2019 Project Peer Review

WBS # 2.5.1.101
Analytical Development & Support (ADS)

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Denver, CO
March 2019

Goal Statement

Goal: Enable biofuel and bioproducts R&D by

1. ensuring high quality analytical data for internal and external stakeholders,
2. development of critical analytical procedures
3. support of NREL's globally adopted procedures

ACTIVITY	OUTCOME	RELEVANCE
Ensure reliable data	High-quality experimental results	Confidence in research results
Develop new analytical methods	Published & disseminated procedures	Consensus Methods for the Community

Quad Chart Overview

Timeline

- Project start date – FY19
- Project end date – FY21
- Percent complete – 17%

	Total Costs Pre FY17	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-Project End Date)
DOE Funds	$3.4M	$1.1M	$750K	$2.4MM

Collaborators: all NREL biofuels researchers, multiple industry, and governmental collaborators

Barriers addressed

Ct-A. Defining metrics around feedstock quality

Ct-J. Identification and Evaluation of Potential Co-products

Objective

The ADS Project supports and enables biofuel and bioproducts R&D by ensuring high quality analytical data for internal and external stakeholders, and makes tools available to the wider community through method development and globally adopted procedures

End of Project Goals

- Continue to ensure and enable the generation of high quality analytical data for internal and external stakeholders
- Publish NREL Laboratory Analytical Procedures (LAPs) for determining the composition of deacetylated and mechanically refined (DMR) pretreated slurries, and DMR/EH hydrolysates

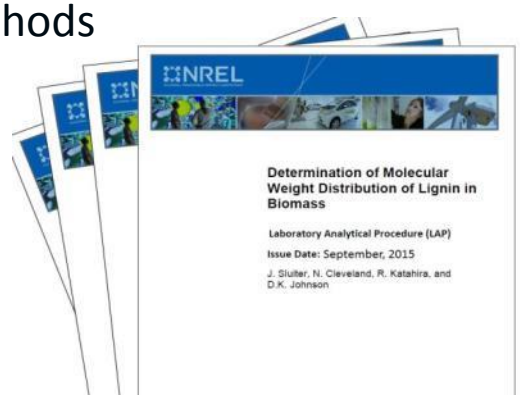
External Collaborators

ADM	Earth Energy	Leaf Resources	Waste Empowered
ASTM International	EdenIQ	KST	WSU
Ciris Energy	Environmental Protection Agency	Purdue	WVU
Cornell	GrainMillers	Shell	
CSU	ICM	Toyota	
Dupont	Kellogg	UT-Austin	

1 - Project Overview

Method Development

Collaborate to implement new analytical methods for improved speciation and mass closure



Sample Coordination and QC

More than 14,600 analyses that support NREL researchers

Laboratory Support

Maintain four analytical laboratories

Maintain existing instrumentation and identify and validate new instruments

Relationships

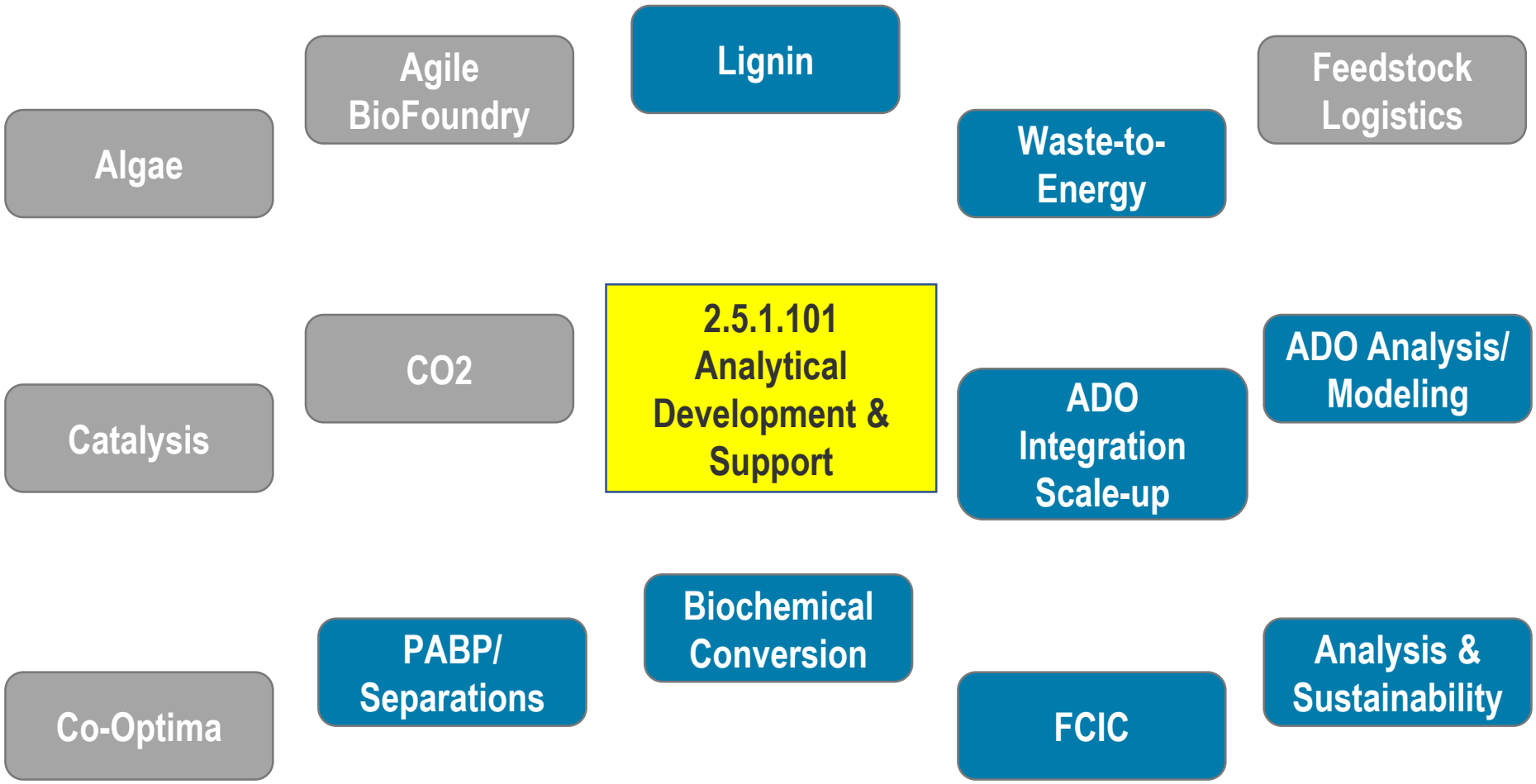
Cultivate relationships with industry, academia, and other laboratories

Maintain NIR calibration models for licensing to partners

2 - Approach

Analytical Development and Support (ADS) Project

2 - Approach: Connections with Other Projects



2 - Approach – Management

We support 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

Collaborate with internal and external stakeholders to identify and develop appropriate analytical methods to address evolving research needs

Task 2: Analytical Support

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

Scientists

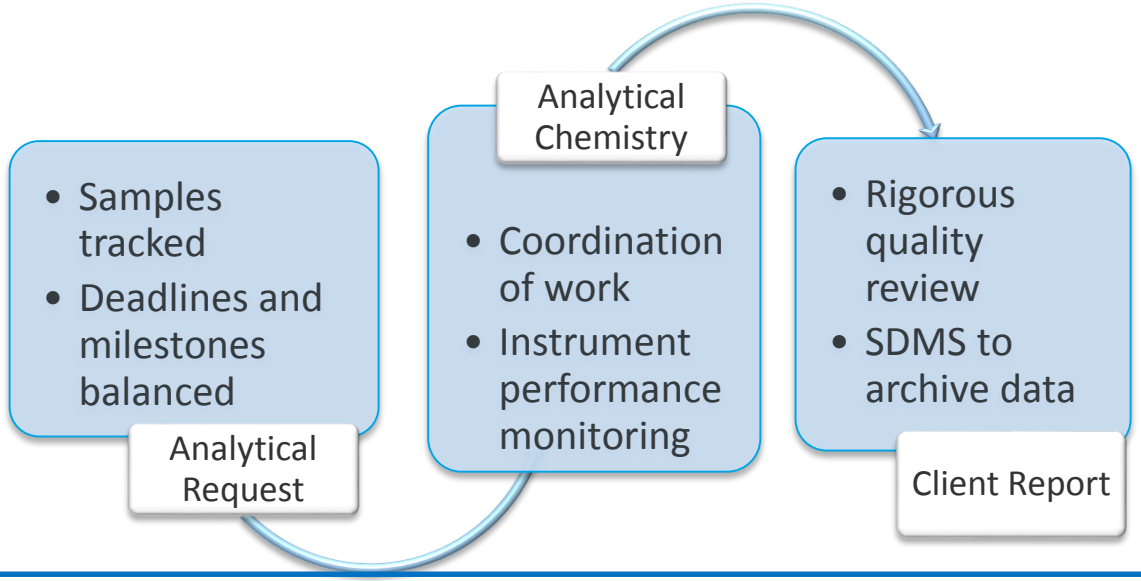
- Communicate and coordinate with PIs from program tasks
- Anticipate analytical needs based on work plans
- Recommend new methods for development
- Lead research, achieve BETO milestones and publish work

Research technicians

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

2 - Approach – Sample Mgmt. & Data Handling

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



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

FY18	
Procedures	**Samples**
HPLC-Routine	6300
HPLC-Development	500
Advanced Analytical	250
HPLC/PAD	400
NIR	3500
FAME	450
Liquors	450
FIS	350
Sample Prep	1700
CHN	1600
Total Solids	800
Feedstock Solids	350
Intermediate Solids	300
Extraction	300
Starch	150
Ashing	220
Hydrolysis	150
UV/Vis	150
Titration	250
Specialized Testing	150
YSI	150
ASE/APL Pretreatment	150
AFDW	40
Specialized Filtering	20
TOTAL	**18,680**
90 TASKS	

> 63,000 discrete analyses

2 – Approach – 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

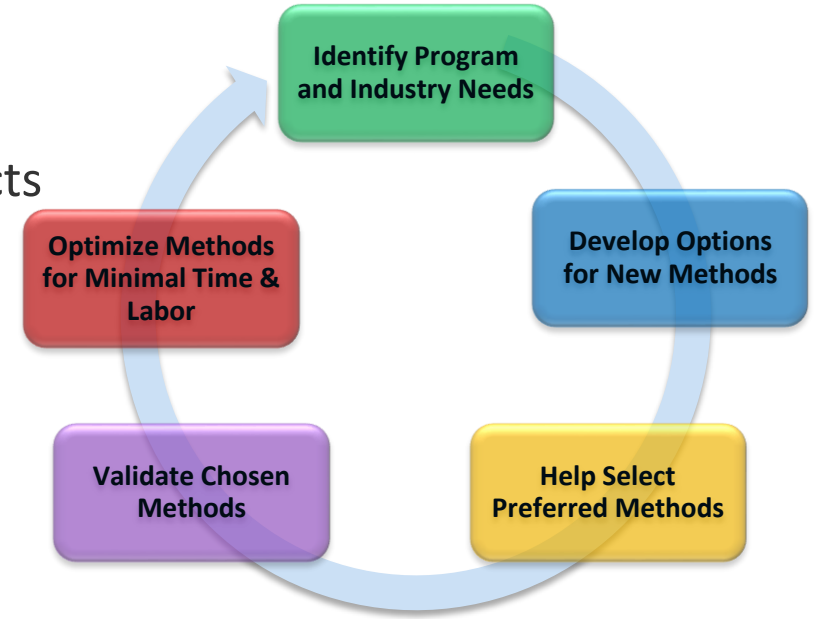
- This project has primary responsibility for instrument upkeep
- Troubleshooting, repairs, and routine maintenance
- We place and monitor subcontracts for instrumentation that supports most program tasks

2 - 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.

Challenges

- Keeping abreast of emerging analytical needs as the program changes
- Developing robust methods quickly
- Managing analysis for multiple platform projects
- Maintaining four laboratories and a multiple analytical instruments



Critical Success Factors

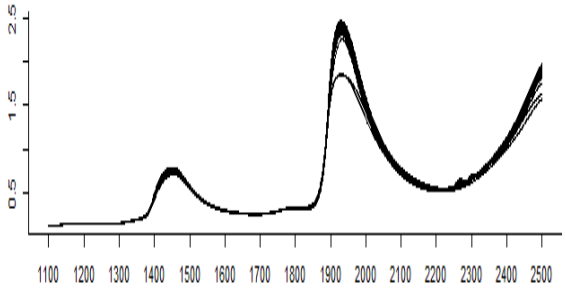
- Provide quality data in a timely manner to multiple projects
- Ensure data are reliable and actionable
- Develop & provide methods to the internal & external stakeholders

3 - Technical Accomplishments (TA)

Analytical Development and Support (ADS) Project

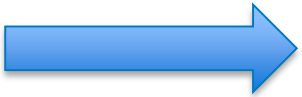
TA – At-line Fermentation Monitoring with NIR 1

- Demonstrated at-line measurement of carbohydrates, ethanol, and 2,3-butanediol (BDO)
- Used a combination of fermentation broth samples and synthetic “spiked” samples to build a robust calibration model using a FOSS XDS NIR spectrometer



+

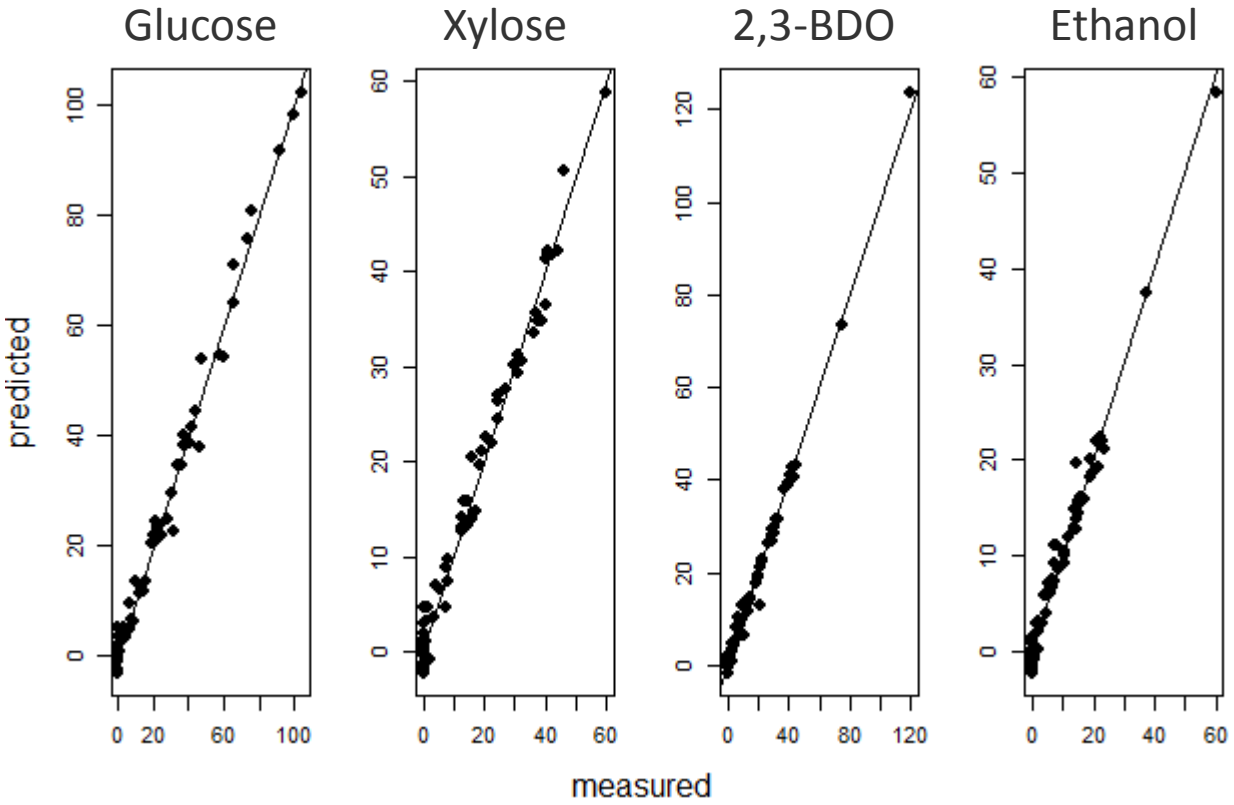
Glucose	Xylose	Ethanol	BDO
0	0	0	37.5
0	0	0	60
33.75	12.75	22.5	10.5
24.75	8.25	3	14.25
6	4.5	12	17.25
25.5	12	0	12
18.75	30.75	21	13.5
6.75	7.5	39	15
21	15	28.5	15



CALIBRATION MODEL

TA – At-line Fermentation Monitoring with NIR 2

- Demonstrated at-line measurement of carbohydrates, ethanol, and 2,3-butanediol (BDO)
- Used a combination of fermentation broth samples and synthetic "spiked" samples to build a robust calibration model using a FOSS XDS NIR spectrometer

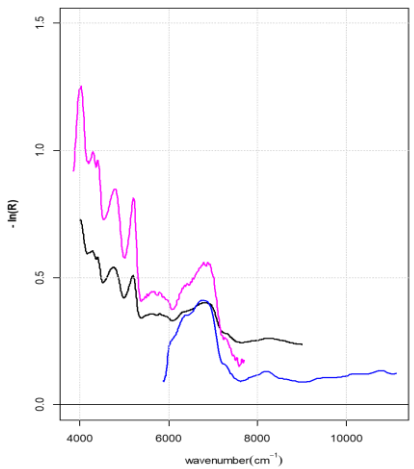


Using the Model

Moving to real-time in-line monitoring is the next step

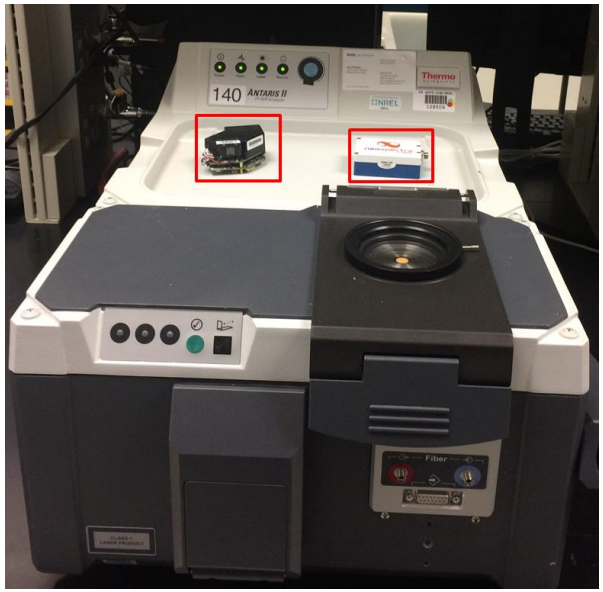
TA – Low-cost NIR Spectrometers for Biomass Analysis

- We investigated low cost portable NIR spectrometers to reduce the time and cost for obtaining quality analytical results – ubiquitous hand-held sensors
- We transferred existing NREL IP to two different low-cost spectrometers.



Preliminary results were encouraging

This work continues as a <u>cost-shared</u> TCF project

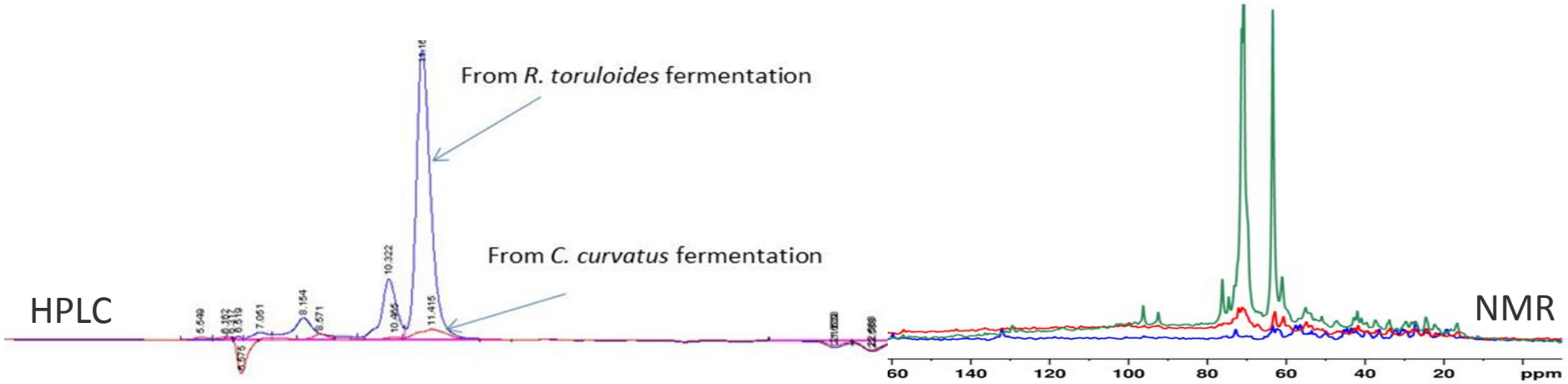


Constituent	Parameter	Thermo-Antaris FT-NIR	TI NIRSCAN Nano EVM	Si-Ware NeoSpectra
	Spectral Range (nm)	1111-2500	900-1700	1300-2619
	# PCs	6	9	4
Glucan	R^2-calibration	0.97	0.89	0.86
	RMSEC (g/L)	**0.30**	**0.60**	**0.70**
	R^2-LOO CV	0.95	0.78	0.73
	RMSECV (g/L)	**0.50**	**0.90**	**1.00**
Xylan	R^2-calibration	0.91	0.73	0.74
	RMSEC (g/L)	0.20	0.40	0.40
	R^2-LOO CV	0.85	0.47	0.58
	RMSECV (g/L)	0.30	0.50	0.50
Lignin	R^2-calibration	0.98	0.91	0.93
	RMSEC (g/L)	0.20	0.40	0.40
	R^2-LOO CV	0.96	0.65	0.84
	RMSECV (g/L)	0.30	0.90	0.60

TA- Quantification of Fermentation By-Products (w/ Bench-Scale R&D)

Lipid-producing fermentations offer a viable opportunity to produce renewable fuels and products, but carbon balances had been incomplete

- We collaborated with the Bench Scale Integration project to investigate the carbon mass balance of various lipid producing fermentations
  - We developed analytical methods suitable for **exopolysaccharide isolation and evaluation** and showed that large amounts of carbon are lost to EPS and sugar alcohols
  - We used **HPLC and NMR analysis** for identification of major compound groups, and LC-MS analysis for qualitative analysis of fermentation broths
- Carbon closure was determined to be 91-92% for *C. curvatus* with 60% unproductive carbon.



TA - Consultation with US EPA and California Air Resource Board

EPA's **Renewable Fuel Standard (RFS)**

- Biofuels are categorized and valued based on the source of carbohydrate
  - Categories are captured in various Renewable Identification Numbers (RINs)
- RIN values are based on category
  - **cellulosic RIN values** varied between $2.00-$3.00 in 2018

California **Low-Carbon Fuel Standard (LCFS)** has similar categories

https://www.epa.gov/fuels-registration-reporting-and-compliance-help/rin-trades-and-price-information

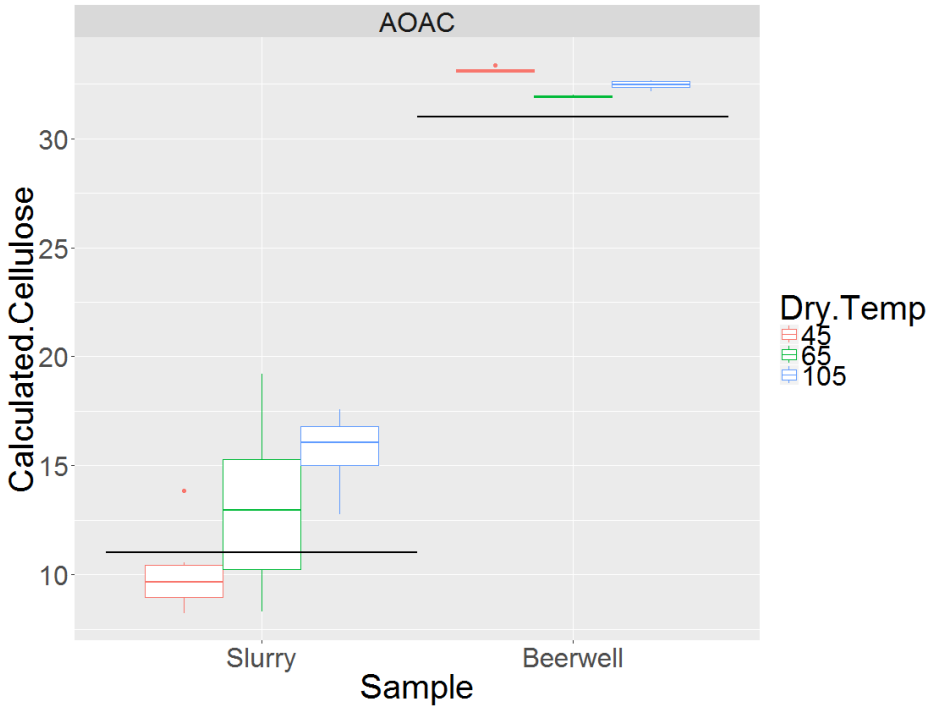
TA - Gen 1.5 Ethanol Industry Collaboration

Because cellulose and starch are both hydrolyzed to glucose for quantification, accurate quantification of either glucan is dependent on the starch assay

In collaboration with ICM, we documented shortcoming with ***current, accepted starch / cellulosic assays*** for accurately assigning the origin of glucans between starch and cellulose.

the energy of innovation™

We showed that **improper drying** and preparation of intermediate corn fermentation slurries resulted in **inaccurately high cellulose measurement**.



TA – ASTM Participation

ASTM is one of the Voluntary Consensus Standard Bodies (VCSB) recognized throughout the biofuels industry. ASTM E48 on Bioenergy and Industrial Chemicals from Biomass holds many of the preferred analytical methods for compositional analysis in biofuels.

- Justin Sluiter of ADS is chair of ASTM subcommittee E48.05 on Biomass Conversion.

Two Critical Methods Currently Under Development

- ***Sample Preparation Method***
  - This method will provide new guidance for improved drying and preparation of corn fiber materials
  - This is in part derived from NREL/ICM research
- **In situ Calculation of Converted Fractions Method**
  - New guidance for calculation of conversion of carbohydrates in complex multi-source products
  - NREL ADS and TEA groups have had significant input into the calculations for carbohydrate conversion during complex, multi-carbohydrate fermentations.

TA - Collaboration with NIST

Currently, there are multiple analytical methods for measuring carbohydrates in feedstocks but no community certified material for evaluation of methods.

We have led the collaboration with NIST and Industry to generate representative samples for evaluation of analytical methods for measuring samples containing Cellulose and Starch

NREL
NATIONAL RENEWABLE ENERGY LABORATORY

NIST

This collaboration resulted in

- **Actual products** from the corn ethanol industry,
- produced with **industrially relevant conditions**,
- that provide appropriate **matrix interactions**

Interlaboratory Round Robin to determine consensus composition to begin shortly

TA - New Method for Measuring Cellulose-specific Carbohydrates

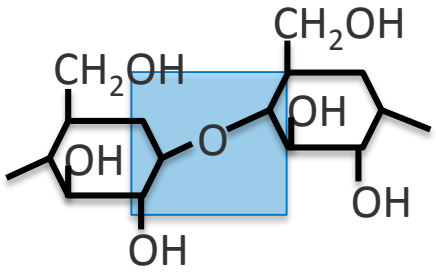
A cellulose specific analytical assay is not currently publicly available. Both industry and government agencies have requested that NREL assist in this area.

We developed an analytical procedure for the quantification of non-starch derived carbohydrates present in high starch samples.

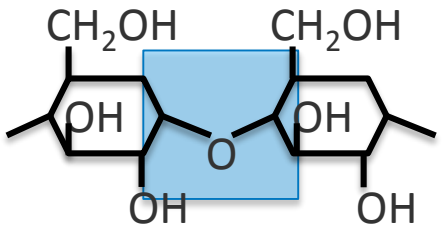
- This procedure will allow for the determination of the ***source of carbohydrates*** and therefore the calculation of cellulosic conversion in Gen1.5 biorefineries.
- The newly developed analytical procedure avoids the key issue of compounding errors associated with cellulosic quantification by sequentially removing the interfering carbohydrates, therefore a direct ***cellulosic carbohydrate content can be more accurately determined***.

This method was used in the certification of the NIST reference materials.

Cellulose



Starch



TA - Analytical Method Development for New Feedstocks

BETO has committed to investigate alternate, low cost feedstock such as wet waste for renewable fuels and products production. There are not currently good methods for quantification of components present in wet wastes.


Biogas Opportunities Roadmap

Voluntary Actions to Reduce Methane Emissions and Increase Energy Independence

U.S. Department of Agriculture, U.S. Environmental Protection Agency, U.S. Department of Energy

August 2014

- Complex feedstocks required new analytical methods
  - ADS has modified existing assays for high-fat, high-protein, diverse carbohydrate sources
- New conversion pathways require new calculations
  - ADS has applied new conversion calculation tools to determine rates of conversion of compound classes during anaerobic digestion

You can not valorize what you do not know is present

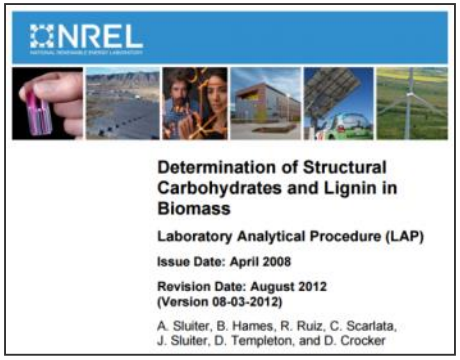
4 - Relevance

Analytical Development and Support (ADS) Project

Goal: The ADS Project supports and enables biofuel and bioproducts R&D by ensuring high quality analytical data for internal and external stakeholders, and makes tools available to the wider community through method development and globally adopted procedures

4 - Relevance – Laboratory Analytical Procedures (LAPs)

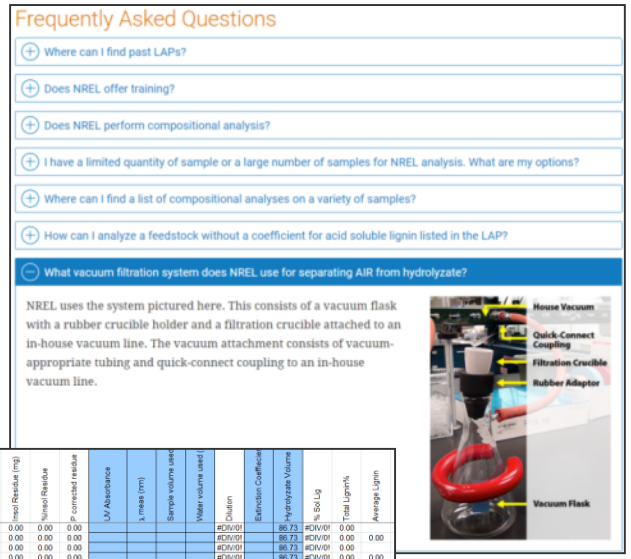
NREL is a leader in biofuels analysis and our methods let the world-wide community "speak the same language"

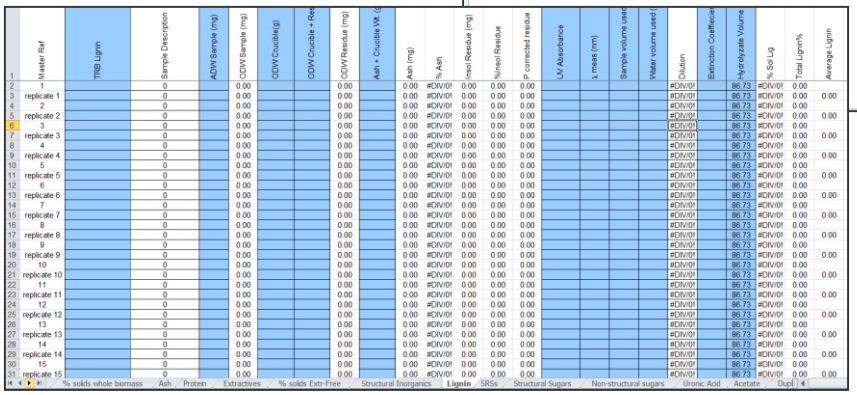


- Regularly evaluated and updated to meet the changing needs of program and industry
- Used world-wide as *de facto* standards for analysis of biomass
- Several methods have been adopted by ASTM
- Recently used for recertification of NIST's Biomass Reference Materials

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

Includes procedures, calculations, and FAQ





- Page Views (unique)
  - FY16 10,500
  - FY17 10,400
  - FY18 8,400
- Procedure Downloads
  - FY16 3,200
  - FY17 8,262
  - FY18 7,232

4 - Relevance – Laboratory Analytical Procedures (LAPs)

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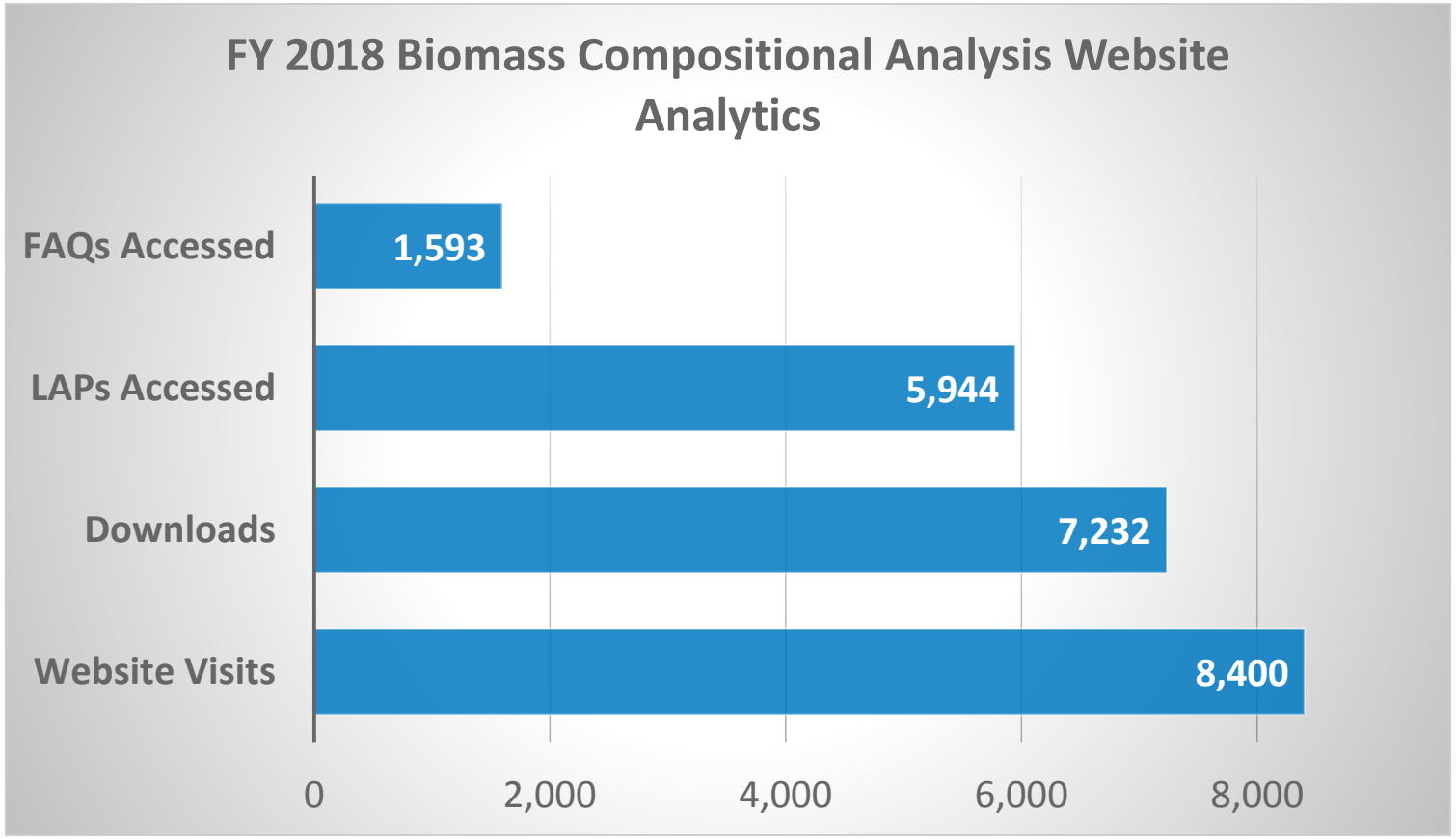


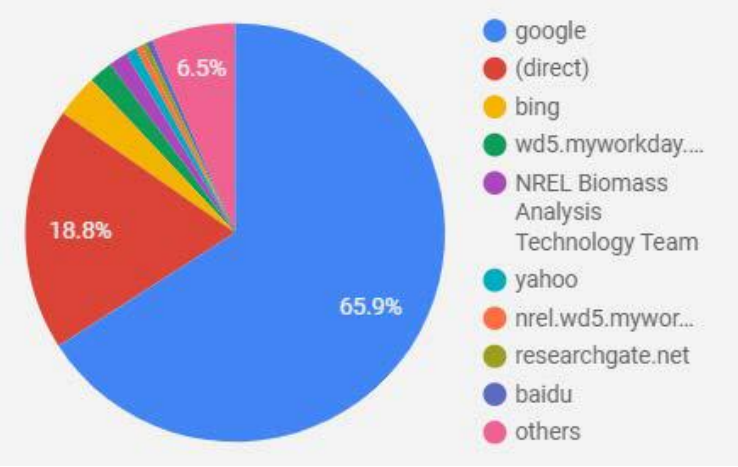
4 - Relevance – Laboratory Analytical Procedures (LAPs)

With business development funds we were able to launch our first Biomass Analysis Team Newsletter

From 10-2018 – 10-2019:

670 Successful deliveries!
Open rate = **28.1%** (vs. avg. **24.1%)**
Click rate = **10.4%** (vs. avg. **3.2%)**

The Newsletter referred **1.4%** of the traffic to the Bioenergy website, 5th highest referrer




NREL
Transforming ENERGY

Biomass Analysis Technology Newsletter
September 2018

In this Issue

Milestone Highlights
Current & Upcoming Work
Tech Talks
Meet Us @
You Should Know
Team Bio
Working with Us

4 - Relevance – Outreach to External Stakeholders

- There is significant uncertainty in the biofuels community regarding quantifying cellulosic ethanol production
- Our 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 developing analytical methods and its strong relationship with ASTM

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

5 - Future Work

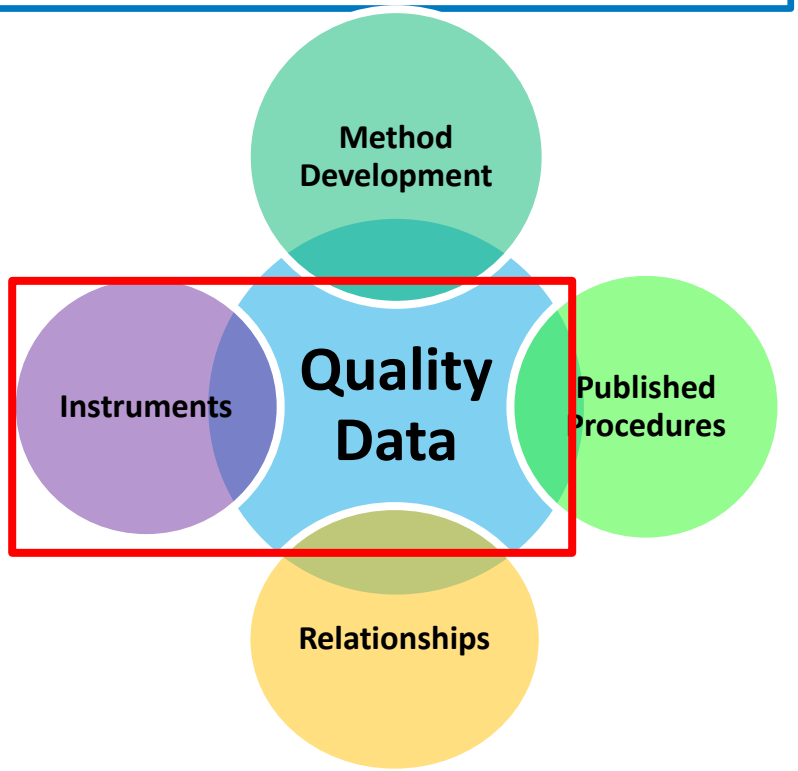
Analytical Development and Support (ADS) Project

5 - Future Work – Quality Data and Maintained Labs

- We will continue to be responsive to the evolving needs of the program
- 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

Continue to provide quality data

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



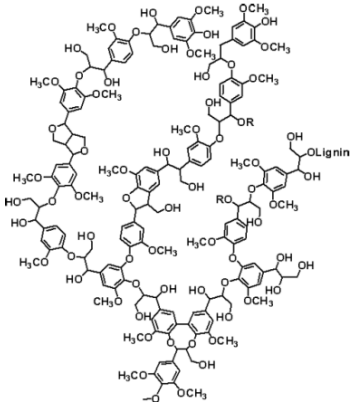
5 - Future Work – Improving Analyses for Stakeholders

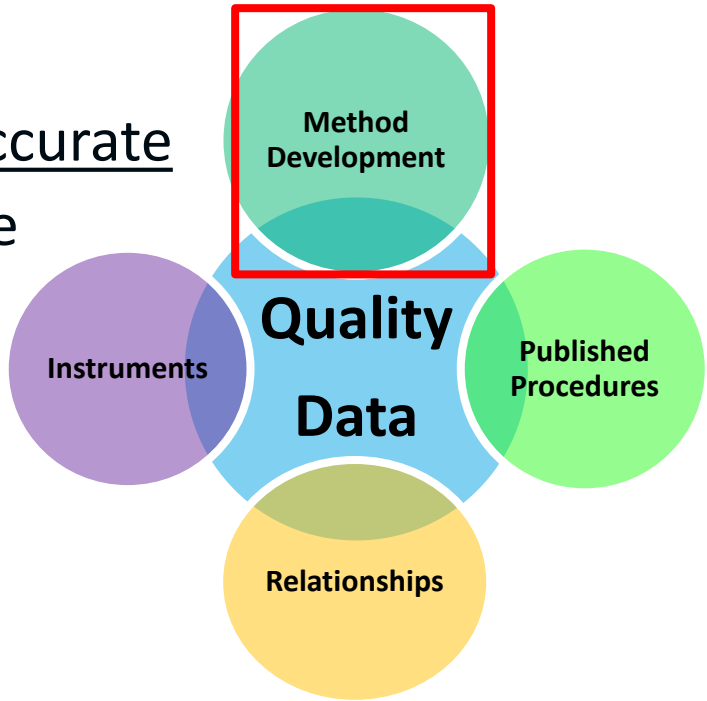
Non-structural carbohydrates and starch

- Generation 1.5 biofuels require differentiation of feedstock structural carbohydrates
- The community needs consensus methods for precisely and accurately measuring process intermediate streams

Lignin

- Process development requires rapid and accurate methods for lignin measurement in alkaline pretreated samples

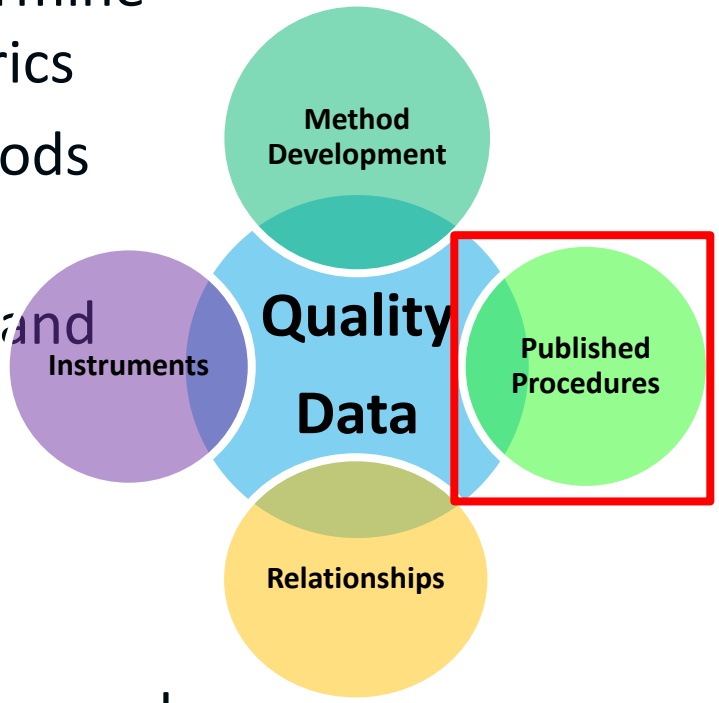




5 - Future Work – External Engagement

Support generation 1.5 biofuels

- Engaging community stakeholders to determine appropriate cellulosic RIN conversion metrics
- Establishing standard starch analysis methods with external collaborators
- Publishing standard procedures for starch and free carbohydrate analysis



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

6 - Summary

Goal: The ADS Project supports and enables biofuel and bioproducts R&D by ensuring high quality analytical data for internal and external stakeholders, and makes 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 methods to measure cellulose when present in complex Gen1.5 matrices
- Investigated Low-cost NIR spectrometers for biomass characterization
- Continued improvements in industrially relevant methods

Relevance

- Industry support
  - Guide standards and references
  - NIR models available for license
- Laboratory Management
- LAPs
- Quality reliable data is crucial for process development work

Future Work

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

Acknowledgements

- Beau Hoffman (BETO)
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- Bill Michener
- Ryan Ness
- Courtney Payne
- Darren Peterson
- Michelle Reed
- Amie Sluiter
- Justin Sluiter
- Brittany Thornton
- Jeff Wolfe

Publications & Presentations 2017-2018

- "High Throughput Screening Technologies in Biomass Characterization", Stephen R. Decker, Renee M. Happs, Anne E. Harman-Ware, Edward J. Wolfrum, Daniel Jacobson, Deborah Weighill, Piet C. Jones, Gerald A. Tuskan, David Kainer, Miguel Rodriguez, Gbekeloluwa Oguntimein, *Frontiers in Energy Research (2018). doi:10.3389/fenrg.2018.00120*
- "Switchgrass and Giant Miscanthus Biomass and Theoretical Ethanol Production from Reclaimed Mine Lands", Steffany Scagline-Mellor, Thomas Griggs, Jeffrey Skousen, Edward Wolfrum, Ida Holásková. *Bioenerg. Res. (2018). doi:10.1007/s12155-018-9915-2*
- "Quantitative trait loci for cell wall composition traits measured using near-infrared spectroscopy in the model C4 perennial grass *Panicum hallii*", E. Milano, C. Payne, E. Wolfrum, J. Lovell, J. Jenkins, J. Schmutz, T. Juenger. *Biotechnology for Biofuels 11:25 (2018). doi:10.1186/s13068-018-1033-z*
- "Evaluation of 15 cool season perennial grasses as biofuel feedstocks using NIR/PLS", C. Payne, E. Wolfrum, N. Nagle, J. Brummer, N. Hanson. *Agronomy Journal (2017). doi:10.2134/agronj2016.09.0510*
- "The buffering capacity of stems: genetic architecture of nonstructural carbohydrates in cultivated Asian rice, Oryza sativa", Diane Wang, Rongkui Han, Edward Wolfrum, Susan McCouch. *New Phytologist (2017). doi:10.1111/nph.14614*
- "The effect of biomass densification on structural sugar release and yield in biofuel feedstock and feedstock blends", Edward J. Wolfrum, Nicholas J. Nagle, Ryan M. Ness, Darren J. Peterson, Allison E. Ray, Daniel M. Stevens. *Bioenergy Research (2017). doi:10.1007/s12155-017-9813-z*
- "Improved sugar yields from biomass sorghum feedstocks: Comparing low-lignin mutants and pretreatment chemistries", Bruno Godin, Nick Nagle, Scott Sattler, Richard Agneessens, Jérôme Delcarte and Edward Wolfrum. *Biotechnology for Biofuels 9:251(2016). doi:10.1186/s13068-016-0667-y*

Responses to Previous Reviewers' Comments

APPROACH

- The structure of considering both method development and direct analytical support for projects makes sense. The critical success factors and identification of challenges look right.
- The team is highly recognized and generating gold standards for regulatory agencies and the industry using "common language" across various analytic chemists and policy makers to generate these.
- Management is well described and organized. The facility requires a high level of rigor during operation as their success is measured by the reliability of their data. High reliability is a result of this well organized operation.
- It is good to see the focus on quality of data as well as the quantity of assays performed.
- It is unusual from an industry perspective not to see a LIMS being used to handle that number of samples, customers, and assay types.

PROGRESS

- The team spends a large amount of time making sure that their equipment is well maintained and up and running so that throughput doesn't suffer.
- In 2016, 14000 samples and >60,000 analysis. Supported many projects, and analytical was never a barrier to reaching milestones.
- The NIR effort is a great example of an initiative through which the group can really help the broader biorefinery community.. Standardized models for Gen 1.5 biofuel feedstocks and lignin will also be invaluable for the industry..
- The high quality data is maintained through the good skills and expertise built in the group and good maintenance of the equipment. The challenges of keeping up with moving targets needs by project teams and quick development of "good enough" robust methods are well addressed.
- It is recommended to have better data repository system to enable data sharing, faster analysis and later perhaps data mining and modeling.

RELEVANCE

- Relevance would have to be high.
- There's really not much to say. This is fully, completely, totally relevant to the BETO program.
- This project/team is well known for developing and sharing new analytical methods that enable the industry. Consistent and high quality data is integral to project TEAs. They continue to identify the most relevant needs, e.g. a D3 RIN credit pathway.
- The development of standardized procedures for other labs to use is another strength of this program.

FUTURE WORK

- Since this is a service function, future work depends on the projects given to them.
- It would be good to see if the group can collaborate with the SCADA effort at PNNL on data handling.
- With the thousands of samples from multiple users and projects, it would seem that large scale data mining could provide some new and interesting insights about biomass.
- This is a service function which has to be agile and with high quality instrumentation and skilled people. The team demonstrated that they are highly equipped to do so in finding novel ways to quickly analyze multitude of analytes required to deliver on BETO's mission and vision.

Overall Impressions (Not Scored)

- The project highlights the role that a National Laboratory can play in doing work central to a range of processes and releasing data into the public domain, thereby saving industry a significant amount of money.
- This is one of the most important parts of the BETO program.
- It is one of the invisible machines that keep everything running smoothly. They have done a commendable job running samples in a timely manner for multiple projects and delivering high-quality data (accurate, precise, consistent, relevant). In addition, the team supports their own instruments and those in other labs.
- I would echo previous reviews on this superb activity: keep doing what you're doing.

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

Analytical Development & Support Milestones

Milestone Name/Description	FY/Q	Type
Measuring Fermentation Byproducts / Identify and quantify at least three side products produced during fermentation for at least 5 different fermentation experiments. Quantify the fraction of non-productive carbon loss because of these side products and document the analytical methods used to make these measurements.	FY17 Q3	Quarterly Progress Measure (Regular)
Development of a procedure for consistent measurement of cellulose conversion in industrial starch ethanol plants in support of RFS-RIN credit generation.	FY17 Q4	Annual Milestone (Regular)
Draft publication comparing micro NIR capabilities and performance on biomass feedstock	FY18 Q1	Quarterly Progress Measure (Stretch)
Annual report on analytical support activities, including status of laboratory instrumentation, service subcontracts, scientific data management system (SDMS), web page, and a review of analytical work performed for the platform.	FY18 Q2	Quarterly Progress Measure (Regular)
Report on ASTM membership and engagement, with a focus on industrial needs	FY18 Q3	Quarterly Progress Measure (Regular)
Develop draft publication procedure to measure cellulose in a generation 1.5 starch ethanol plant (capstone)	FY18 Q4	Annual Milestone (Stretch)
Q1 - Collaborate with NIST, ASTM, and other external stakeholders to produce a reference material for method accuracy and precision evaluation of cellulosic quantification methods when both cellulosic- and non-cellulosic sources of glucans are present.	FY19 Q1	Quarterly Progress Measure (Regular)
Annual report on analytical support activities for NREL Biochemical Platform documenting laboratory instrumentation status, scientific data management, summary of analyses performed, and outreach activity to external stakeholders.	FY19 Q2	Quarterly Progress Measure (Regular)