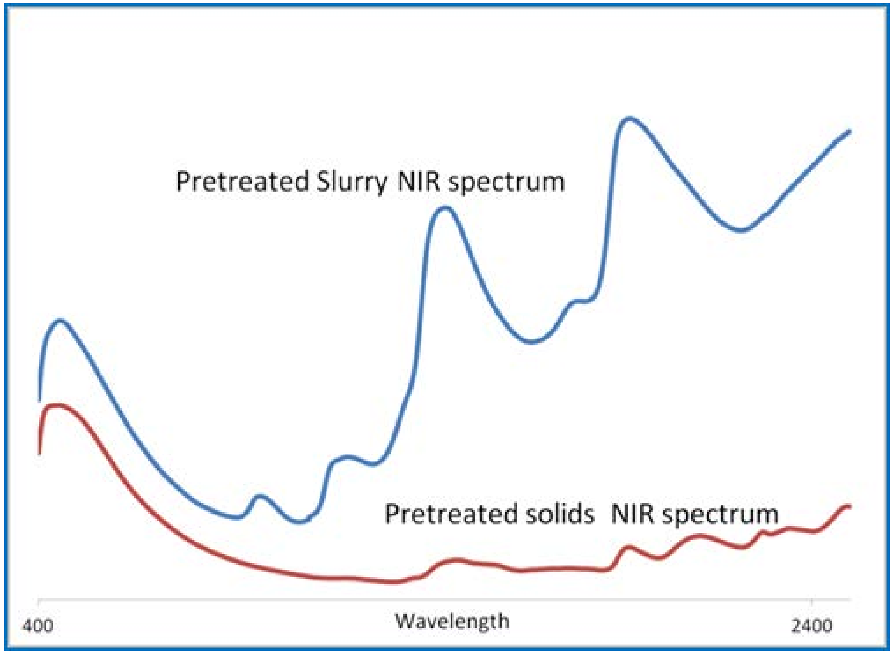


Thermo FT-NIR

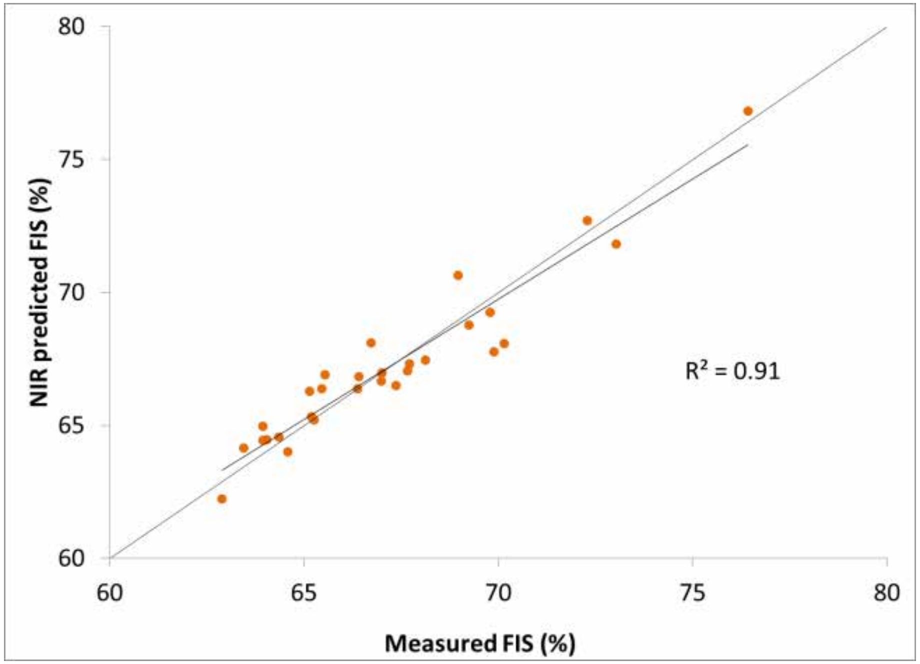
Photos by Dennis Schroeder

NIR Models for Rapid Compositional Analysis

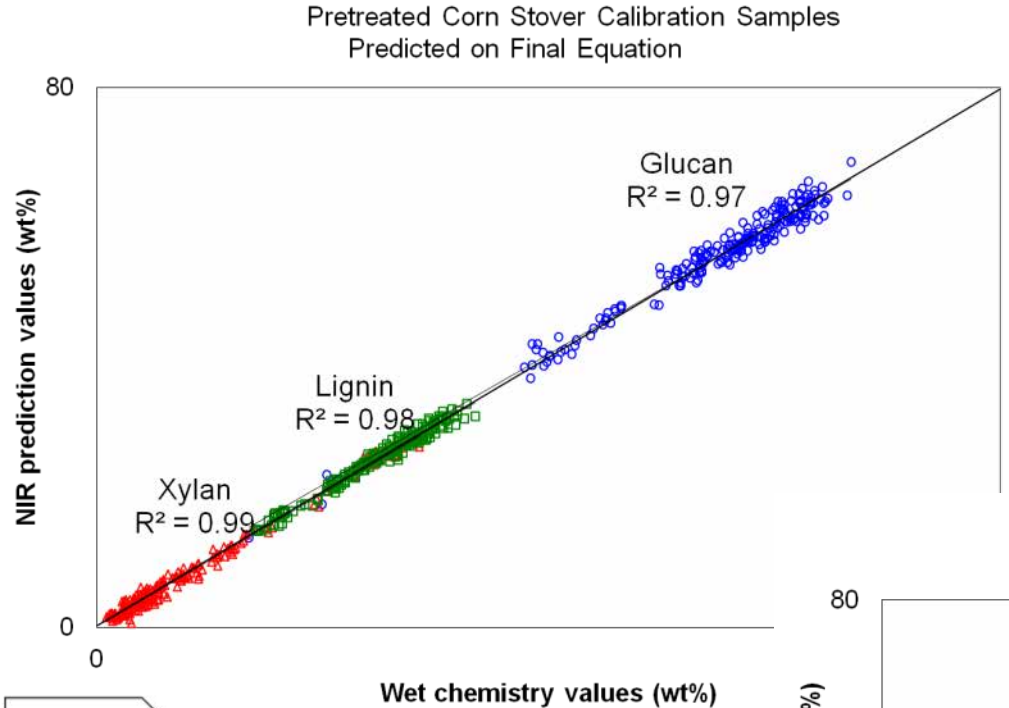
Corn stover feedstock
Mixed herbaceous feedstock
Pretreated corn stover solids
Pretreated corn stover solids in-situ
Carbohydrate release during pretreatment
Solid matter in pretreated slurry

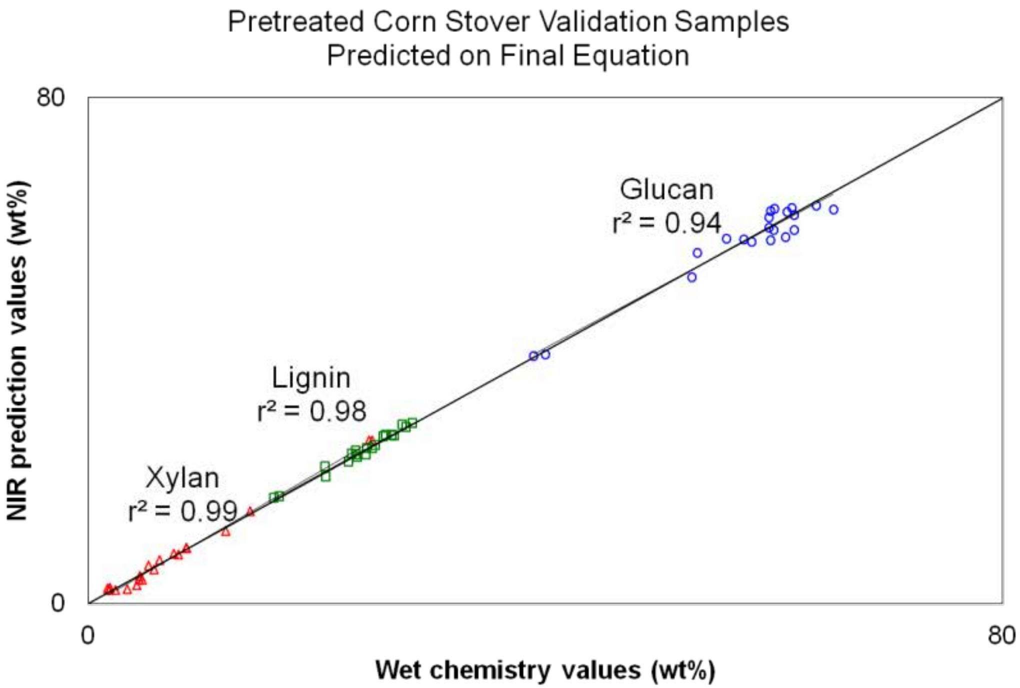


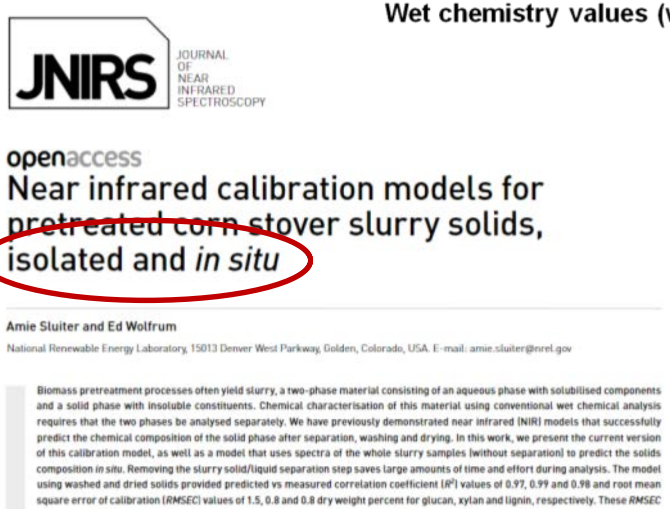
NIR predicted vs. measured FIS values for pretreated slurry samples



Pretreated Corn Stover Solids NIR Model







JNIRS JOURNAL OF NEAR INFRARED SPECTROSCOPY

openaccess

Near infrared calibration models for pretreated corn stover slurry solids, isolated and *in situ*

Amie Sluiter and Ed Wolfrum

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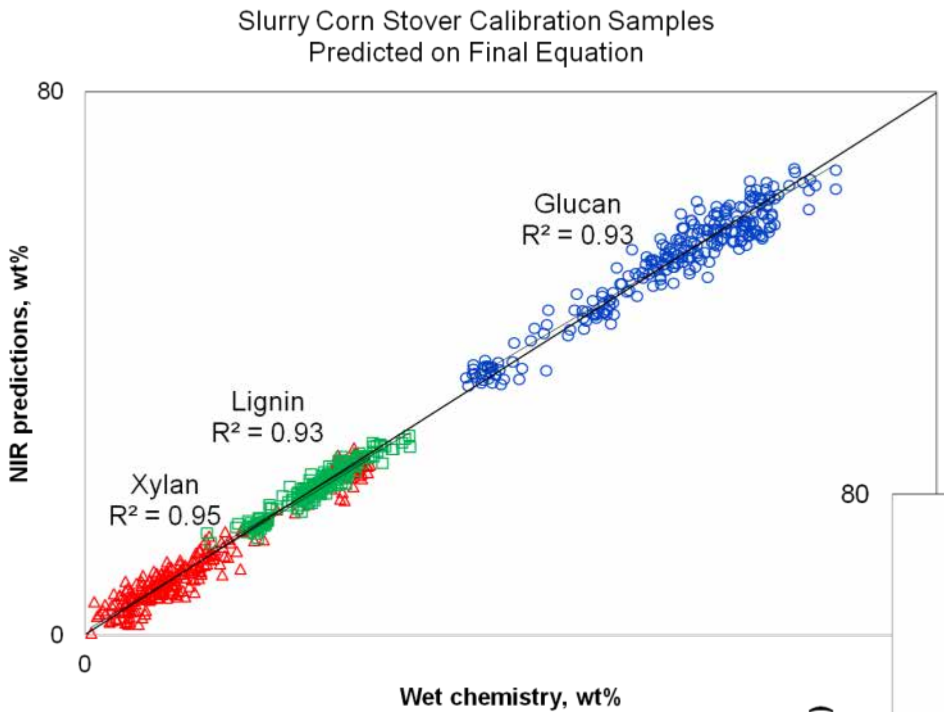
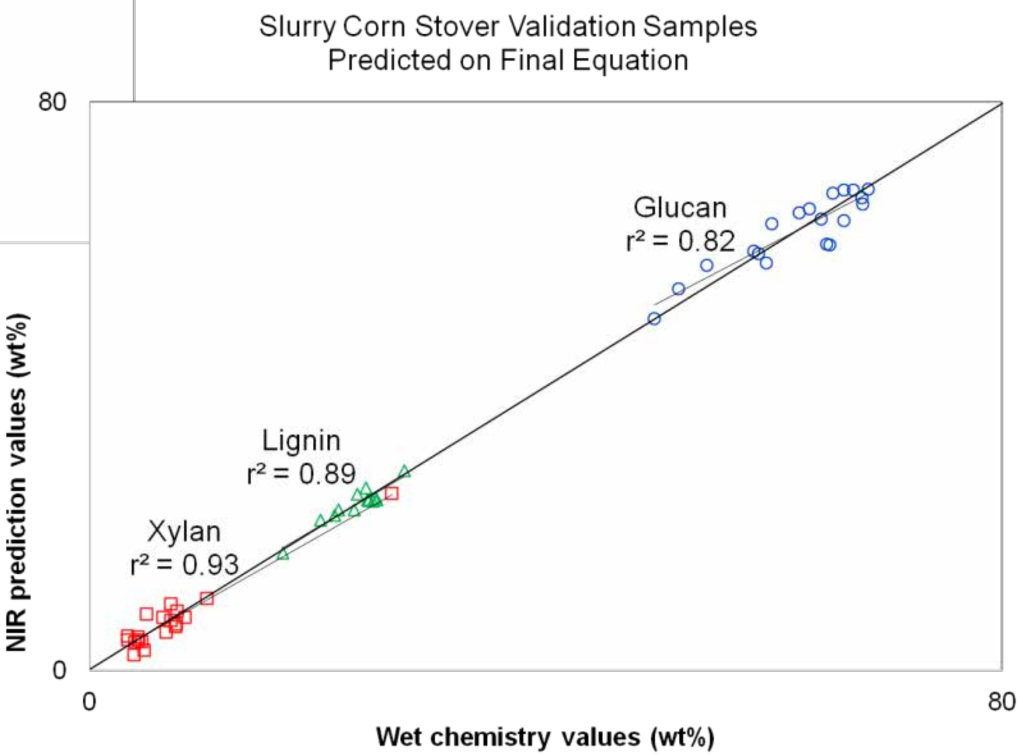
Biomass pretreatment processes often yield slurry, a two-phase material consisting of an aqueous phase with solubilised components and a solid phase with insoluble constituents. Chemical characterisation of this material using conventional wet chemical analysis requires that the two phases be analysed separately. We have previously demonstrated near infrared (NIR) models that successfully predict the chemical composition of the solid phase after separation, washing and drying. In this work, we present the current version of this calibration model, as well as a model that uses spectra of the whole slurry samples (without separation) to predict the solids composition *in situ*. Removing the slurry solid/liquid separation step saves large amounts of time and effort during analysis. The model using washed and dried solids provided predicted vs measured correlation coefficient (R^2) values of 0.97, 0.99 and 0.98 and root mean square error of calibration (*RMSEC*) values of 1.5, 0.8 and 0.8 dry weight percent for glucan, xylan and lignin, respectively. These *RMSEC*

Pretreated Corn Stover Slurry NIR Model



- 262 calibration samples- acid, steam, DA
- Scans on whole slurry
- No liquor background removed
- More process relevant



Next Generation NIR – Low Cost Instruments

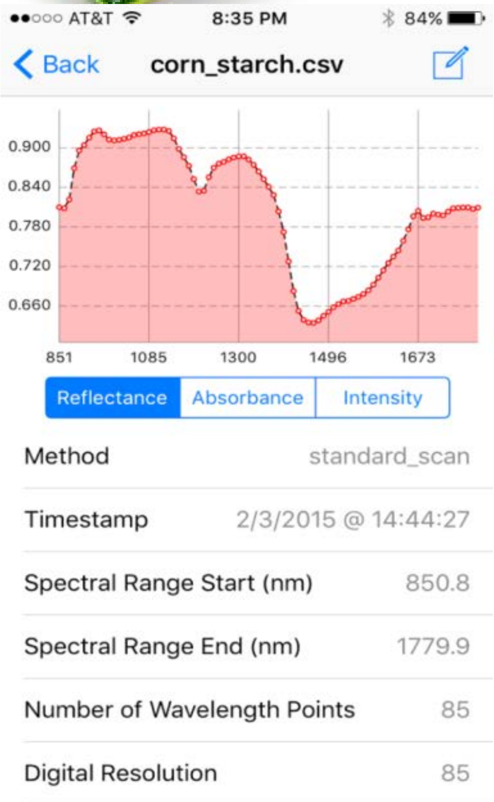
- A new generation of NIR spectrometers are about to become commercially available
- A step change in technology & cost
- How well would a low-cost spectrometer work compared to the standard laboratory system for our applications?
  - Thermo Antares FT-NIR (~$95,000)
  - NIRSCAN NANO (~$1,000)

NIRSCAN NANO

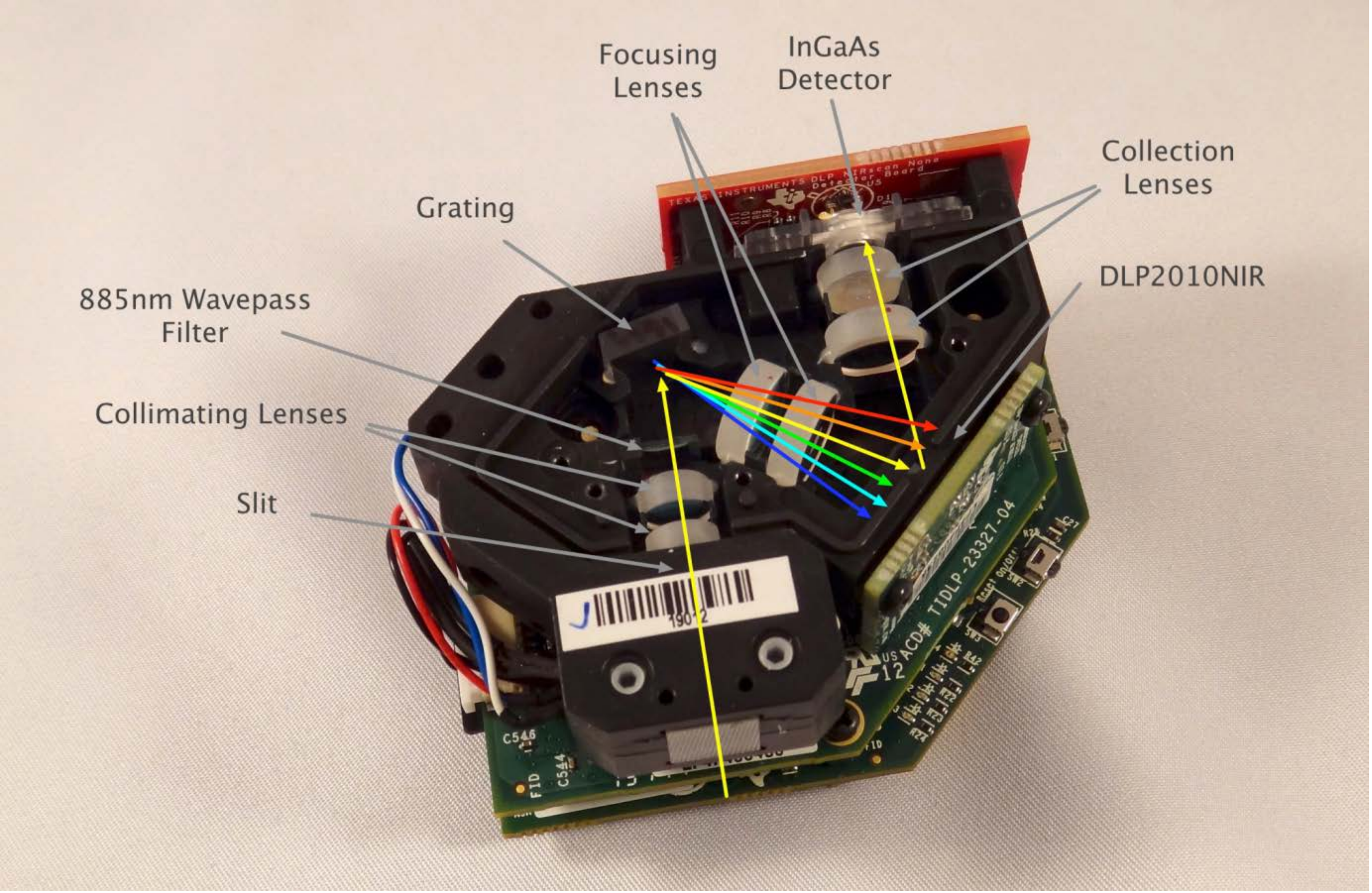
- Prototype NIR spectrometer designed by Texas Instruments using a Digital Micromirror Device (DMD)
- Same technology as in LCD projectors; a 2-D array of mirrors that can be individually moved – allows replacement of the expensive array detector with a single sensor
- The DMD in this spectrometer (DLP2100NIR) has over 460,000 mirrors on 6*um* pitch
- Referred to as a “Reference Design”. TI is looking for markets for their DMDs beyond LCD projectors!

NIRSCAN NANO (2)

- Built-in broadband emission lamp
- Single GaAs detector
- DMD acts as a post-dispersive monochrometer
- 900-1700nm wavelength range
- Operable in reflectance or transmittance mode
- Micro USB & Bluetooth
- Open source reference design and simple software interface
  - Windows GUI
  - Android, iPhone apps



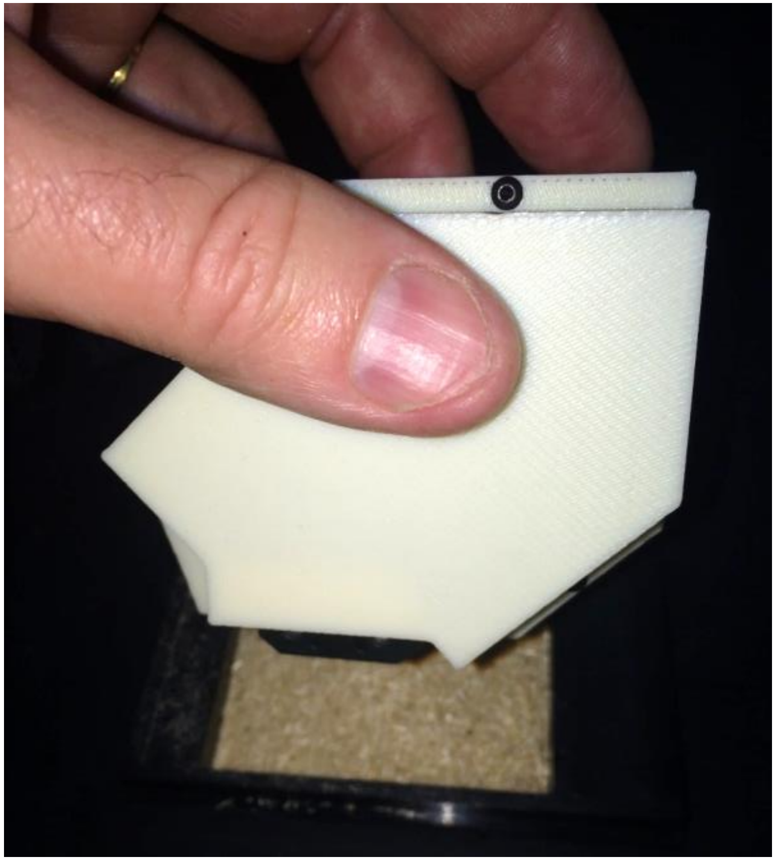
NIRSCAN NANO (3)

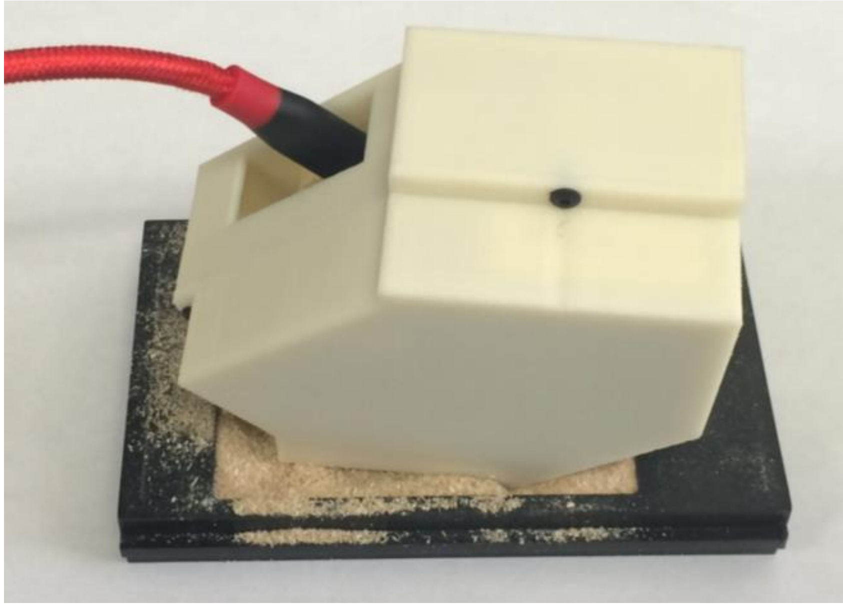


Comparing Models from both Spectrometers

- 37 miscanthus samples taken from existing biomass feedstock calibration model
- Thermo-Antares spectra taken using a standard cell holder - previous calibration experiment
- Milled samples poured into ¼ cup cell (used only as a holder) and smoothed
- NIRSCAN NANO spectrometer placed on top of milled material
- 3 replicate spectra taken with spectrometer rotated between samples
- Replicate spectra averaged

Pictures (~3000 words)

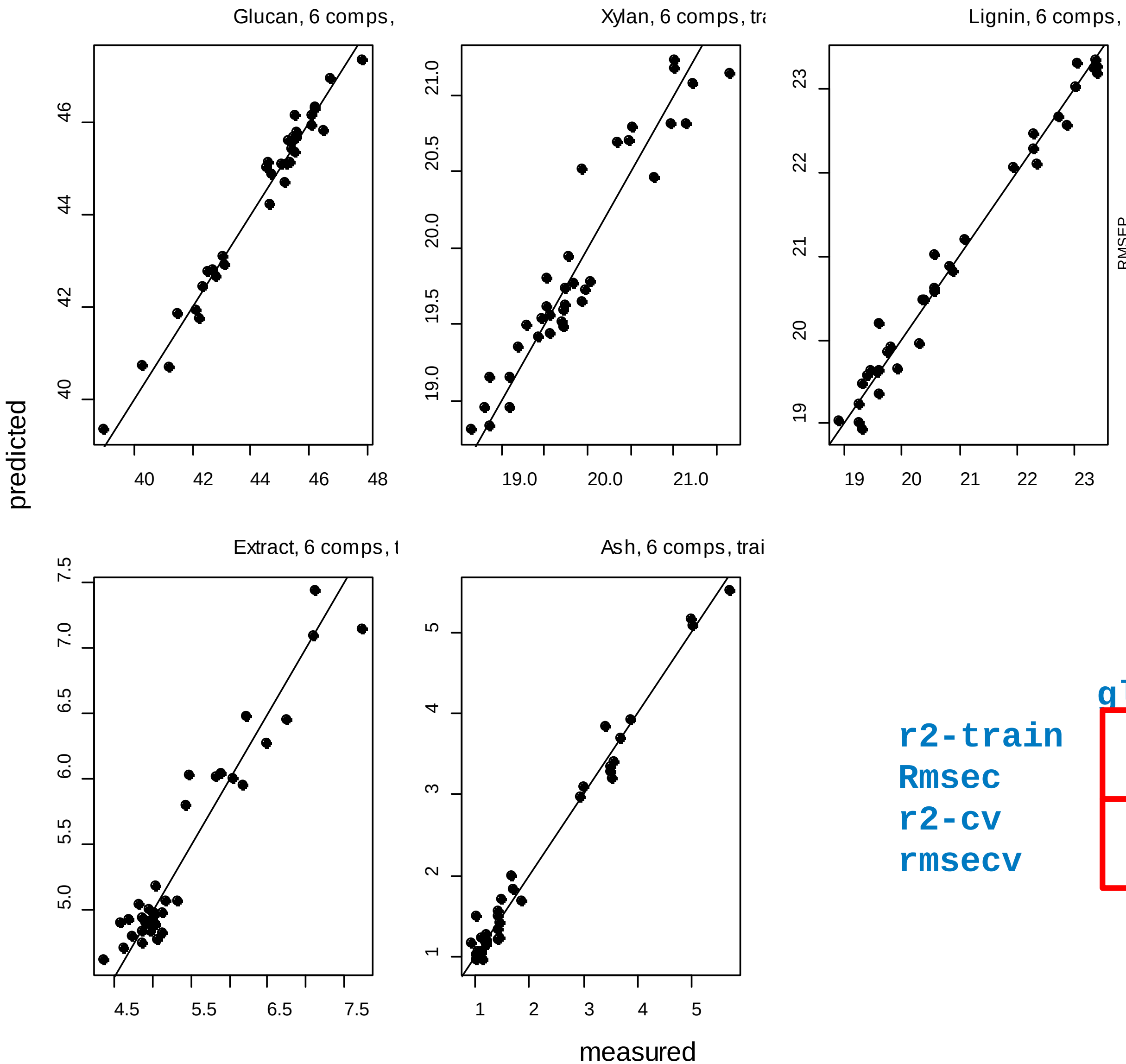


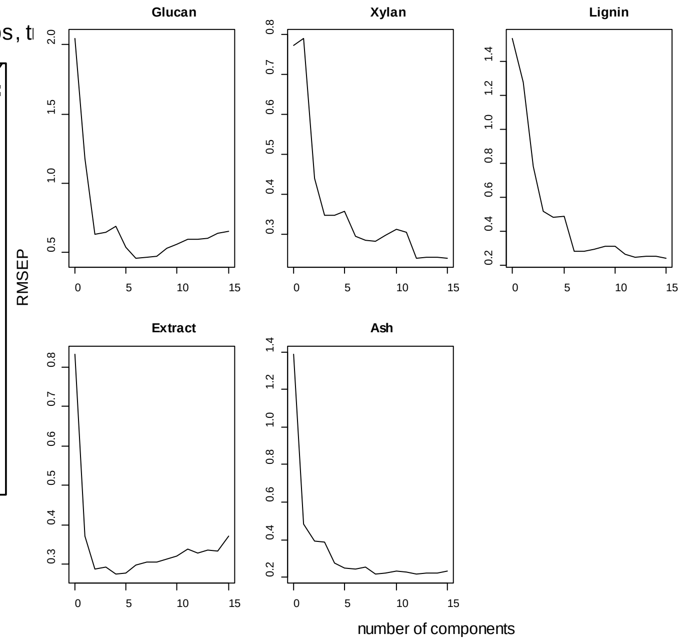


Experimental Details

- Spectra collected with NIRSCAN NANO Windows application
- Files exported as individual text files
- Used R to process spectral files & build models
- Spectra transformed (SNV_DT_SG) prior to modeling
- Performance based on PLS2 model (calibration & cross-validation)
  - correlation coefficient (R^2)
  - root mean squared error (RMSE)
  - # of Principal Components used

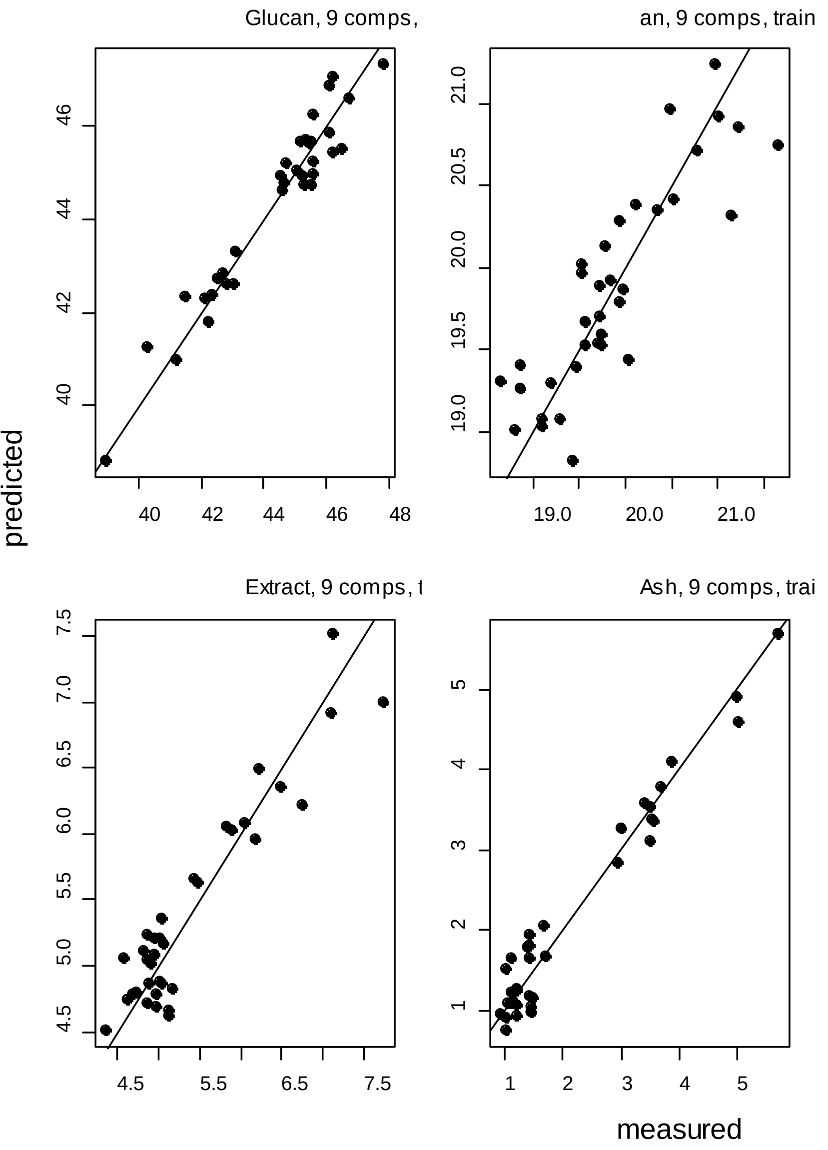
Results - Thermo-Antares Spinning Ring Cup - SRC

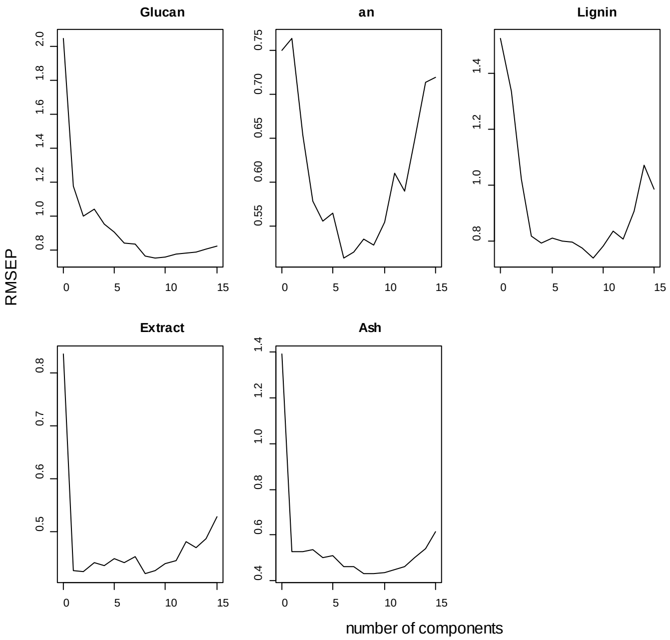




	glucan	xylan	lignin	ext	ash
r2-train	0.97	0.91	0.98	0.92	0.98
Rmsec	0.30	0.20	0.20	0.20	0.20
r2-cv	0.95	0.85	0.96	0.86	0.97
rmsecv	0.50	0.30	0.30	0.30	0.20

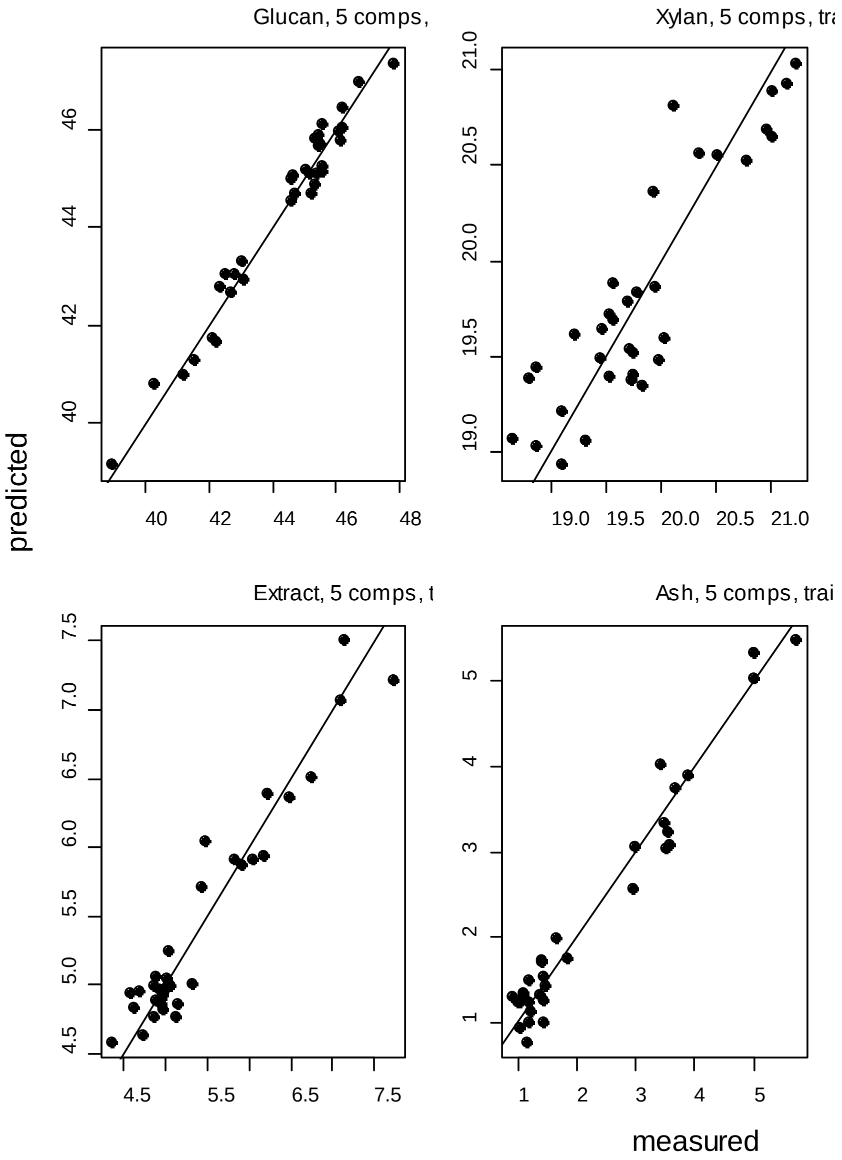
Results - NIRSCAN NANO Spectrometer

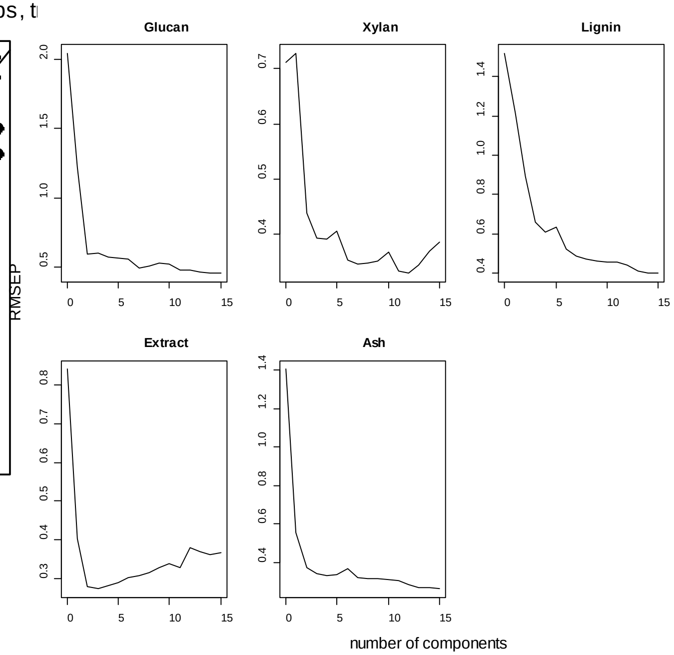




	glucan	xylan	lignin	ext	ash
r2-train	0.94	0.75	0.91	0.88	0.96
rmsec	0.50	0.40	0.50	0.30	0.30
r2-cv	0.86	0.47	0.75	0.72	0.90
rmsecv	0.80	0.50	0.70	0.40	0.40

Results - Thermo SRC – Reduced Spectral Range





	glucan	xylan	lignin	ext	ash
r2-train	0.97	0.79	0.92	0.92	0.96
rmsec	0.30	0.30	0.40	0.20	0.30
r2-cv	0.92	0.66	0.81	0.87	0.94
rmsecv	0.60	0.40	0.60	0.30	0.30

Summary – Low-cost NIR Spectrometer

- Calibration model for biomass compositional analysis built with NIRSCAN NANO spectra is not quite as good as model built with Thermo Antaris spectra
  - Lower R^2 values
  - Approximately 2-fold larger RMSE values
  - More PCs needed to fit model
- Decreased performance of NIRSCAN NANO appears to be due to a more narrow spectral range rather than a SNR issue – fixable problem in the longer term
- Model using NIRSCAN NANO spectra should be adequate for certain applications at a ~30x cost savings
- Working with an OEM manufacturer to move this work forward