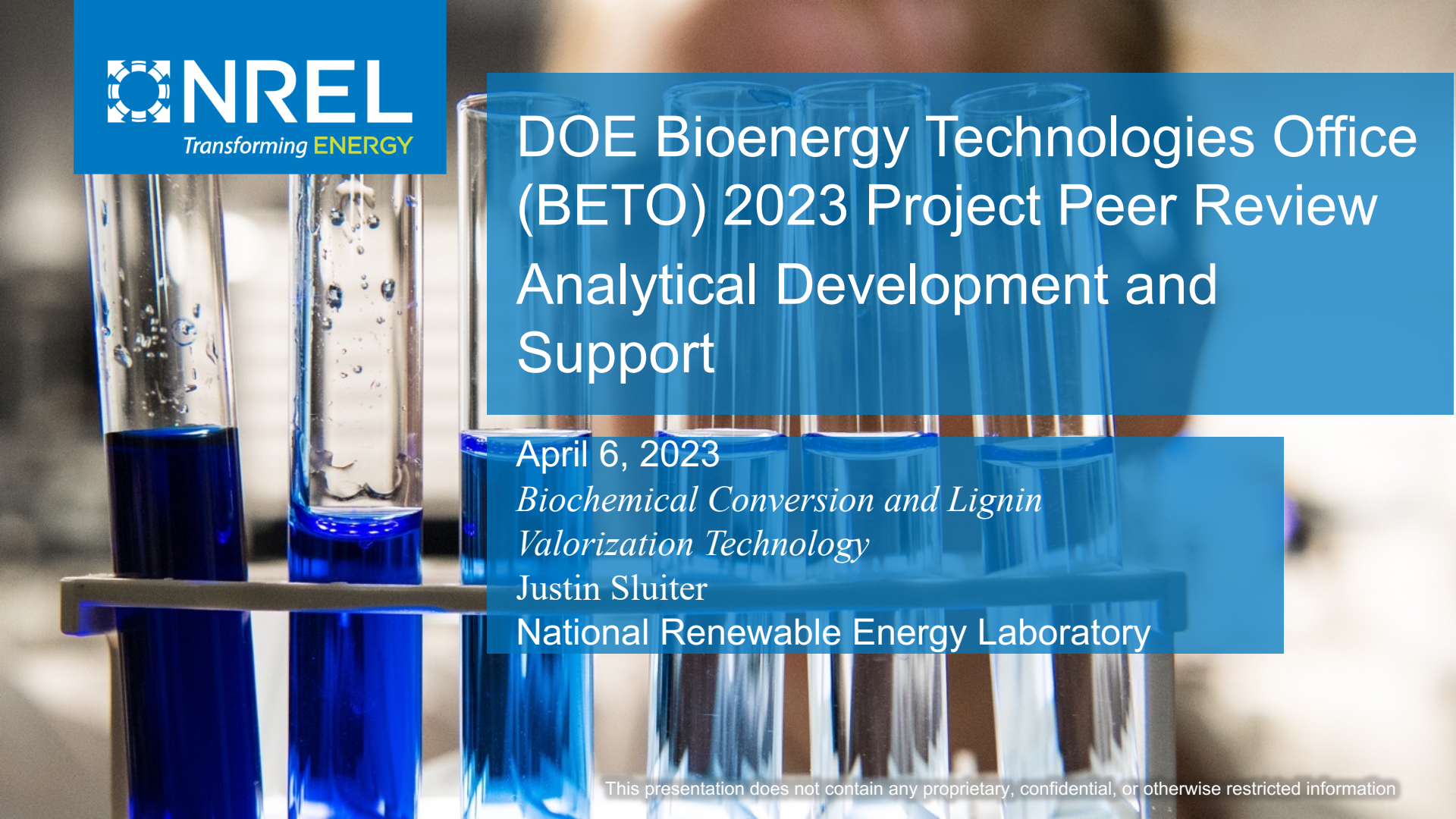


DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

Analytical Development and Support

April 6, 2023

Biochemical Conversion and Lignin Valorization Technology

Justin Sluiter
National Renewable Energy Laboratory

Project Overview

ADS supports BETO's goal of sustainable fuel production by developing new analytical methods and ensuring that project evaluation data is the highest caliber. This allows for sound technoeconomic analysis of all projects.
External projects directly address industrial needs, increasing BETO impact.

- Analytical Development
  - Develops novel methods to support developing technology, at NREL and in industry
- Analytical Support
  - Provides analytical and QA/AC support to many BETO projects
  - Maintains shared user laboratories and instrumentation

External projects
- Industry-led research at NREL
- Initiated through a directed funding opportunity with this project

* Novozymes / Poet
* ADM/ Mesa, Arizona
* Prenexus Health, LLC
* Shell International

Project Leaders
- Justin Sluiter
- Darren Peterson
- Katie Michel

Approach: Quality Data

Quality analytical data is the foundation for making sound scientific decisions

- Coordinate samples analysis across programs
- Provide Quality Control on every analysis

- Maintain multiple analytical laboratories with shared instrumentation
  - HPLCs, moisture analyzers, ovens, furnaces, etc.

- Provide training to staff across the program on analytical instruments and procedures
  - Skilled staff produce quality data
  - Trained staff work safely

Progress: Analytical Support

ADS staff collaborate with & process data for projects across the program to ensure every project is provided the highest quality data in a timely fashion.

20+ program projects

Data

- Algal Biofuels and Bioproducts
- Pretreatment and Biological Upgrading
- Lignin Valorization

Data Request

Analytical Chemistry

Coordination of work

Instrument performance monitoring

Client Report

Composition Intermediate		3350
Fraction Insoluble solids	375	2250
HPLC	2762	2762
Liquor analysis	698	3490
Neutralization Capacity	12	12
NIR prediction	1422	1422
Starch	87	174
Total	**5,993**	**17,688**

Approach: Method Development

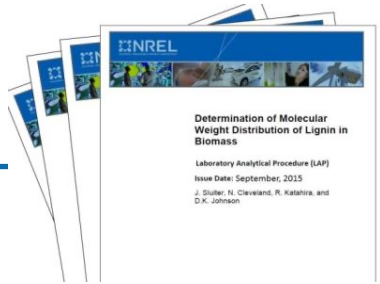
Existing, widely adopted methods serve as a foundation for development of new methods.

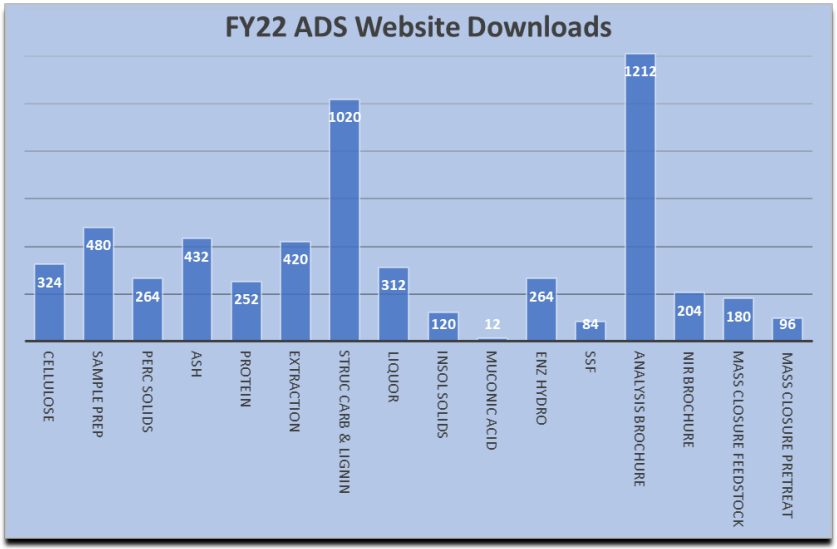
Background

- 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

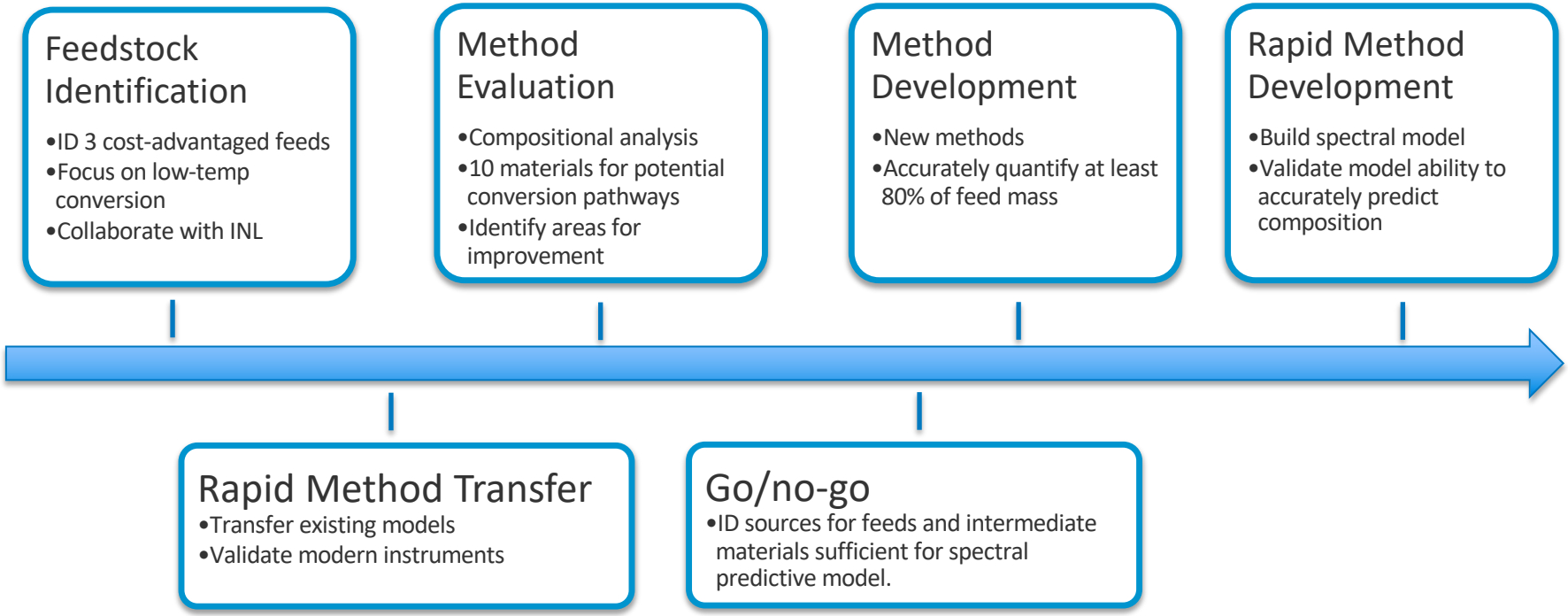




Our internationally recognized Laboratory Analytical Procedures (LAPs) serve as a basis for development of novel methods.

Approach: Project Milestones and Vision

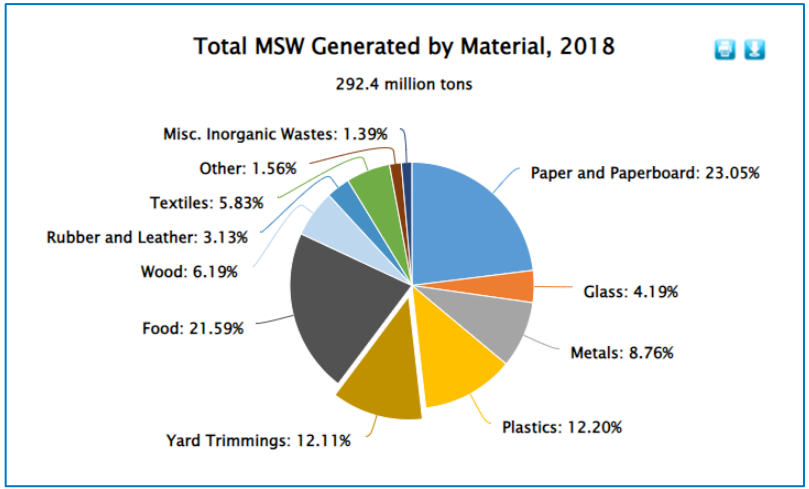
Development of new analytical capabilities: A story through project milestones



We Identify new materials where existing methods lack, use existing literature to target components not well characterized, and modify methods to target key compounds.

Progress: Milestone progress

Collaboration with INL to identify and characterize low-cost feeds



Cost-advantaged feedstocks were identified based on INL research, literature, and EPA data

- Existing analytical methods are acceptable for some feeds, some need improvement for full mass closure and speciation
- Constituent improvements needed
  - Pectin
  - Non-structural
  - Fats

Sample Description	%Total Ash	%Total Protein	% Lignin	% Glucan	% Xylan	% Galactan	% Arabinan	% Fructan	% Acetyl	% Total
Cellulosic										
Office Paper	17.69	0.00	1.59	63.68	15.65	1.26	1.11	0.00	0.00	100.98
Cardboard	3.05	0.00	18.96	62.72	9.37	1.46	6.02	0.00	0.00	101.58
Glossy Paper	26.81	0.00	3.23	53.80	12.88	0.00	1.72	0.00	0.00	98.45
Yard Waste	5.52	0.00	28.71	17.28	5.94	2.73	5.32	0.00	2.14	67.63
Textile	0.26	0.00	2.14	98.48	0.00	0.00	0.00	0.00	0.00	100.88
Foods										
Protein	11.84	53.94	26.68	3.00	0.00	0.00	0.00	0.00	0.00	95.46
Dairy	7.68	37.06	26.52	10.28	0.00	4.22	0.00	0.00	0.00	85.77
Vegetables	11.72	16.69	8.06	24.76	1.98	3.48	2.92	2.27	0.00	71.87
Fruit	3.14	3.31	11.11	32.07	2.03	2.06	2.90	2.96	0.00	59.59
Mixed Food Waste	6.17	13.00	19.62	37.51	1.09	2.06	1.15	1.76	0.00	82.36

Approach: Risks and DEI

Risk	Mitigation Strategy
Methods are not industrially relevant	Maintain relationships with industrial partners and standards organizations (ASTM) to allow external method review
Expert staff departs NREL	Cross train existing staff
Instruments are not operational	Produce redundant procedures that utilize different instruments

- Diversity, equity, and inclusion activities
  - Ensure unrestricted access to Laboratory analytical procedures (LAPs)
    - Published online, accessible worldwide at nrel.gov/bioenergy/biomass-compositional-analysis.html
    - FAQs and training videos and to assist users
    - Website contacts for answers from NREL experts
  - Actively cultivating relationship with Metro State University (HSI)

Impact: BETO

- Supported SAF research with novel analytical techniques
  - Biomass extraction methods
  - Rapid lignin quantification
  - On-line NIR BDO fermentation monitoring
  - Anerobic digestion solids methods
  - Diode array analysis of acetic acid

- Ensured quality analytical data was available to evaluate developing SAF technologies
  - QA/QC
  - Training
  - Analytical instrument care and maintenance

Through quality analytical data, new conversion processes to produce SAF and products can be evaluated for technoeconomic viability.

Impact: Support of Gen 1.5 Ethanol

June 2019: EPA requested BETO assistance to develop a cellulosic assay that was scientifically validated and publicly available capable of differentiating cellulose glucans form starch glucans.

- This work directly supports generation of D3 RIN credits for the Renewable Fuels Standard with a value of hundreds of million of dollars.

January 2021: The resulting method and associated validation are summarized in a publication in *Cellulose* released

- Sluiter, J.B., Michel, K.P., Addison, B. *et al.* Direct determination of cellulosic glucan content in starch-containing samples. *Cellulose* **28**, 1989–2002 (2021). https://doi.org/10.1007/s10570-020-03652-2

February 2021: Published an NREL LAP adaptation to support the Corn Ethanol industry (2021)

Impact: Support of Gen 1.5 Ethanol

August 2022: Method adopted by US EPA as preferred method for measuring cellulose in corn grain (RIN credits)

2022 Guidance Document (pdf) (September 2022, EPA-420-B-22-041)

Ongoing: Support of industry using the NREL cellulose method

- Participation in ASTM working group for Cellulose Assay
- Collaborating with Megazyme to adapt the method for known biases
- Helping industry and academia understand and perform the procedure, including a training video

NEOGEN | Megazyme

"This letter serves to state our support for the work of Justin Sluiter and his Biomass Analytical Technologies (BAT) team at NREL."

"The value of this publication [Sluiter *et al*.] cannot be overstated"

Impact: Funded Industrial Collaborations

With the success of the collaboration with the EPA and industry for cellulosic method development, ADS looked for new areas where we could use our knowledge to generate value for industry where they may struggle with limited access to expertise of instrumentation.

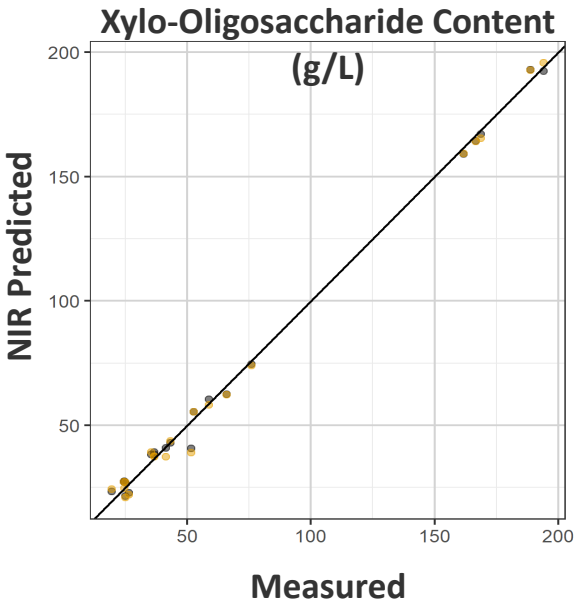
- Direct industrial impact
  - Competed funding opportunities awarded in 2022
  - Industry leads research to address issues
  - Provides industry partners with access to NREL expertise and equipment
  - Agreements will generate new analytical methods for public use (not protected by IP)
  - Projects are ongoing

Prenexus Health, Inc

Challenge: Prenexus was a small nutritional supplement startup using slow wet chemical techniques to monitor their production of xylo-oligomers

Solution: Use ADS expertise in Near-Infrared (NIR) and chemometrics to develop at-line predictive models for xylo-oligosaccharide

Progress: Concluded. Robust models for xylo-oligomers, total solids, and total soluble xylose developed and validated. Manuscript on this work in process.



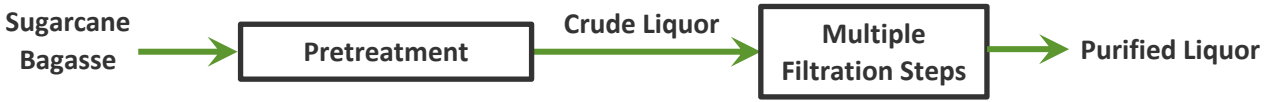


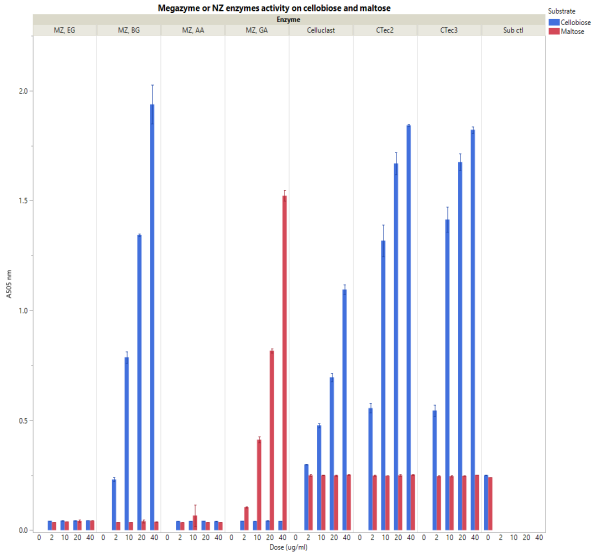
Image from www.prenexushealth.com/prenexos

Novozymes and Poet

Challenge: The existing NREL Cellulose method for Gen 1.5 ethanol has known biases and does not quantify hemicellulose

Solution: Use the ADS developed method as base to develop alternatives method(s) that address mixed linkage beta-glucans, along with hemicellulose content while ensuring access to all resistant starches

Progress: In progress.



Comparison of various enzymes for cellulose (blue) and starch (red) activity

	DMSO		NaOH	
Sample	**Starch,** Average Value	**Cellulosic Glucan**, Average Value	**Starch,** Average Value	**Cellulosic Glucan**, Average Value
NIST 8644 BC	74.39	1.91	75.30	1.94
NIST 8645 AC	3.87	6.25	5.03	5.38
Avicel Cellulose	0.00	93.37	0.00	97.91
Resistant Starch	96.84	0.53	97.46	0.39
Corn Starch	102.73	0.42	99.56	0.41

Initial comparison of the NREL published NaOH method with the AOAC DMSO method, shows slightly higher values for starch and lower values for cellulose when using the NaOH method.

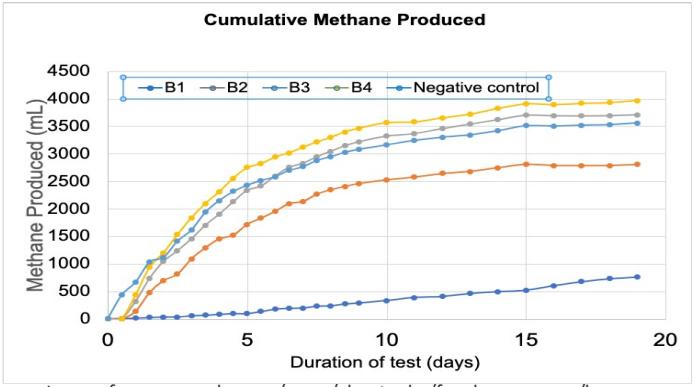
Images from Novozymes.com and poet.com

ADM & City of Mesa, AZ

Challenge: The existing analytical techniques for characterization of food wastes and anaerobic digestion products are insufficient for calculation of D3 RINs.

Solution: Apply existing ADS analytical methods and validation techniques as a base for new method development.

Progress: In progress.



Images from www.adm.com/en-us/about-adm/f and mesaaz.gov/home

Feed/substrate: Thickened Mixed Sludge (TMS) – mixture of 67% WAS and 33% primary sludge from the Kansas City Blue River wastewater facility

This work has experienced some delays and we are in the process of generating digestates for analysis.

AD[illegible] LA[illegible] support conversion into valuable renewable fuels and support D3 RIN during an[illegible].

KANSAS STATE UNIVERSITY

ADM Unlocking Nature. Enriching Life.

mesa·az

Shell

Shell wants to develop standardized analytical procedures for chemical composition of wet manure feedstocks and the digestate formed following anaerobic digestion of organic waste to produce renewable natural gas (RNG)

Build and validate near-infrared (NIR) models for high throughput analysis, potentially deployable at commercial facilities

The output will be a new analytical method and publication for manure analysis and NIR analysis of both dry and wet slurries.

This work has just begun.
Shell has delivered the initial samples for analysis.

Summary

- Develop new analytical methods and support existing procedures
- Ensure quality data for evaluation of SAF BETO conversion processes
- Address industrial needs via external projects, increasing BETO impact

Approach:

- Develop novel analytical techniques to support developing research at NREL and in industry
- Ensure quality analytical data is utilized to evaluate developing technologies
- Mitigate risks through industrial partnerships, training, and redundant procedures
- Increase access to analytical methods through publicly available Laboratory Analytical Procedures and relationships with minority serving institutions

Impact:

- Industrial support through development and publication of neutral third-party method to measure cellulose in corn grain
- Direct industrial support through Directed Funding Opportunity projects with four industrial partners or partner groups
- Continuing support of industry through method improvement and assisting industry and academia with method performance
- Ensuring quality analytical data results in fair and accurate technology assessment

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
- New benchtop and rapid methods to support cost advantaged feeds

Quad Chart Overview

Timeline
- 10/1/2021
- 9/30/2024

	FY22 Costed	Total Award
DOE Funding		
Project Cost Share*		

TRL at Project Start: 4
TRL at Project End: 5

Project Goal
The goal of this project is to support evaluation of conversion technologies by developing benchtop and rapid analytical methods.

End of Project Milestone
Demonstrate the ability of a spectral predictive model to provide accurate chemical compositional data for glucose, xylose, galactose, arabinose, lignin, protein, pectin, and acetyl content for at least 3 different feedstocks across a variety of low temperature conversion technologies.

Funding Mechanism
BETO Conversion Lab Call 2021

Project Partners*
- Partner 1
- Partner 2

**Only fill out if applicable.*

www.nrel.gov

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Additional Slides

Project Milestones

- 3/2022. Collaborate with INL to identify a list of at least 3 feedstocks that are being considered for low-temperature conversion
- 6/2022.0Transfer existing legacy NIR/PLS models to modern NIR instruments
- 9/2022. Perform compositional analysis of at least 10 different materials that represent the potential conversion pathways & identify areas for improvement
- 9/2023. Produce a procedure that is capable of accurately quantifying at least 80% of mass present in feedstocks
- 9/2024. Demonstrate spectral predictive model's ability to provide accurate chemical compositional data low temperature conversion technologies

- 3/2023. Go/No-Go: Establish sources for required feeds and conversion intermediate materials sufficient for development of spectral predictive model.

Megazyme Letter of Support

NEOGEN | Megazyme

Megazyme,
IDA Business Park,
Bray,
Wicklow
A98YV29,
Ireland

NREL,
15013 Denver West Parkway,
Golden,
CO 80401

Dear Sir/Madam,

This letter serves to state our support for the work of Justin Sluiter and his Biomass Analytical Technologies (BAT) team at NREL.

The US bioethanol sector is reliant on a federal subsidy system, the renewable fuel standard (RFS), which is administered by the Environmental Protection Agency (EPA). A significant boost to existing subsidies is available through the use of a technology termed in-situ corn kernel fiber processing. Unfortunately, while a number of proprietary analytical methods have been developed to measure the ethanol produced this approach, and have been accepted by the Californian regulator (California Air Resources Board) for their low carbon fuel standard (LCFS) program, the EPA have not accepted these, and the federal RFS subsidy program remains gridlocked with respect to D3 RIN generation from 1.5G processes.

Sluiter *et al.* sought to address this problem through their seminal publication in 2021 entitled "Direct determination of cellulosic glucan content in starch containing samples". In a complex market that is typified by confidential proprietary information and trade secrets, Justin provided an open access research article that addressed the challenges and proposed solutions for this fundamental analytical problem facing the bioethanol industry. This has generated interest and momentum within the scientific community to solve the remaining analytical challenges. The value of this publication cannot be overstated.

I have collaborated with Justin wherever possible over the last 3 years, and currently co-chair a working group with him under the auspices of ASTM. I have found him to be a scientific expert of the highest calibre. His experience in method development for the compositional analysis of biomass materials is unrivalled and his impartial, balanced contributions demonstrate absolute integrity with his only goal being to advance the science and increase the available body of knowledge in the field.

Yours sincerely,

David Mangan

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Directors: J. Adent (USA), J. Lilly (USA), S. Quinlan (USA), S. Donnachie (UK)

Responses to Previous Reviewers' Comments

- The previous comments from the 2021 Peer Reviewers were largely very positive.

- Areas for improvement:
  - A risk is that potential adoption of the developed methods will depend on the use of instrumentation that is accessible to other potential users, so a focus on inexpensive and user-friendly methods is critical.
    - ADS has been working with industry through the DFOs and informal communications to better understand the instruments that are widely available.
    - One example is our investigation of a benchtop extraction method intended as equivalent to the ASE extractions.

Publications, Patents, Presentations, Awards, and Commercialization

- List any publications, patents, awards, and presentations that have resulted from work on this project.
- Use at least 12-point font.
- Describe the status of any technology transfer or commercialization efforts.