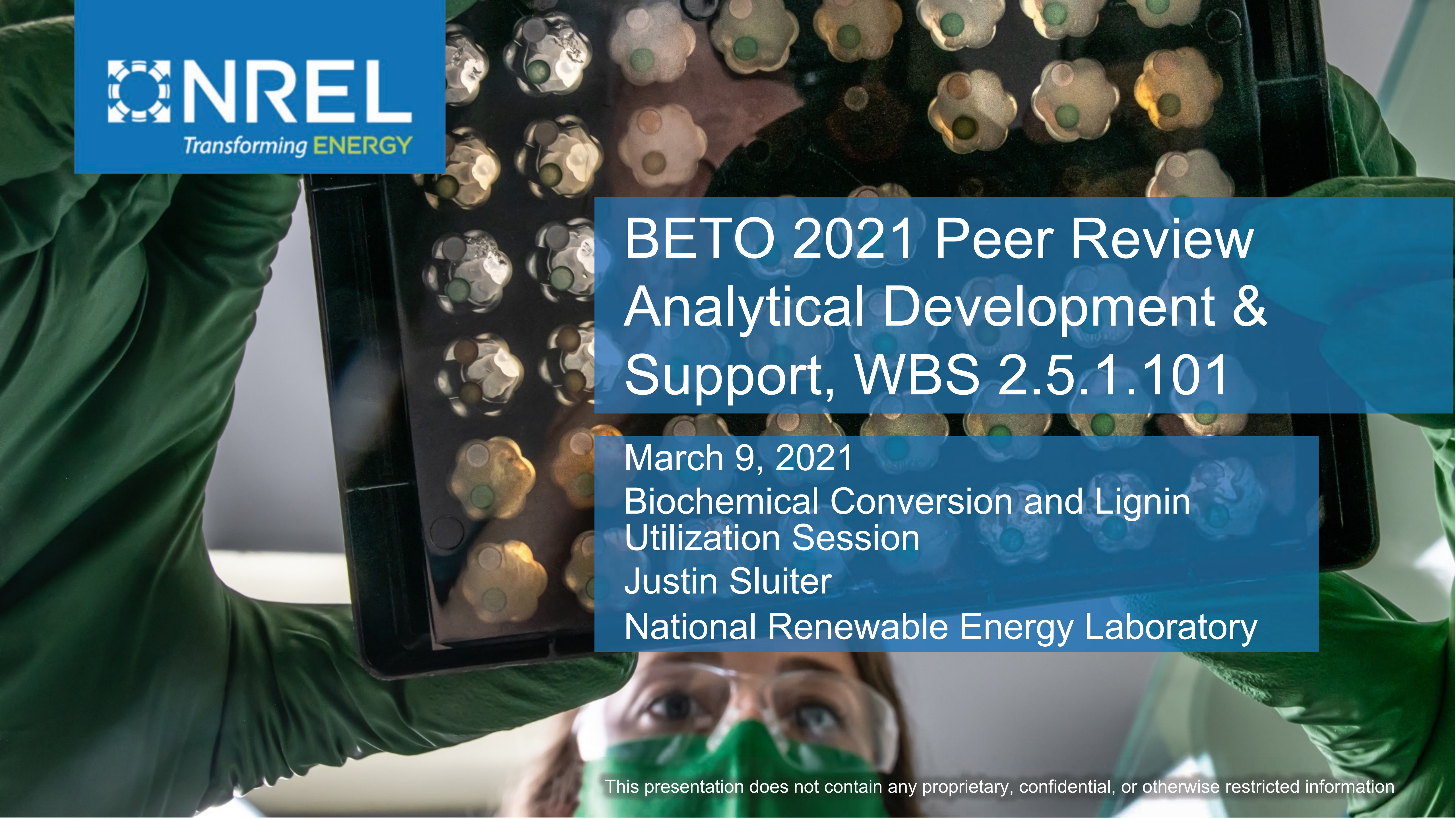


NREL
Transforming ENERGY

BETO 2021 Peer Review Analytical Development & Support, WBS 2.5.1.101

March 9, 2021

Biochemical Conversion and Lignin Utilization Session

Justin Sluiter
National Renewable Energy Laboratory

Market Trends

Product	Anticipated decrease in gasoline/ethanol demand; diesel demand steady
	Increasing demand for aviation and marine fuel
	Demand for higher-performance products
	Increasing demand for renewable/recyclable materials
Feedstock	Sustained low oil prices
	Decreasing cost of renewable electricity
	Sustainable waste management
	Expanding availability of green H_2
	Closing the carbon cycle
Capital	Risk of greenfield investments
	Challenges and costs of biorefinery start-up
	Availability of depreciated and underutilized capital equipment
Social Responsibility	Carbon intensity reduction
	Access to clean air and water
	Environmental equity

NREL's Bioenergy Program Is Enabling a Sustainable Energy Future by Responding to Key Market Needs

Value Proposition

- Enabling industry through development of new analytical procedures and ensuring high-quality research data

Key Differentiators

- Unbiased analytical procedures based on sound science respond to industry needs and provide public methods

- Reliable research results offer solid foundations to build on current technology

Project Overview

- **What:** The goal of the ADS project is to develop critical analytical procedures, and support NREL's published and globally adopted procedures that ensure high quality analytical data for internal and external stakeholders

- **Today:** Often researchers rely on unvalidated procedures to determine technology success, causing gaps in consistency

- **Importance:** Industry requires unbiased, public analytical procedures based on sound science, as well as high quality research results to evaluate technology for investment

- **Risks:** Analytical methods are crucial for evaluation of developing technologies and must be in place as new technologies are emerging.

1. Management

Challenge: Generating large volume of high-quality analytical data for multiple platform projects

- Maintaining multiple laboratories and analytical instruments

Employ online tools for sample handling, data storage, and instrument care

Provide training for methodology and instrumentation

Research technicians

- Produce consistent quality data and maintain laboratories and equipment

Challenge: Keeping abreast of emerging analytical needs, internally & externally

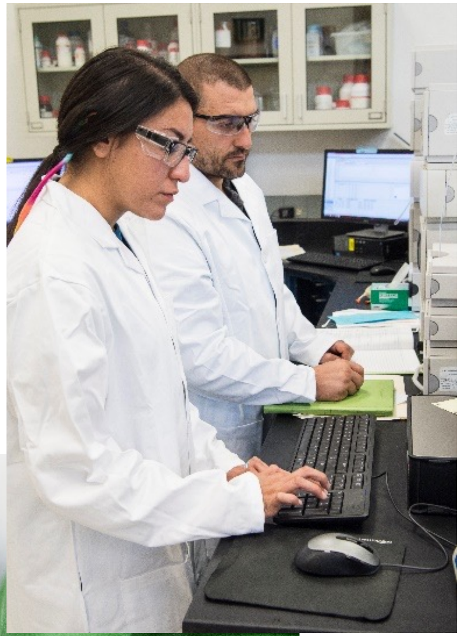
- Developing robust methods quickly

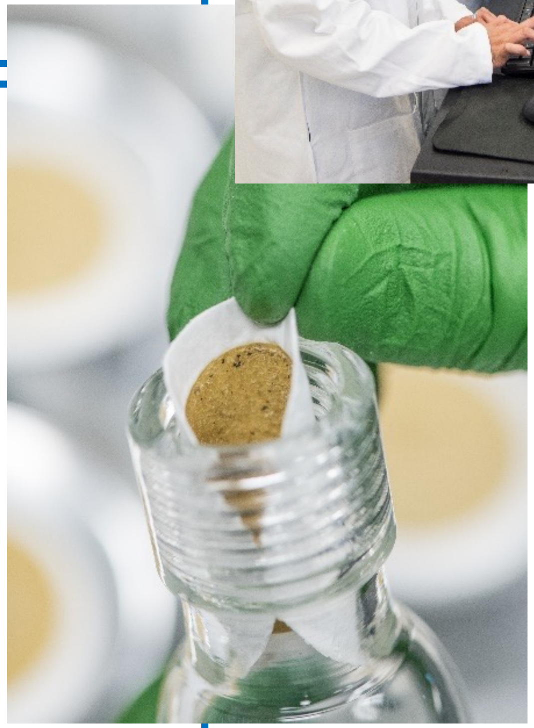
Proactively communicate with internal and external stakeholders

Build from established procedures as foundation for new methods

Scientists

- Communicate and coordinate with PIs from program tasks
- Recommend and lead new method development
- Lead research, achieve BETO milestones and publish work





1. Management

We focus on the evolving needs of R&D, always a moving target

We are responsive to new analytical needs across multiple projects and industries

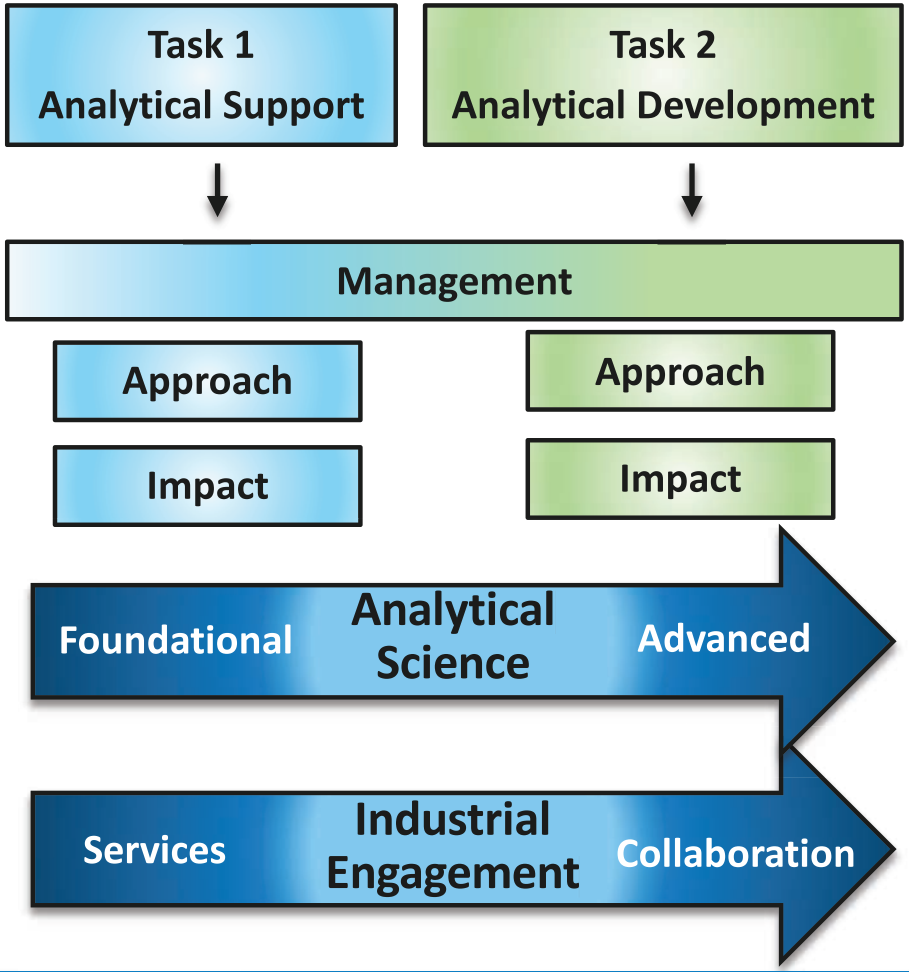
Task 1: Analytical Support

Ensure consistent data, provide functional labs, and handle sample management. External engagement

Task 2: Analytical Development

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

Photos by Pat Corkery and Dennis Schroder / NREL



2. Approach- Analytical Support

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

Approach

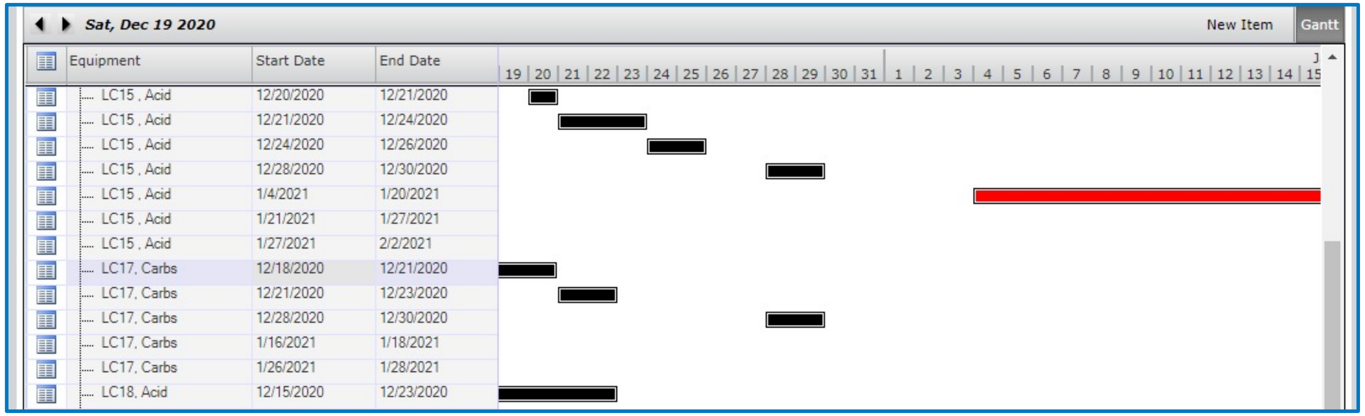
- Ensure researchers are well-trained on procedures and safety to support platform tasks
- Utilize project members in the lab, providing training and oversight for shared instrumentation
- Take primary responsibility for instrument: troubleshooting, repairs, and routine maintenance

Metrics

- Operational laboratories with functional instrumentation
- Well trained staff that operate safely and produce high quality data

Risk/mitigation strategy

- *Risk*: Poorly trained staff produce subpar data or present a safety risk
- *Mitigation*: Ensure proper training with documented results
- *Risk*: Instrument downtime
- *Mitigation*: Practice active upkeep and preventative maintenance



Equipment	Start Date	End Date
LC15 , Acid	12/20/2020	12/21/2020
LC15 , Acid	12/21/2020	12/24/2020
LC15 , Acid	12/24/2020	12/26/2020
LC15 , Acid	12/28/2020	12/30/2020
LC15 , Acid	1/4/2021	1/20/2021
LC15 , Acid	1/21/2021	1/27/2021
LC15 , Acid	1/27/2021	2/2/2021
LC17, Carbs	12/18/2020	12/21/2020
LC17, Carbs	12/21/2020	12/23/2020
LC17, Carbs	12/28/2020	12/30/2020
LC17, Carbs	1/16/2021	1/18/2021
LC17, Carbs	1/26/2021	1/28/2021
LC18, Acid	12/15/2020	12/23/2020

3. Impact- Analytical Support: Programmatic Support

Challenge

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

Transparent communication of these results to researchers

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
- No missed deadlines
- Satisfied clients and successful projects
- No wasted research time

Analytical Request

Samples tracked

Deadlines and milestones balanced

Analytical Chemistry

Coordination of work

Instrument performance monitoring

Client Report

Rigorous quality review

SDMS to archive data

Two-week turnaround for analysis

Analysis	FY19	FY20
ASE Extraction	600	450
CHN	3300	2100
Compositional Analysis	500	400
FIS	1200	1500
Intermediate Solids	300	800
Near-Infrared (NIR)	400	800
Liquors Analysis		2200
UV-VIS	1800	1200
%Solids/Ash	4500	1500
Starch	300	200
Unique Samples	**5,772**	**4,608**
Total Analyses	**21,862**	**16,156**
Projects Served	**61**	**41**

2. Approach- Method Development

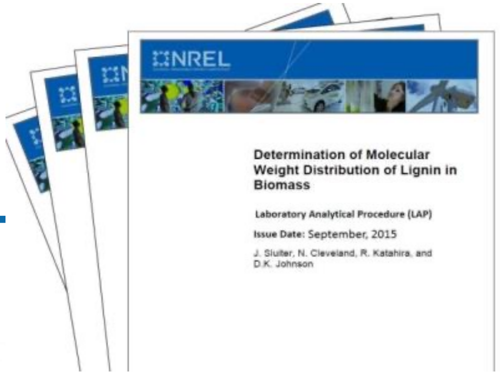
Industry requires unbiased public analytical procedures based on sound science to build on and implement current technology

Background

- Industry uses proprietary procedures to determine technology success, causing gaps in consistency

Approach

- Use our current public methods as starting point to develop novel methods for specific needs


NREL

Determination of Molecular Weight Distribution of Lignin in Biomass

Laboratory Analytical Procedure (LAP)

Issue Date: September, 2015

J. Sluiter, N. Cleveland, R. Katahira, and D.K. Johnson

Metrics

- Peer reviewed publication and/or publicly released Laboratory Analytical Procedure (LAP)
- Validation of method through round robin
- Quantified precision and accuracy
- Verification of method with advanced analytical (e.g., NMR, LCMS)

Risk/mitigation strategy

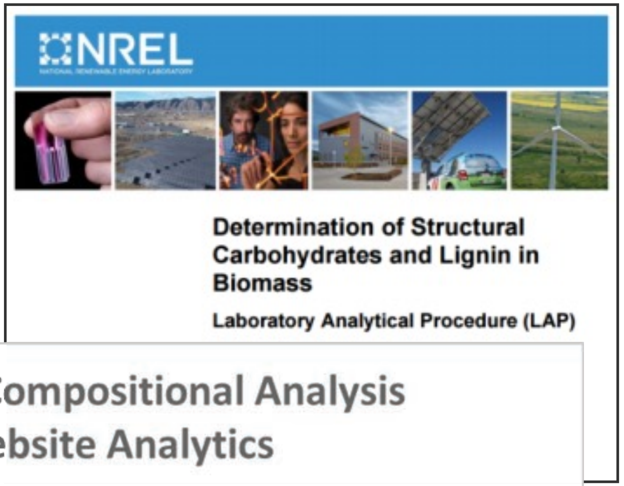
- *Risk*: Methods are not available when they are needed
- *Mitigation*: Proactive evaluation of research directions
- *Risk*: Methods are so complex they are not accessible to everyone
- *Mitigation*: Verify methods with advanced analytical, make core methods simpler

Photo by Dennis Schroeder / NREL

3. Impact- Method Development: External Engagement

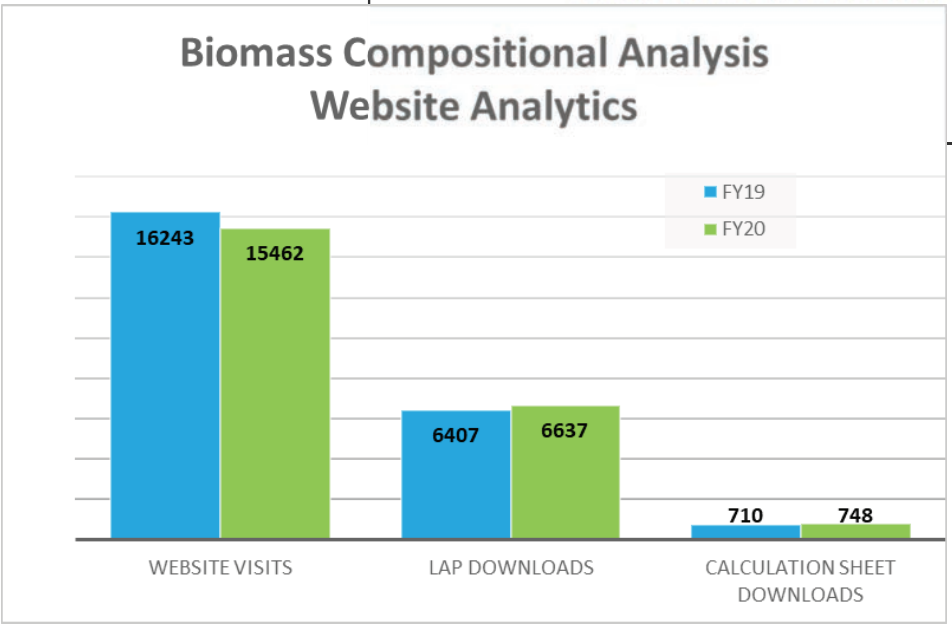
Laboratory Analytical Procedures

- 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
- Used for recertification of NIST’s Biomass Reference Materials



Biomass Compositional Analysis Website

- http://www.nrel.gov/biomass/analytical_procedures.html
- Downloadable LAPs
- Downloadable calculation sheets
- Contact information
- Videos and FAQs
- Newsletter



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

3. Impact- Method Development: External Engagement

We are in continual communication with community stakeholders to ensure the availability of precise, accurate, and robust analytical methods that will enable R&D int the biofuels community.

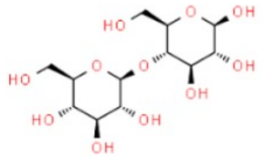
Our partnerships allow us to be responsive to new analytical needs across multiple projects and industries

NREL is a chair of Subcommittee E48.05 on Biomass Conversion
NREL Laboratory Analytical Procedures (LAPs) were adopted by ASTM almost 10 years ago

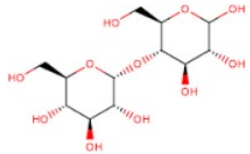
3. Impact- Analytical Method Development

Simultaneous conversion of starch and cellulose in a corn ethanol plant:

Analytical challenges



β -Glucan
Cellobiose



α-Glucan
Maltose

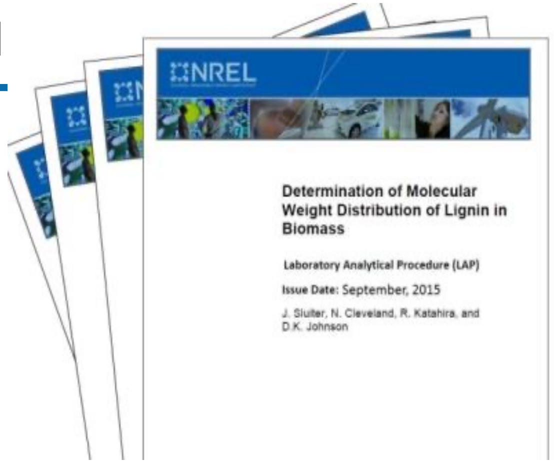
Background

The Renewable Fuels Standard established a financial incentive for generation and use of renewable fuels that were generated from renewable feeds.

- Most of the renewable ethanol generated in the US is from corn starch (1st gen), but a small amount is also from the cellulose (2nd gen) present in the corn grain
- The burden of proof that cellulose is converted is on the analytical data generated at the conversion facility.
- The corn ethanol industry has little experience in measurement of cellulose and the EPA has been concerned about the precision and accuracy of proprietary methods.
- Industry uses proprietary procedures to determine technology success, causing issues with transparently of data

Approach

- Engage with the community to determine the maximum benefit that NREL can provide
- Use our current public methods as a starting point to develop novel methods for to meet the need



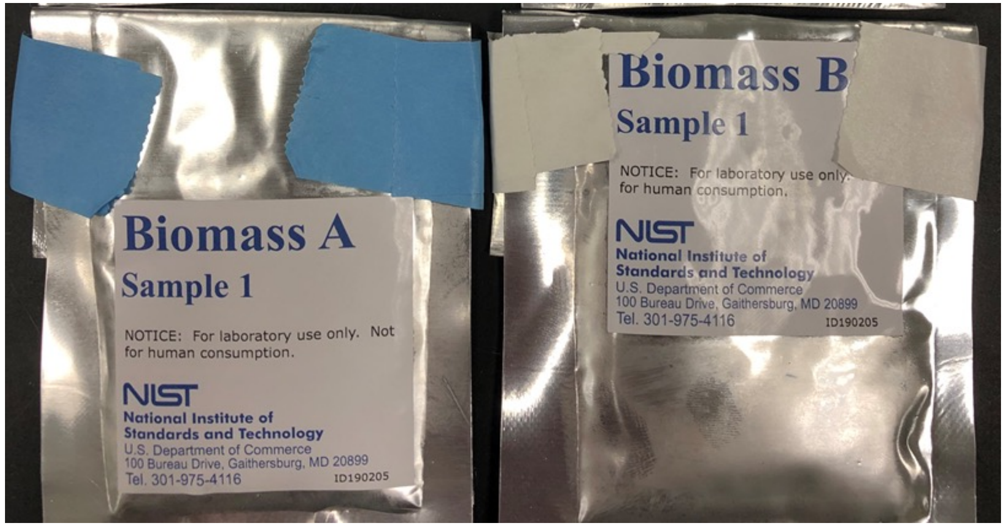
3. Impact- Analytical Method Development

EPA requested BETO assistance to develop a cellulosic assay that was scientifically validated and publicly available

- ADS has a reputation for reliable analytical procedures and experience with carbohydrate chemistry
- ADS has an established relationship with stakeholders
- ADS has access to advanced analytical techniques to validate the developed method

The Challenge:
Develop an accurate and precise method that measures cellulose when there is starch present.

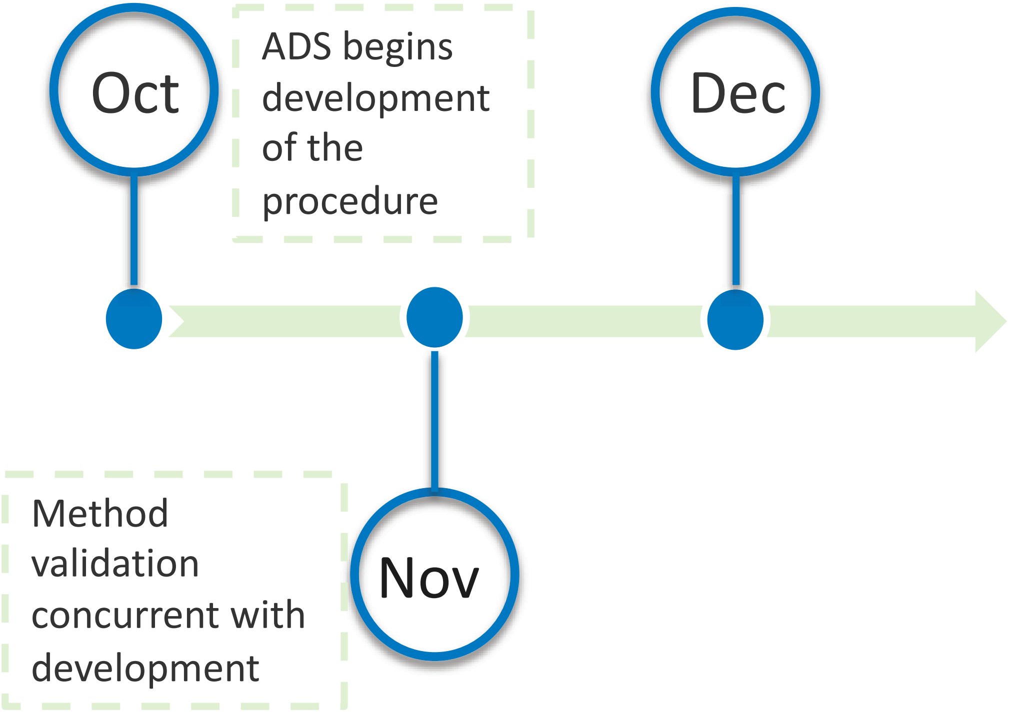
- There are no native standards with known concentrations
- Do not use synthetic matrices as standards to ensure appropriate fit for native materials



Our Objective:

- Develop a suitable method
- Validate with sister national labs in round robin
- Do it in a year

3. Impact- Analytical Method Development



FY19Q3 Milestone:

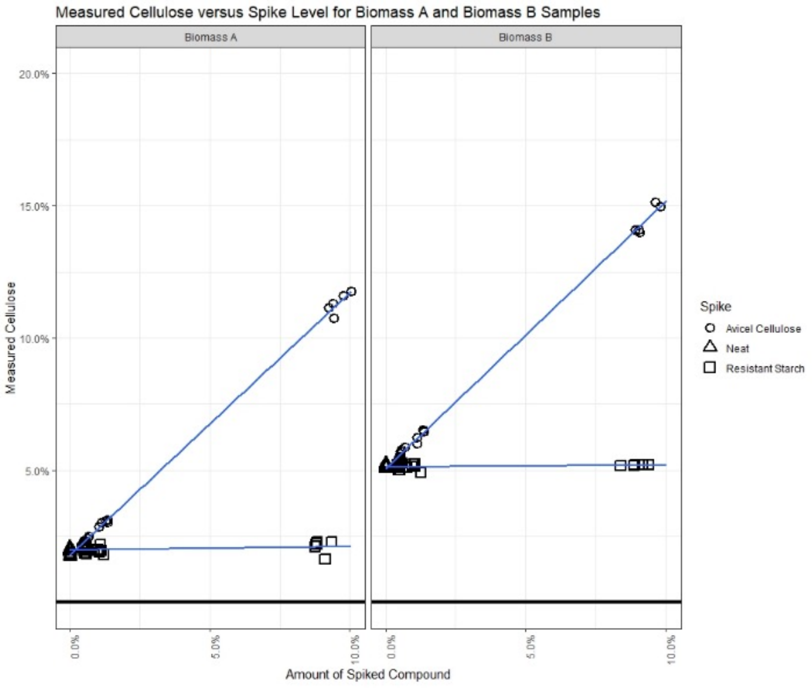
"Document improvements to starch assay for high starch corn fiber samples"

Determination of Structural Carbohydrates and Lignin in Biomass

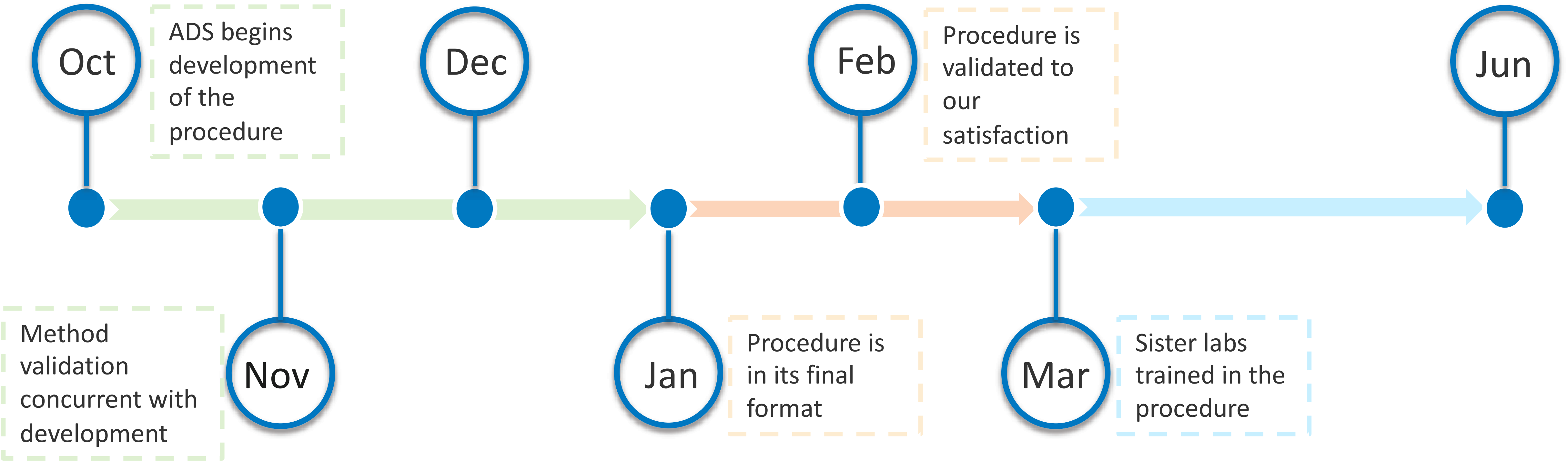
Laboratory Analytical Procedure (LAP)

3. Impact- Analytical Method Development

- **Oct** — ADS begins development of the procedure
- **Nov** — Method validation concurrent with development
- **Dec**
- **Jan** — Procedure is in its final format
- **Feb** — Procedure is validated to our satisfaction



3. Impact- Analytical Method Development



A very aggressive timeline for method development

In June the final report with the method and accuracy obtained at NREL was submitted to the EPA and for publication.

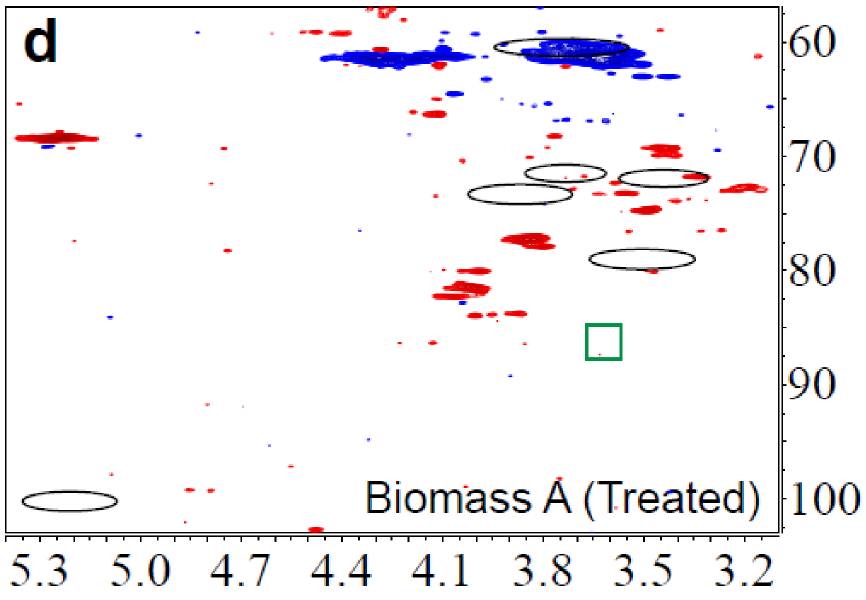
4. Progress and Outcomes

ADS leveraged our experience with carbohydrate analysis to design a robust analytical procedure.

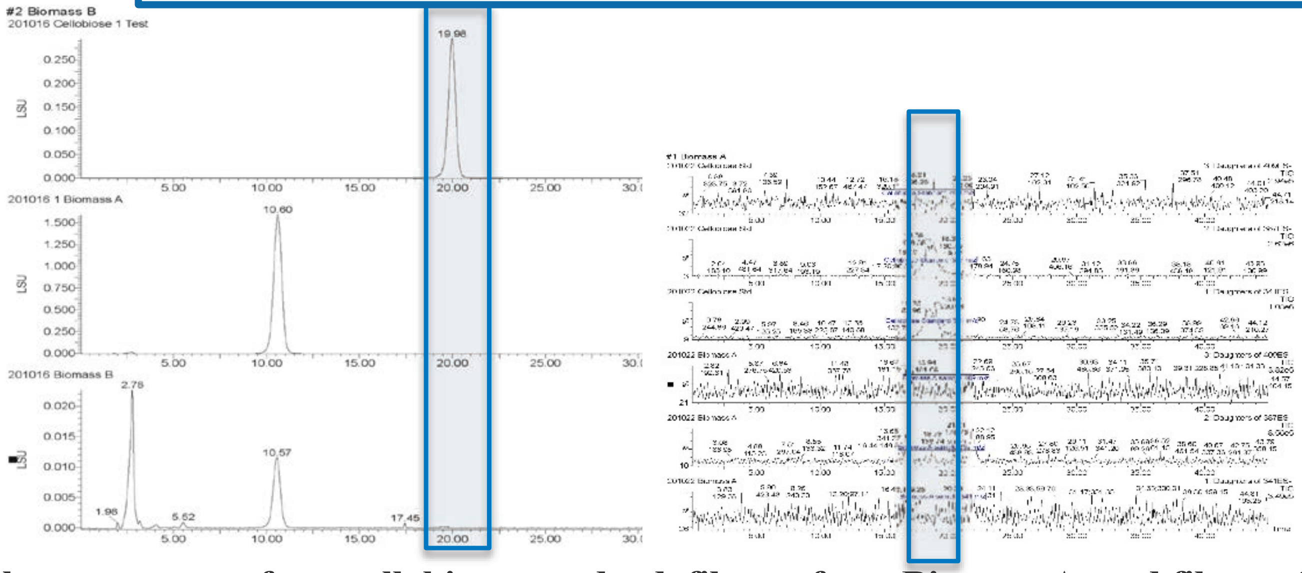
- NREL starch procedures and lignocellulosic procedures formed a base for this new analytical procedure.

This procedure was validated using advanced techniques.

- Our experience with LC/MS techniques to quantify carbohydrate oligomers was leveraged to validate the method without reliance on standards.
- This prevents similar burdens for industry



Gel-state 1H-13C HSQC NMR on ball-milled biomass samples



Chromatograms for a cellobiose standard, filtrate from Biomass A, and filtrate from Biomass B; ELSD response scaled to maximum peak height. b Mass spectra of the peak of interest at 19.8 min

4. Progress and Outcomes

Direct determination of cellulosic glucan content in starch-containing samples

Justin B. Sluiter · Katie P. Michel · Bennett Addison · Yining Zeng · William Michener · Alexander L. Paterson · Frédéric A. Perras · Edward J. Wolfrum

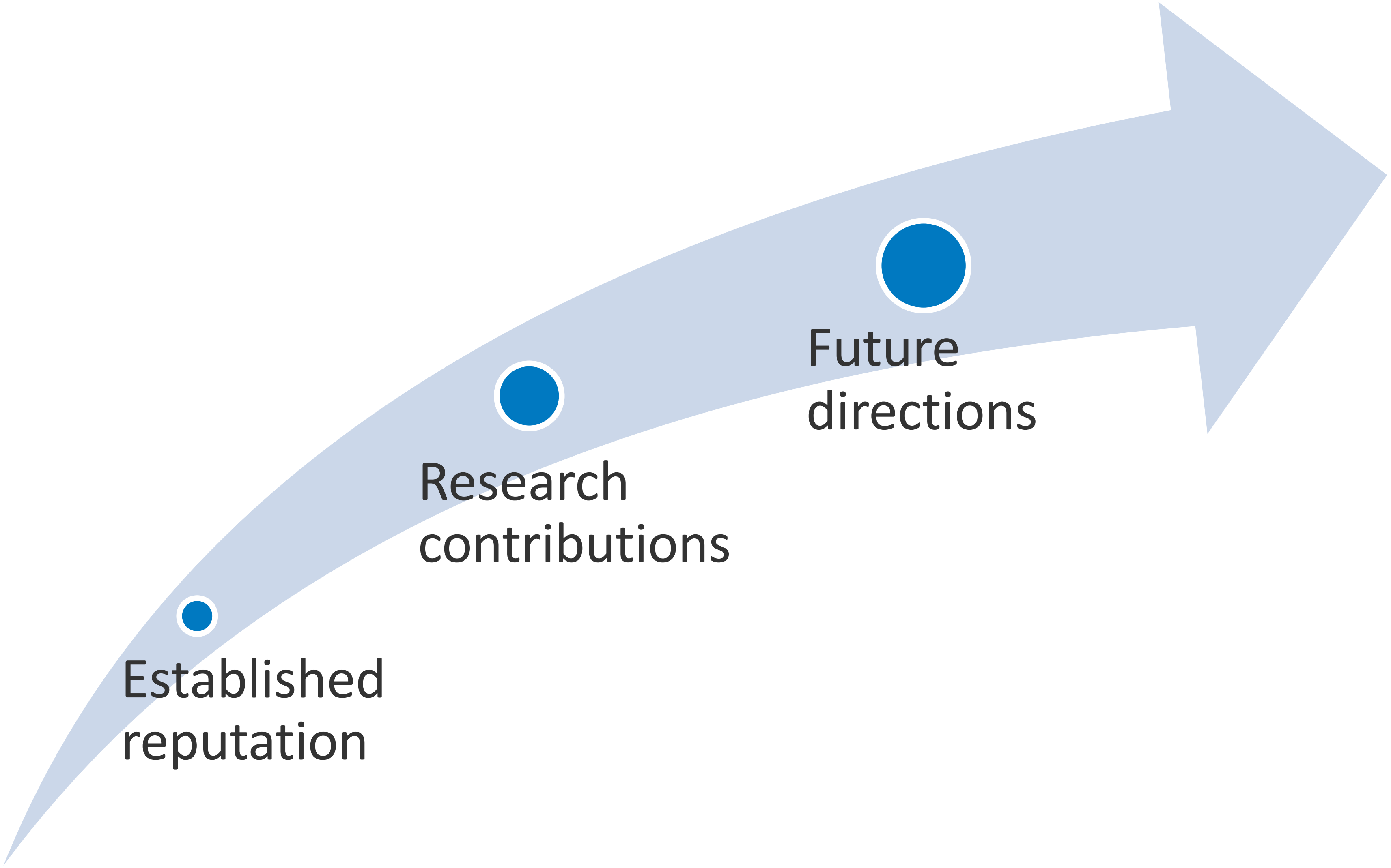
Spike	Level (wt%)	N	Simple estimates			Mixed effect regression model		
			Mean (wt%)	SD	CV (%)	Mean (wt%)	SD	CV (%)
Biomass A								
No spike	0	18	1.95	0.13	6.5	1.9	0.04	2.1
Avicel cellulose	0.5	9	2.34	0.09	3.7	2.4	0.04	1.7
Avicel cellulose	1	5	3.03	0.09	3.1	2.9	0.04	1.5
Avicel cellulose	10	5	11.34	0.34	3.0	11.2	0.11	1.0
Resistant starch	0.5	9	2.02	0.11	5.7	2.0	0.06	3.0
Resistant starch	1	5	1.97	0.14	7.1	2.0	0.06	2.8
Resistant starch	10	5	2.22	0.07	3.1	2.2	0.04	1.8
Biomass B								
No spike	0	16	5.21	0.06	1.2	5.2	0.038	0.7
Avicel cellulose	0.5	9	5.63	0.17	3.0	5.7	0.039	0.7
Avicel Cellulose	1	5	6.32	0.17	2.7	6.1	0.040	0.7
Avicel cellulose	10	6	14.69	0.50	3.4	14.8	0.115	0.8
Resistant starch	0.5	9	5.17	0.10	1.9	5.2	0.058	1.1
Resistant starch	1	5	5.14	0.13	2.5	5.2	0.056	1.1
Resistant starch	10	5	5.21	0.05	1.0	5.2	0.039	0.8

Exceptional precision

- The EPA released a guidance memo that requires a 10% CV for replicate analysis of cellulose.
  - at the time this was a challenging target
  - This could potentially require many replicates
- **The NREL method has measured CV's of ≤ 3.0%**

The low CV% allows for fewer replicates required to reach EPA targets, saving time and money.

Analytical Developments



4. Progress and Outcomes

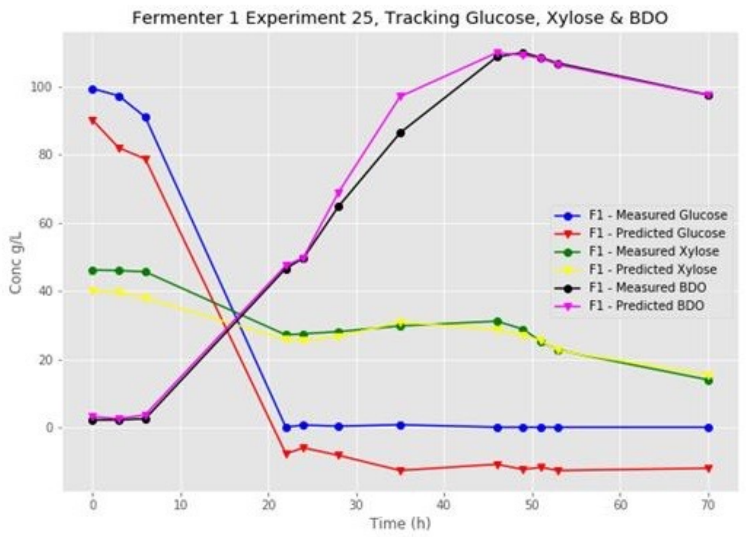
Near-Infrared instruments and models

- We currently maintain two Thermo-Fisher Antares II FT-NIR spectrometers and one Metrohm XDS instrument.
- We also continued to maintain calibration models for

Corn stover	Panicum hallii	Slurry insoluble solids
Sorghum	Herbaceous feedstocks	Reactivity prediction
Miscanthus	Slurry constituents	

NREL has licensed these copyrighted models to external industry stakeholders.

Photo by Dennis Schroeder / NREL



Near-Infrared support of programmatic directions

- Calibration model to predict the concentrations of key components during the microbial conversion of cellulosic sugars to 2,3-Butane-diol

Glucose	2,3-butane-diol (BDO)	Acetoin
Xylose	Glycerol	

– The PLS model is currently being used to monitor BDO fermentation by NREL researchers.

4. Progress and Outcomes

Novel Feedstock Composition

Biofuels research is moving towards cost-advantaged feedstocks which present unique challenges

- Many publicly used compositional analysis methods rely on gross measurements such as total organic weight or total carbon percentage of the feedstocks.
  - Food wastes and anaerobic digestion feeds present unique challenges as they have high levels of fats, proteins, pectins and other components not present in traditional energy crops.
- ADS has been developing analytical procedures for these feedstocks to anticipate the direction that BETO is moving
  - Areas of current research include pectin analysis, fats and oils quantification, refinement of lignin quantification to account for high protein contents.

Summary

Management:

Actively engage with researchers and stakeholders to anticipate research needs. Maintain state of the art, functional laboratories to ensure highest quality data.

Approach:

Use existing procedures and tools as a foundation to develop novel analytical techniques to support developing research.

Impact:

Excellent reputation with stakeholders for quality analytical chemistry methodology. Well maintained laboratories and high-quality data.

Progress and Outcomes:

High precision and accuracy analytical procedure for monitoring cellulose conversion in ethanol plants, which will result in money saved.

New analytical methods for real-time monitoring of BDO

First stages of new methods to support cost advantaged feeds

Photo by Dennis Schroeder / NREL

Quad Chart Overview, WBS 2.5.1.101

Timeline

- October 1, 2018
- September 30, 2021

	FY20	Active Project
DOE Funding	FY19: $750K FY20: $950K FY21: $671K	$2,371K

Project Partners*

- N/A

Barriers addressed

Ct-A: Defining Metrics around Feedstock Quality

Ct-B: Efficient Preprocessing and Pretreatment

Ct-C: Process Development for Conversion of Lignin

Project Goal

The goal of the ADS project is to ensure consistent, high quality analytical data for internal and external stakeholders, develop critical analytical procedures that respond to program and industry needs, and support NREL's globally adopted procedures

End of Project Milestone

Comparison of Machine-Learning Algorithms as Prediction Models of Biomass Composition: Use machine learning techniques to improve spectroscopy-based, rapid biomass compositional analysis approaches. Use at least two different machine-learning algorithms to develop new rapid biomass compositional analysis prediction models and compare their performance to existing "industry standard" linear models for prediction accuracy and uncertainty. (End of project milestone was adjusted for funding decrease.)

Funding Mechanism: Core BETO funding.

**Only fill out if applicable.*

Thank you

www.nrel.gov

This work was authored by the National Renewable Energy Laboratory, operated by Alliance for Sustainable Energy, LLC, for the U.S. Department of Energy (DOE) under Contract No. DE-AC36-08GO28308. Funding provided by U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office. The views expressed in the article do not necessarily represent the views of the DOE or the U.S. Government. The U.S. Government retains and the publisher, by accepting the article for publication, acknowledges that the U.S. Government retains a nonexclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this work, or allow others to do so, for U.S. Government purposes.

NREL Transforming ENERGY

Additional Slides

Method Development Details

Method Development, Improvement or Validation

Black Liquor and Alkaline Pretreated Carbohydrate Quantification in the Liquor Fraction: Quantification of black liquors and alkaline pretreated biomass has been problematic because our carbohydrate HPLC columns are not rated for samples with a pH above 8. Also, the acidified the black liquor or alkaline liquor forms lignin precipitates which can force free sugars to precipitate as well. While we can determine sugar concentrations for total sugar analysis with our standard 4% hydrolysis, we are currently unable to quantify free monomeric sugars. Using a Dionex ICS-5000+ with an HPAE-PAD set up we are able to quantify monomeric sugars in the black liquors without compromising the samples. The HPAE-PAD with an Dionex SA-10 column runs at a pH of 11 so the soluble lignin remains in solution and sugars like: glucose, xylose, arabinose, & galactose can be quantified.

Quantification of Avicel during Fermentation: Avicel is an insoluble glucose polymer that can be utilized for fermentation. Our standard 2-step hydrolysis method determines glucose but cannot differentiate the source of the glucose. Because avicel is insoluble, it cannot be injected on our HPLC's. Therefore, we developed a curve that analyzed dried yeast used in a fermentation spiked with known quantities of avicel. These mixtures were analyzed using a 2-step hydrolysis as well as analyzed on our Elementar CHN analyzer. While both methods produced a reliable curve, the CHN analysis produced more reliable results as well as a quicker turnaround. Developing a curve of yeast mass and avicel mass against the %N, which we assume is protein, was able to quantify utilized avicel during fermentation and provide biologists with a novel metho for tracking carbohydrate consumption.

Novel Feedstock Composition

In FY19 we performed composition on a large variety of feedstocks. Each feedstock has its own challenges. A few of the novel feedstocks we quantified in FY19 include, pine, cork bark, pistachio hulls, almond hulls, pistachio and almond shells. Pine was challenging because its composition has oil and wax that are not extracted with ethanol or hexanes and lead to false high quantification of lignin content. To quantify pine we had to first extract the material with acetone, then water and ethanol. Almond and pistachio hulls presented new challenges as well. Both are very high in extractives and contain pectin. While we don't have a universal method to quantify pectin, we are able to identify the galacturonic acid present in the hull extracts. Pectin analysis and quantification has been identified as a potential research growth area.

While birch wood and poplar are not novel feedstocks for ADS to analyze, we did receive quite a few samples that contained large amounts of inorganic impurities. The inorganic impurities were present due to a mechanical failure while milling the samples resulting in addition of, mostly Iron(III) oxide, rust. It was important to confirm that our 2-step hydrolysis procedure could still accurately quantify carbohydrates and lignin with the presence of the impurity. While we were successful with quantifying the composition of the feedstock, we were also able to quantify the amount of Iron(III) oxide that had contaminated the original samples and the neutralization effect the Iron (III) oxide had during analytical hydrolysis.

Method Development Details

Starch

The accurate quantification of starch present in mixed starch-cellulose feeds and intermediate products has been of increased interest to the biofuels community since the EPA has incentivized the conversion of cellulose as part of the Renewable Fuel Standard Program. ADS has worked, in recent years, to collaborate with the industry to better understand the state of analytical chemistry for starch quantification. The FY19Q1 milestone "NIST Standard development – Collaborate with NIST and other external stakeholders to produce a reference material that contains both cellulose and starch for evaluation of accuracy of cellulosic quantification methods when non-cellulosic sources of glucans are present" was the capstone the research to improve the quantification of starch in corn grain and related products.

The accurate quantification of starch present in mixed starch-cellulose feeds and intermediate products has been of increased interest to the biofuels community since the EPA has incentivized the conversion of cellulose as part of the Renewable Fuel Standard Program. ADS has worked, in recent years, to collaborate with the industry to better understand the state of analytical chemistry for starch quantification. The cellulosic glucan present in samples that also contain substantial amounts of starch was an improved methodology for starch quantification. ADS had previously made recommendation to improve our starch assay procedure and this year's work made additional procedural improvements. Specifically, a procedural change recommended by Megazyme, the enzyme manufacturer, to switch from boiling DMSO to chilled sodium hydroxide as the agent to access resistant starch allows us to increase the number of samples analyzed per batch while eliminating the hazard of boiling water baths.

Anaerobic Digestion

Analytical Development and Support has been collaborating in research to better utilize cost advantaged feedstocks such as food wastes or municipal solid wastes. Many existing compositional analysis methods rely on gross measurements such as total organic weight or total carbon percentage of the feedstocks. These methods have proven sufficient for anaerobic digestion bioreactor operators to track total conversion and reduction of organic matter in the production of methane but are not granular enough to perform optimization of biological pathways in support of enhancements that lead to higher value fuel products.

Feedstocks typical of anaerobic digestion present new challenges for determination of chemical composition as they contain components that are not present in significant concentrations in traditional terrestrial feedstock. We adapted existing procedure to quantify increased concentrations of oils and fats, to speciate some carbohydrates specific to foods and wastes, and to handle intermediate conversion products which are unlike the material for which the methods were originally developed.

Training

This task covers the time necessary to train staff on new instruments and procedures, both the instructor and trainee. Training not only develops staff capabilities but allows the center to have a more diverse base of well-trained personnel. We also ensure that multiple individuals are trained for all the different method we perform to ensure deadlines are appropriately met. It is this essential cross-training that allows for the consistent delivery of analytical data for projects.

Method Development Details

Deacetylation with Radleys Reactor

The ADS project researchers collaborated with other projects to develop a bench-scale method for NaOH-catalyzed deacetylation using a Radleys Reactor-Ready Duo system. Developing a bench-scale deacetylation method allows researchers to vary parameters for sample preparation and optimize control settings. The Radleys Reactor also allows researchers to collect time-course samples. Samples collected are analyzed for lignin, acetate, and sugars using our standard LAPs.

- ***Peer-Reviewed Publications***
- Sluiter, J.B., Michel, K.P., Addison, B. *et al.* Direct determination of cellulosic glucan content in starch-containing samples. *Cellulose* (2021). https://doi.org/10.1007/s10570-020-03652-2
- Salavachua, D., Rydzak, T., Auwae, R., De Capite, A., Black, B.A., Bouvier, J.T., Cleveland, N.S., Elmore, J.R., Furchess, A., Huenemann, J.D., Katahira, R., Michener, W.E., Peterson, D.J., Rohrer, H., Vardon, D.R., Beckham, G.T., & Guss, A.M. "Metabolic engineering of *Pseudomonas putida* for increased polyhydroxyalkanoate production from lignin." *Microbial biotechnology*, Jan. 2020.
- Sievers, D.A., Kuhn, E.M., Thompson, V.S., Yancey, N.A., Hoover, A.N., Resch, M.G., & Wolfrum, E.J. "Throughput, Reliability, and Yields of a Pilot-Scale Conversion Process for Production of Fermentable Sugars from Lignocellulosic Biomass: A Study on Feedstock Ash and Moisture." *ACS Sustainable Chemistry & Engineering*, Jan. 16, 2020.
- Spiller, R., Knoshaug, E.P., Nagle, N., Dong, T., Milbrandt, A., Clippinger, J., Peterson, D.J., Van Wychen, S., Panczak, B., & Pienkos, P.T. "Upgrading brown grease for the production of biofuel intermediates." *Bioresource Technology Reports*, Feb. 1, 2020.
- Ray, A.E., Williams, C.L., Hoover, A.N., Li, C., Sale, K.L., Emerson, R.M., Klinger, J., Oksen, E., Narani, A., Yan, J., Beavers, C.M., Tanjore, D., Yunes, M., Bose, E., Leal, J.H., Bowen, J.L., Wolfrum, E.J., Resch, M.G., Samelsberger, T.A., & Donohoe, B.S. "Multiscale Characterization of Lignocellulosic Biomass Variability and Its Implications to Preprocessing and Conversion: a Case Study for Corn Stover." *ACS Sustainable Chemistry & Engineering*, Feb. 11, 2020.
- Wolfrum, E.J., Payne, C., Schwartz, A., Jacobs, J., & Kressin, R.W. "A Performance Comparison of Low-Cost Near-Infrared (NIR) Spectrometers to a Conventional Laboratory Spectrometer for Rapid Biomass Compositional Analysis." *BioEnergy Research*, May 12, 2020.
- Nagle, N.J., Donohoe, B.S., Wolfrum, E.J., Kuhn, E.M., Haas, T.J., Ray, A.E., Wendt, L.M., Delwiche, M.E., Weiss, N.D., & Radtke, C. "Chemical andstructural changes in corn stover after ensiling: influence on bioconversion." *Frontiers in bioengineering and biotechnology*, Aug. 14, 2020.

- ***Peer-Reviewed Publications***
- Decker, S.R., Harman-Ware, A.E., Happs, R.M., Wolfrum, E.J., Tuskan, G.A., Kainer, D, Oguntimein, G.B., Rodriguez, M, Weighill, D, Jones, P, & Jacobson, D, "High Throughput Screening Technologies in Biomass Characterization." *Fronteirs in Energy Research*, Nov. 17, 2018.
- Knoshaug, E.P., Wolfrum, E.J., Laurens, L.M.L., Harmon, V.L., Dempster, T.A., & McGowen, J. "Unified field studies of the algae testbed public-private partnership as the benchmark for algae agronomics." *Scientific Data*, Nov. 27, 2018.
- Chen, X, Katahira, R, Ge, Z, Lu, L, Hou, D, Peterson, D.J., Tucker, M.P., Chen, X, & Ren, Z.J. "Microbial electrochemical treatment of biorefinery black liquor and resource recovery." *Royal Society of Chemistry*, Dec. 6, 2018.
- Johnson, C.W., Salvachua, D, Rorrer, N.A., Black, B.A., Vardon, D.R., St. John, P.C., Cleveland, N.S., Dominick, G, Elmore, J.R., Khanna, P, Martinez, C.R., Michener, W.E., Peterson, D.J., Ramirez, K.J., Singh, P, Vander Wall, T.A., Wilson, A.N., Yi, X, Biddy, M.J., Bomble, Y.J., Guss, A.M. & Beckham, G.T. "Innovative Chemicals and Materials from Bacterial Aromic Catabolic Pathways." *Joule*. Jun. 19, 2019.
- Payne, C.E., Sluiter, J.B., and Wolfrum, E. published, "Assaying Sorghum for Fuel Production," in the book Sorghum: Methods and Protocols edited by Zhao, ZY, and Dahlberg, J. This book chapter describes the compositional analysis procedures developed by NREL specific to sorghum. This includes references to the publicly available NREL LAPs themselves and specific comments on handling the quantification of starch and other nonstructural carbohydrates in this species.

Selected comments from 2019 Peer Review

General Comment	
Reviewer Comments	**RESPONSE TO COMMENTS**
Well needed project (both from running and keep labs and developing new methods) and it will provide more predictability to bio-fuels and bioproducts. It would be great to see a comparison between methods for fermentation parameter monitoring PI developed and on-the-shelf equipment.	We thank the reviewers for their comments. We will "keep doing what we are doing", and we will try to improve our performance with closer collaborations with internal and external collaborators
The goal of this project is to provide analytical services for various NREL and BETO projects, along with large group of external collaborators. Overall, the project and performance are outstanding. A key strength is that they are posting procedures for biomass analysis and standards. The proposed transition to low-cost NIR spectroscopy is very promising. The only recommendation is to keep doing what you are doing.	We strongly believe that there is substantial value in establishing, supporting, and communication common analytical procedures to ensure a "common language" among biomass researchers, and the this is a unique role that BETO and NREL can play in moving the community forward
ADS plays a vital role in developing, providing, and standardizing analytical services for national labs and collaborators in academia and industry. They perform tens of thousands of analyses a year, supporting dozens of research groups, and have emerged as an important reposity of knowledge regarding laboratory analytical proceedures. The greater community increasingly relies on this important resource.	
This project is need driven. NREL should continue to focus on the development of cost effective methods that can be deployed by research, test and industrial labs. More attention should be paid to cost effective user friendly methods that can be successfully implemented by potential industrial end users.	
This project helps ensure the generation of high quality data for both internal and external stakeholders. The team develops and improves analytical methods that are used across projects, and manages sample workflow so that the project teams can operate efficiently without having to wait on data. There is a strong emphasis on standardization, consistency, and quality control. The ADS team is also developing new methods, and has reported development of a low cost at-line NIR method for real time analysis of fermentation broth that is nearly as accurate as a much higher cost instrument. One gap in their tools is the analysis of intracellular metabolites, for metabolomics and 13C-flux analysis applications.	
Weakness: Strategy needs to focus on future adoption of methods at industrial scale. Cost effectiveness and user friendly format, timeliness and ease of use will inusre commerical adoption of methods being developed. The researchers are encouraged to highlight these goals and work towards them.	Our short-term work with industry is focused on Gen1.5 biorefineries, and we appreciate the need for robust but "user friendly" methods that are practical in an industrial environment.