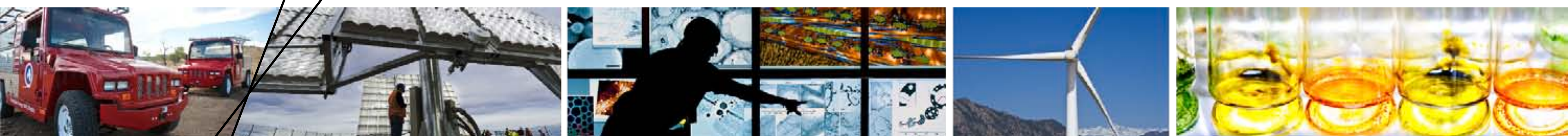
Analytical Development and Support



DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

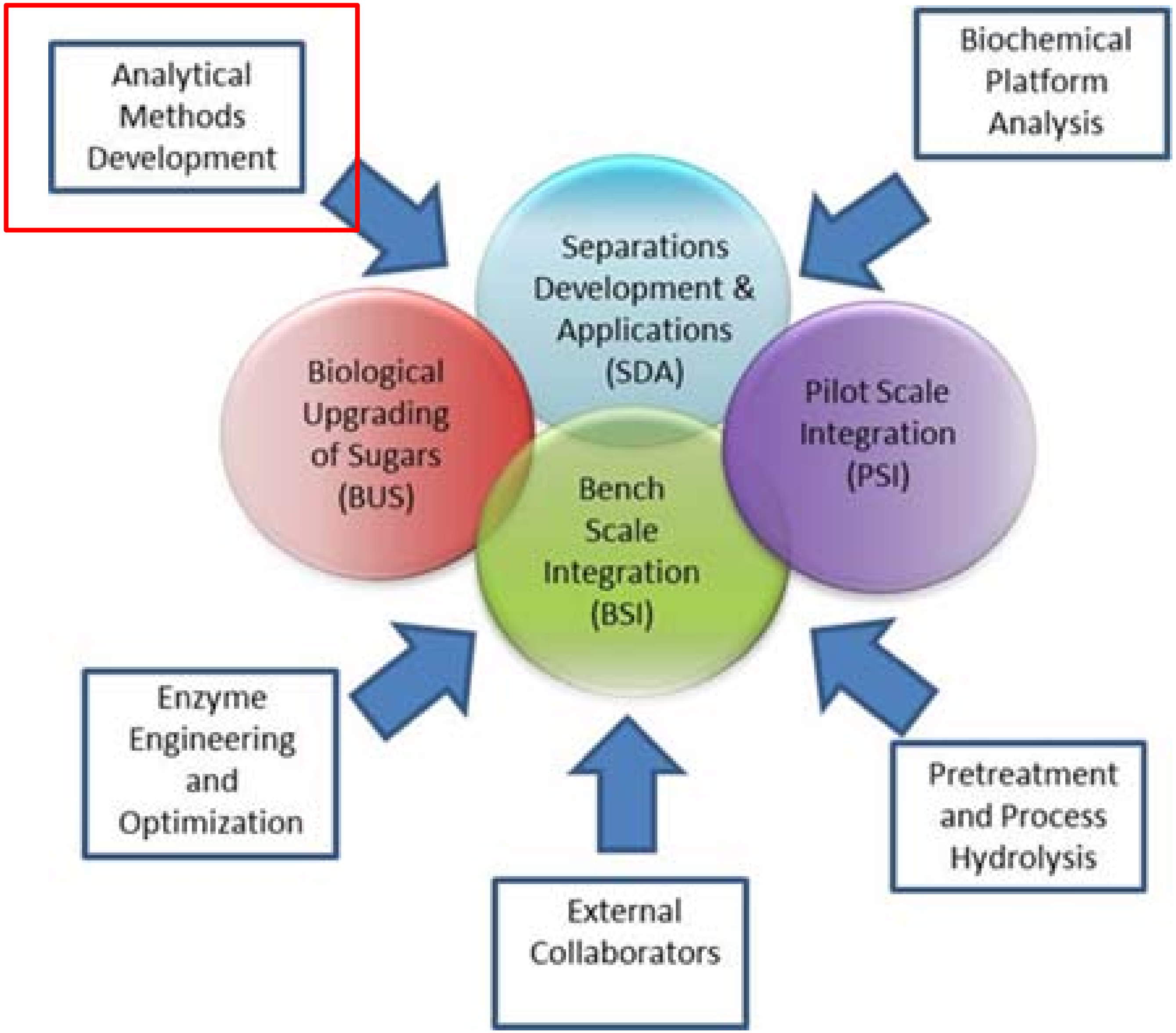
Date: March 26, 2015

Technology Area Review: Biochemical Conversion

Principal Investigator: Ed Wolfrum

National Renewable Energy Laboratory

Role in the Overall Platform



Goal Statement

Goal: Support and enable DOE/BETO biofuel and bioenergy research and development tasks with high quality analytical data and advance the tools available to the biofuels industry through method development and globally adopted procedures

We actively cultivate partnerships with industry, academia, and sister government laboratories, based largely on NREL's reputation for excellence in analysis

Quad Chart Overview

Timeline

- **Project start date- FY14**
- **Project end date- FY17**
- **Percent complete-50%**

Barriers

- **Bt-B. Biomass Variability**
- **Bt-C. Biomass Recalcitrance**
- **Bt-D. Pretreatment Processing**

Budget

	Total Costs FY10 – FY12	FY 13 Costs	FY 14 Costs	Total Planned Funding (FY15-Project End Date
DOE Funded	$5.9M*	$1.3M*	$1.3M	$3.9M FY15-17

*** Activities in this project were part of former BPI Project**

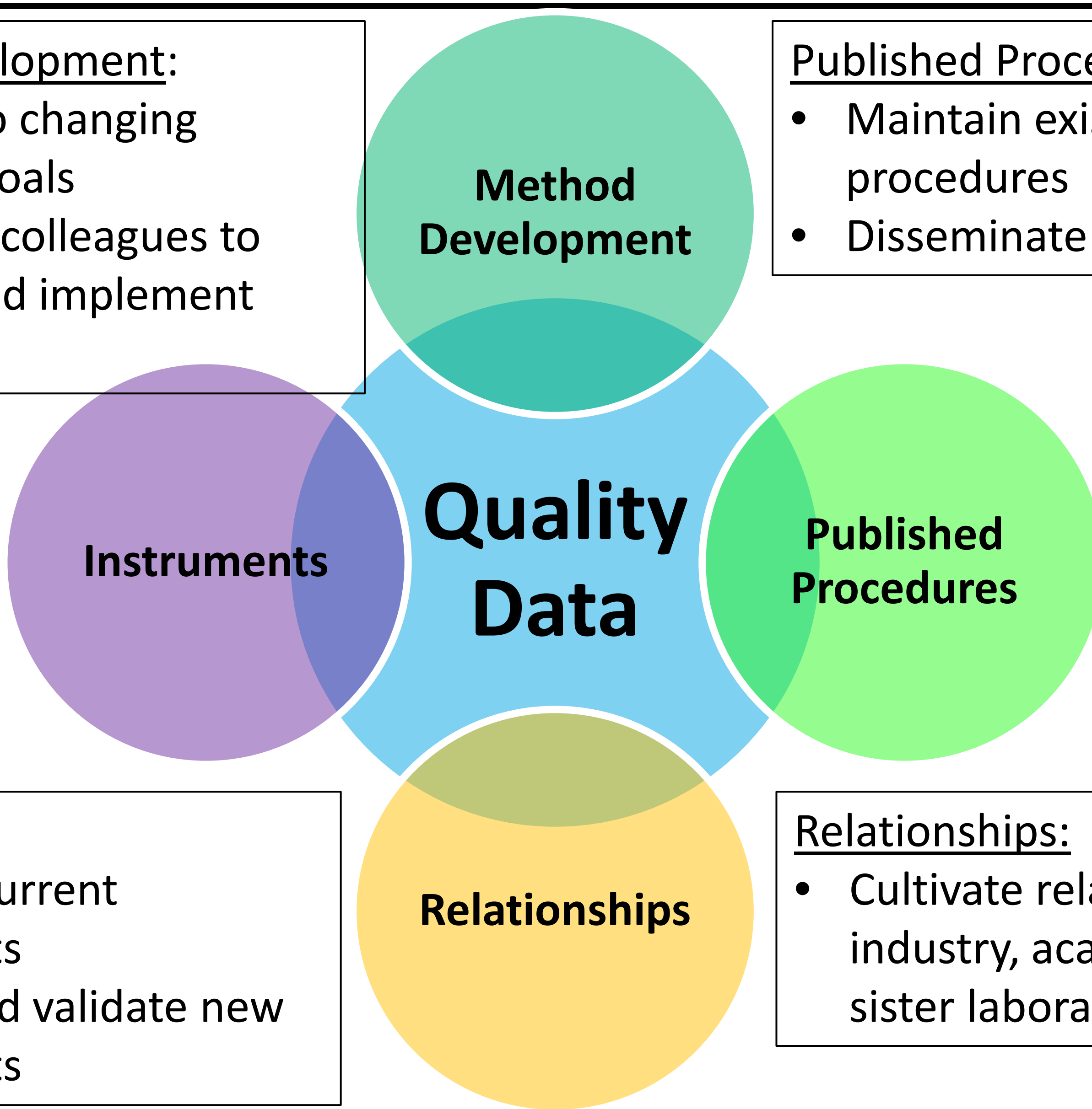
Partners

- Rapid prediction models and analytical work facilitated partnerships with:
  - INL
  - Shell
  - Toyota
  - DuPont
  - Abengoa
  - Chromatin
  - Colorado State University
  - University of Illinois at UC
  - Washington State University

Project Overview

<u>Method Development</u>:
- Respond to changing technical goals
- Work with colleagues to develop and implement methods

<u>Published Procedures</u>:
- Maintain existing procedures
- Disseminate new knowledge



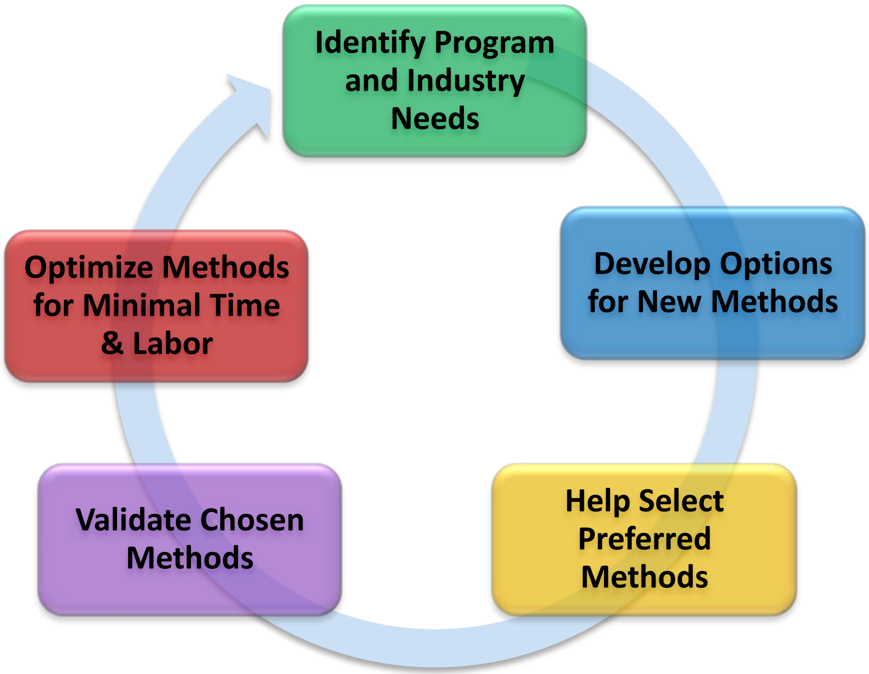
<u>Instruments:</u>
- Maintain current instruments
- Identify and validate new instruments

<u>Relationships:</u>
- Cultivate relationships with industry, academia, and sister laboratories

Approach - Technical

Critical Success Factors

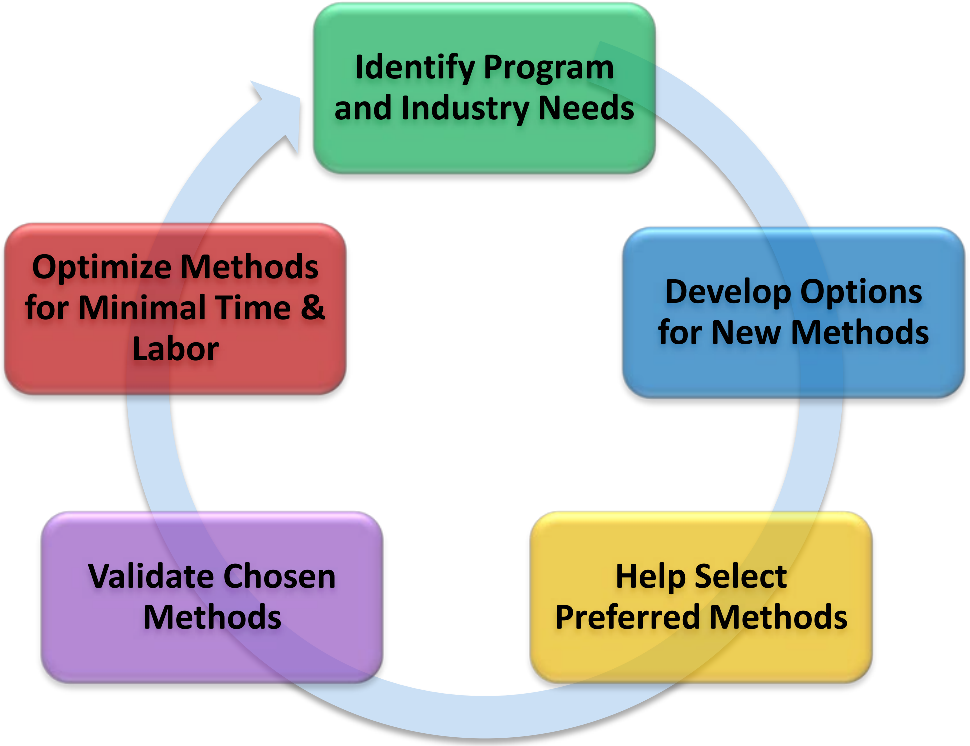
- We need to provide analytical chemistry support and expertise to our colleagues when they need it
- Our success measure is simple: are the clients getting the data they need to meet their research goals?



Challenges

- Keeping abreast of changing program needs
- Supporting multiple projects with multiple deadlines
- Developing robust methods under tight deadlines
- Maintaining a large number of analytical instruments

Approach - Management



We work side by side with research teams to respond to new and changing analytical chemistry needs

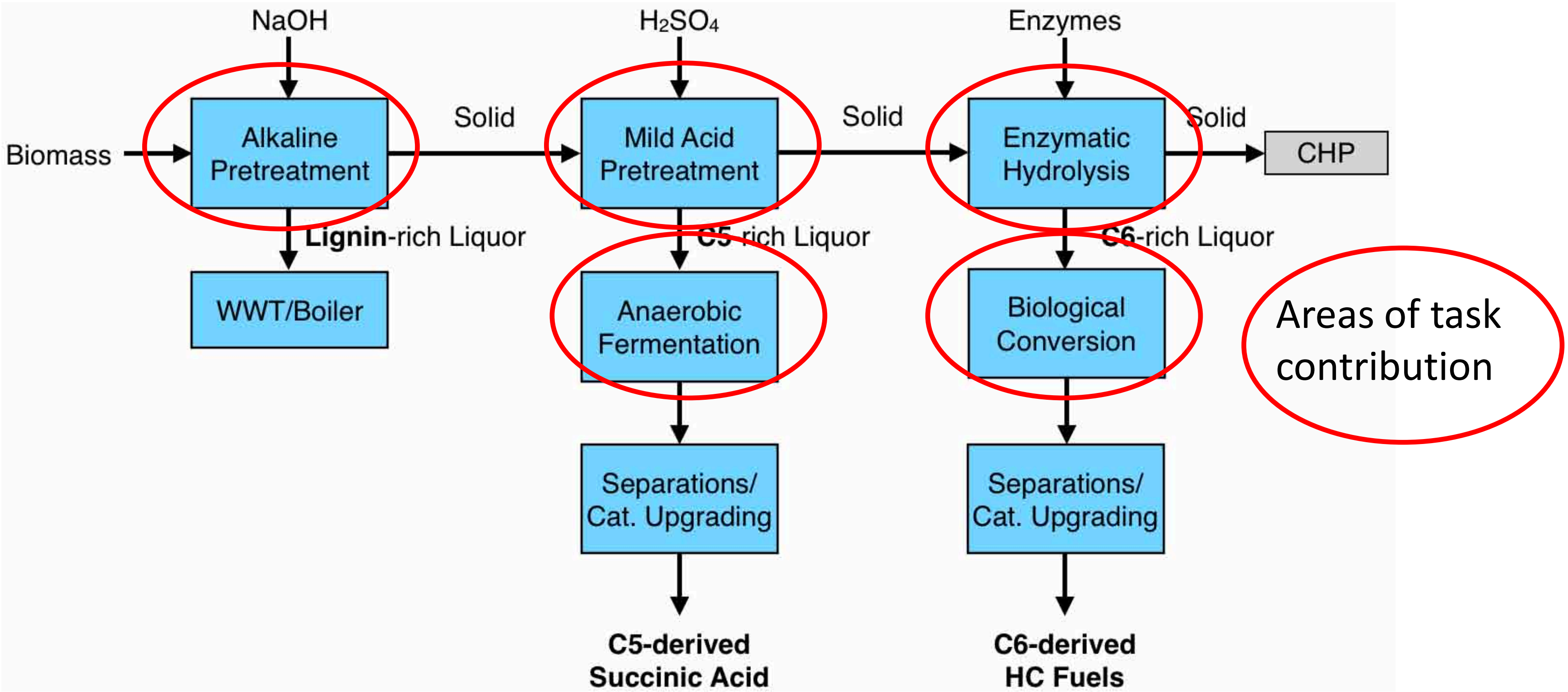
FY15 Milestones

Q1 Milestone: Identify new methods required to meet FY17/FY22 technical program goals

Q2 Milestone: Report on analytical work, including service subcontracts and review of analytical work performed for the platform

Q4 Milestone: Implement validated methods to meet FY17/FY22 technical program goals

Technical Accomplishments



FY17 Technology Demonstration contains 2 pathways, each with analytical challenges

FY15 Q1 milestone to identify challenges and present solutions

FY15 Q4 to author procedures for each new method

Technical Accomplishments - Quality Data

Ensure Quality Laboratory Analytical Data for BETO projects

Data provided for BETO projects	
Analysis	**FY14 Samples**
NIR	2900
Liquor	2400
FAME	500
FIS	460
Intermediate solids	390
Feedstock solids	180
Enzymatic Hydrolysis	160
Ash	125
Gravimetric	120
Starch	120
Reactivity Screening	50
Titration	25

- Analytical results also provided to 18 Work for Others or CRADA projects

- We coordinate analyses and provide QA/QC for many BETO projects
- We have developed lightweight software tools to track:
  - Sample Identity
  - Compositional analysis results
  - Instrument usage/reservation
  - Analysis queue/scheduling
- These tools save time and effort tracking samples, results, and instrument usage
- Some of these tools have been adapted for use in other BETO-funded work (SABC, ATP^3)

Technical Accomplishments - PSI

Determination of fraction insoluble solids (FIS) in pretreated, enzymatic hydrolysis, and fermentation slurries

- *Identified as the major analytical contributor of process model uncertainty*
- *Newly developed method allows **6 times** as many samples per day at increased precision*
- *Alkaline pretreated samples retain base during washing*
- *New procedures specific for alkaline conditions*
- *DMR post-enzymatic hydrolysis and fermentation samples are not well-characterized by current methods*

- *Developed a near-infrared (NIR) calibration model based on this new method*

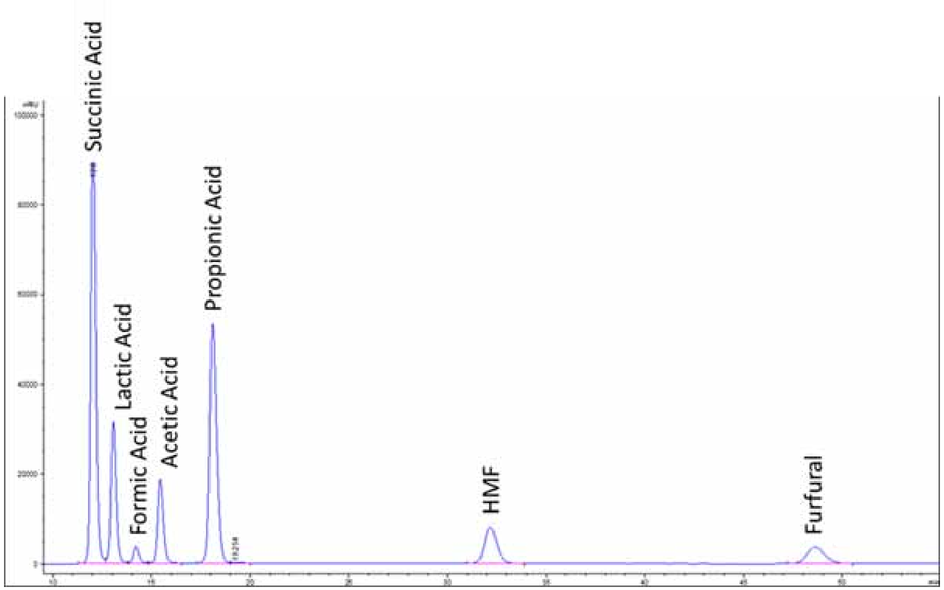
Technical Accomplishments – BUS - C5 to Succinic

Validate and implement methods to quantify products and intermediates

- *Existing protocols were not capable of providing <u>quantitative data for assessment of conversion streams</u>*

- *Identified and developed methods to capture current and possible future products and intermediates*

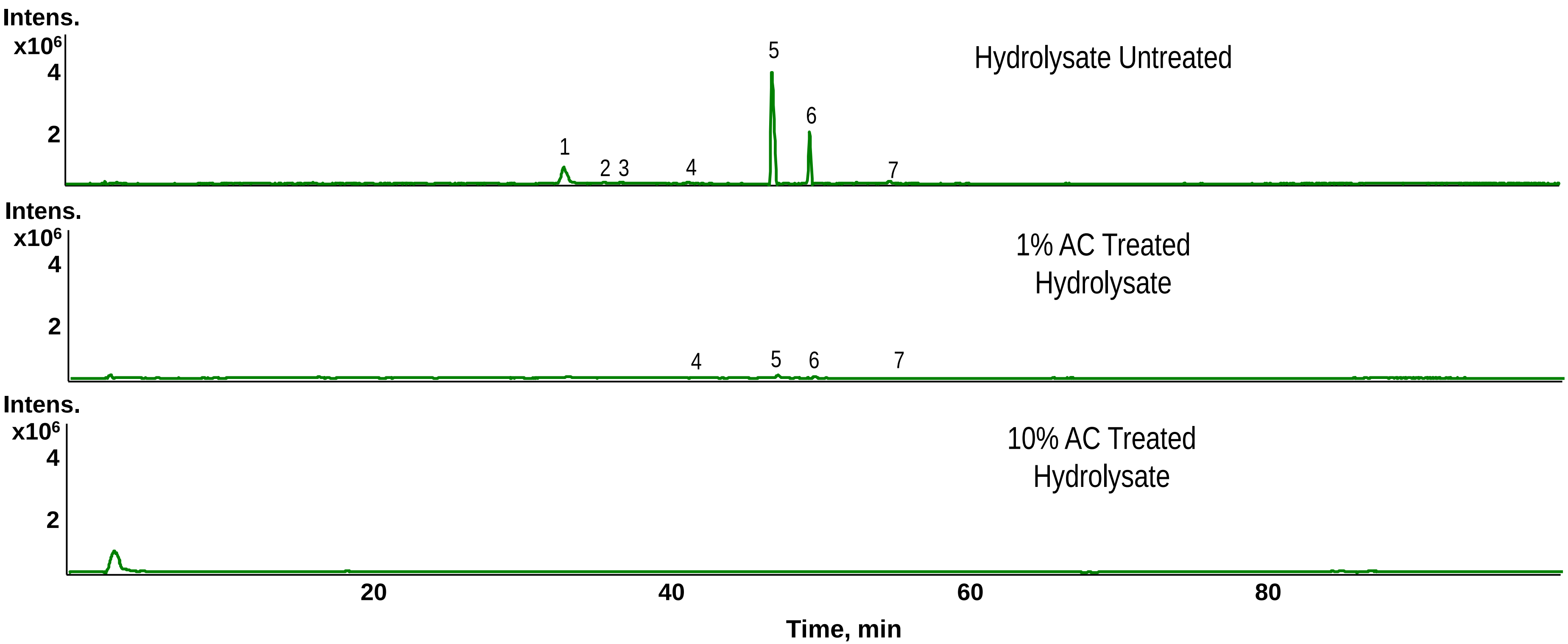
- *Methods and protocols reassessed and redeveloped every time changes occur in processes*



Technical Accomplishments – BUS - C5 to Succinic

Quantify phenolic detoxification of C5 stream

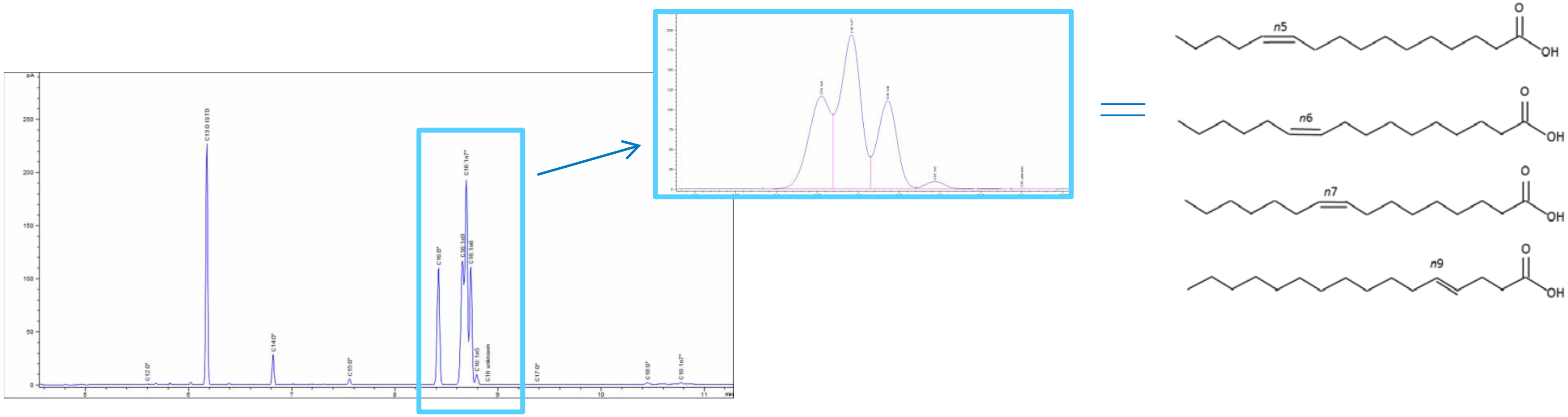
- *Phenolics are toxic to fermentation organisms and removal by activated carbon (AC) was a path forward*
- *Methods were developed identify, resolve and quantify phenolics that may be inhibiting the growth of A. succinogenes*



Technical Accomplishments - BUS - Lipid analysis

Optimized protocols to quantify FAME

- *Previous BETO work reduced GC analysis time from 36 to 23 minutes*
- *Demonstrated FAME method is applicable to current yeast strains*
- *Verified that in-situ method is superior to classic lipid extraction methods*
- *Investigating MACH (modular accelerated column heater) to reduce <u>GC run to 3 minutes</u>*
- *Investigating the use of microwave derivatization*
- *Changing GC carrier gas to reduce cost and increase gas velocity capability*



Technical Accomplishments – BUS - C6 to Lipids

Developed new protocols to quantify C6 loss during conversion

- *Lipid producing organisms also create exopolysaccharides ("slime coat"), which significantly inhibit analysis and represent carbon loss*
- *Necessary sample cleanup introduced contaminant compounds which required further method development*
- *Methods were implemented in real time, allowing for continuous conversion evaluation*
- *Bridge methods were utilized for uninterrupted research*

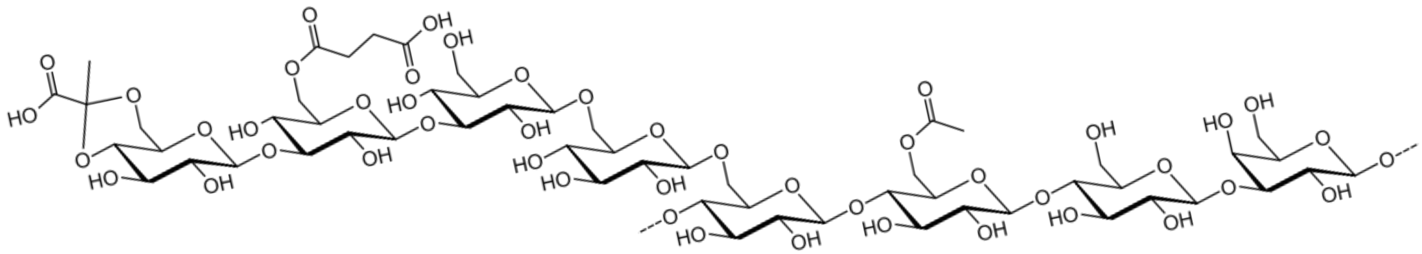
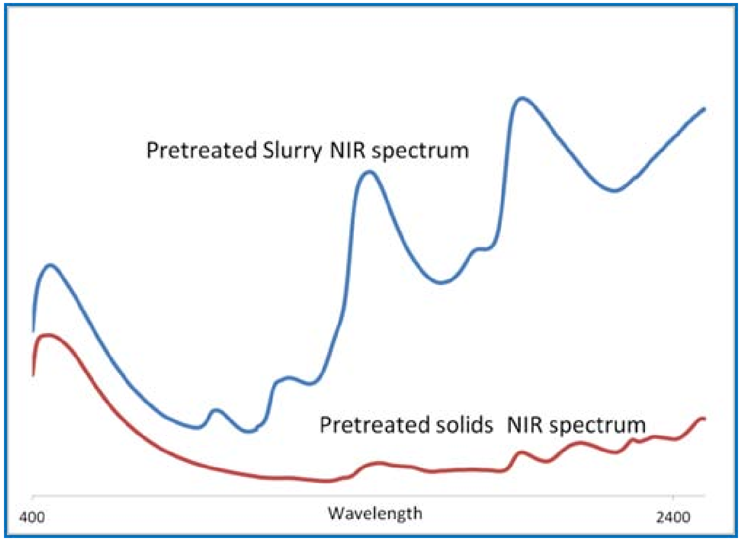


Image courtesy of Wikipedia

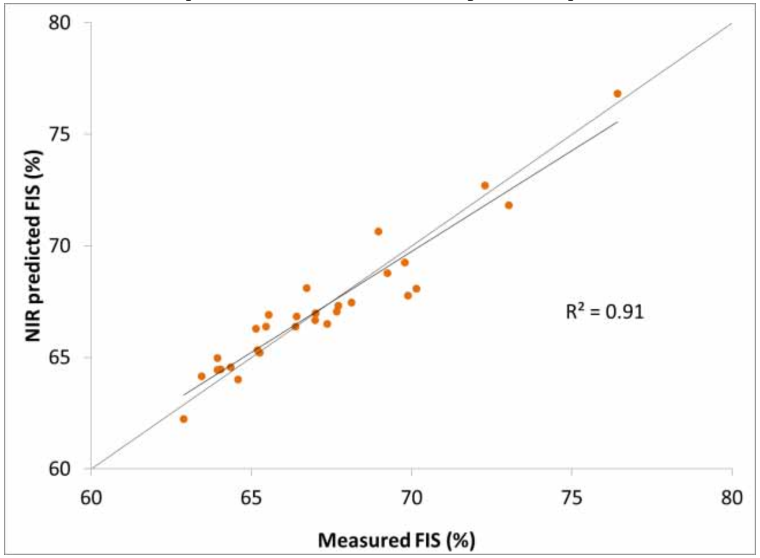
Technical Accomplishments - NIR Models

Near Infrared (NIR) Calibration 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



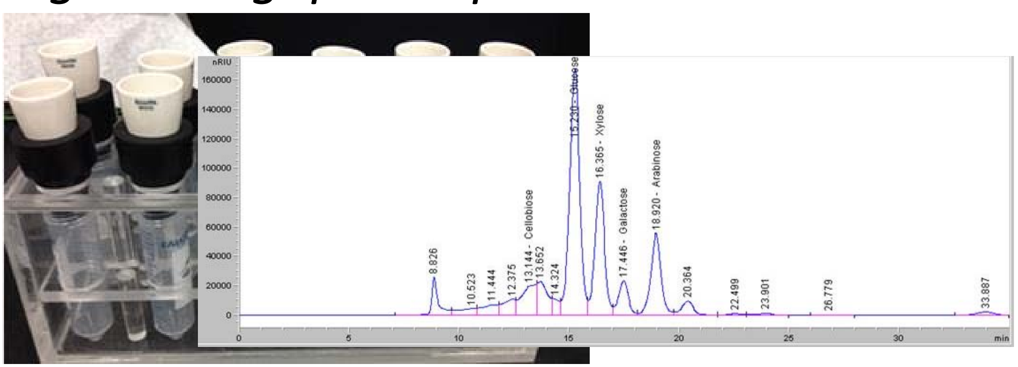
Technical Accomplishments - Increased Throughput

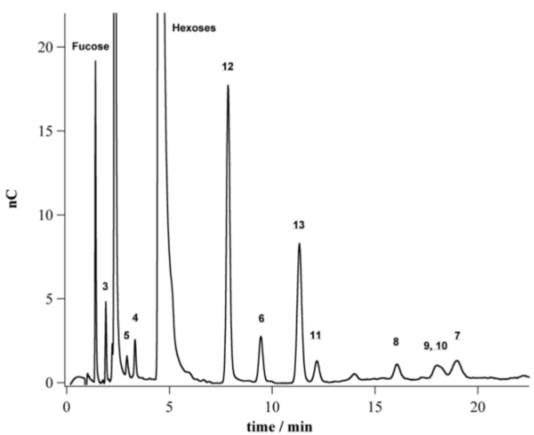
High Throughput Compositional Analysis

- *Traditional methods too slow and expensive*
- *Increases analytical hydrolysis throughput by a factor of 6*
- *New lignin measurements 8 times faster*
- *FY15 milestone will capture more high throughput steps*

Investigation of new chromatographic techniques

- *New products require better chromatographic separation, throughput, and accuracy*
- *Looking forward to new constituents, such as mannose*



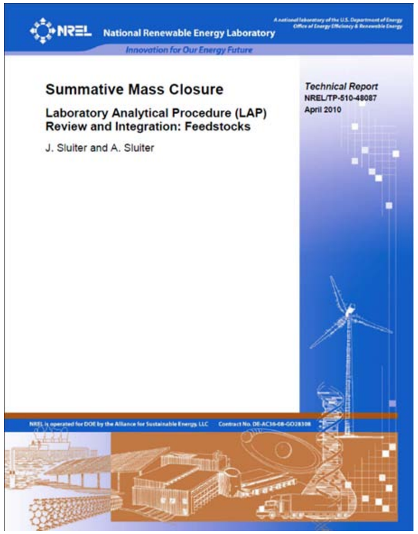


Disaccharides from glucose reversion reactions

Technical Accomplishments - Published Procedures

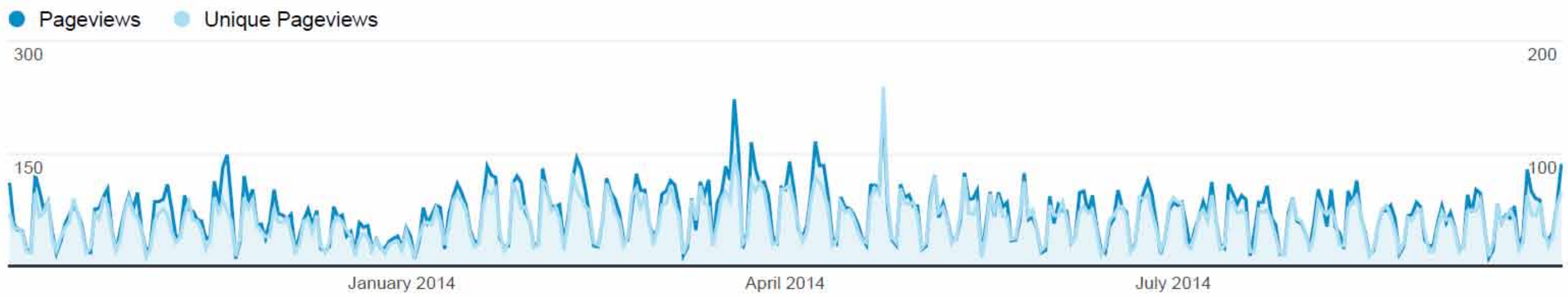
Laboratory Analytical Procedures (LAPs)

- *De facto* standards for biofuels industry worldwide
- Free download for community resource
- Adopted by ASTM
- Newly revamped website
- Community email update with new postings



Website (nrel.gov/biomass/analytical_procedures)

- Over 25,000 page views (FY14)
- Over 6,100 procedure downloads (FY14)



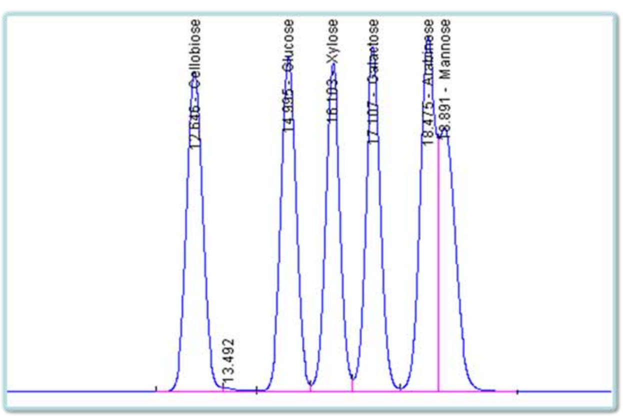
Technical Accomplishments - Instrumentation

Service subcontracts

- *Place and monitor subcontracts for instrumentation that supports most program tasks*
- *Assess instruments for work load, age, and anticipated future use*
- *Make financially prudent decisions on coverage level*
- *Monitor vendors to ensure quality service*
- *Schedule preventative maintenance to minimize lost research time*

Instrument maintenance

- *Analytical chemists have primary responsibility for instrument upkeep*
- *We are responsible for troubleshooting, minor repairs, and routine maintenance*
- *Project members are in the lab, and able to provide training and oversight for shared instrumentation*



Technical Accomplishments - Challenges

Challenges identified for development and optimization for the FY17 /FY22 demonstration targets

Team and analytical project must remain <u>agile and responsive</u>

- Without good data, researchers cannot effectively evaluate changes to processes or unit operations
- Changes to these processes or unit operations will often require new analytical methods or improved detection limits of existing methods

Method Development has to support other projects in real time

- We develop "bridge methods" to provide data during final method development and validation
- We do this work at the same time with the same resources

We are supporting <u>multiple projects simultaneously</u>, working closely with other projects to share knowledge and balance resources

Relevance

- ***Analytical Development and Support is crucial to meet BETO technical goals and to advance the biofuels industry***
- ***Individual BETO Projects are ultimately responsible for their results; our job is to support and enable our colleagues' work***

Quality Data

- *Robust, consistent, and reliable analytical data is absolutely critical for research and development*
- *We support and enable many BETO projects with high quality analytical data*
- *We maintain shared laboratories and equipment for many BETO projects*

Community Support

- *Maintaining the Laboratory Analytical Procedures (LAPs) is critical for the industry to "speak the same language"*
- *NREL-maintained NIR predictive models give industry and academia the inexpensive analysis tools they require*

Method Development

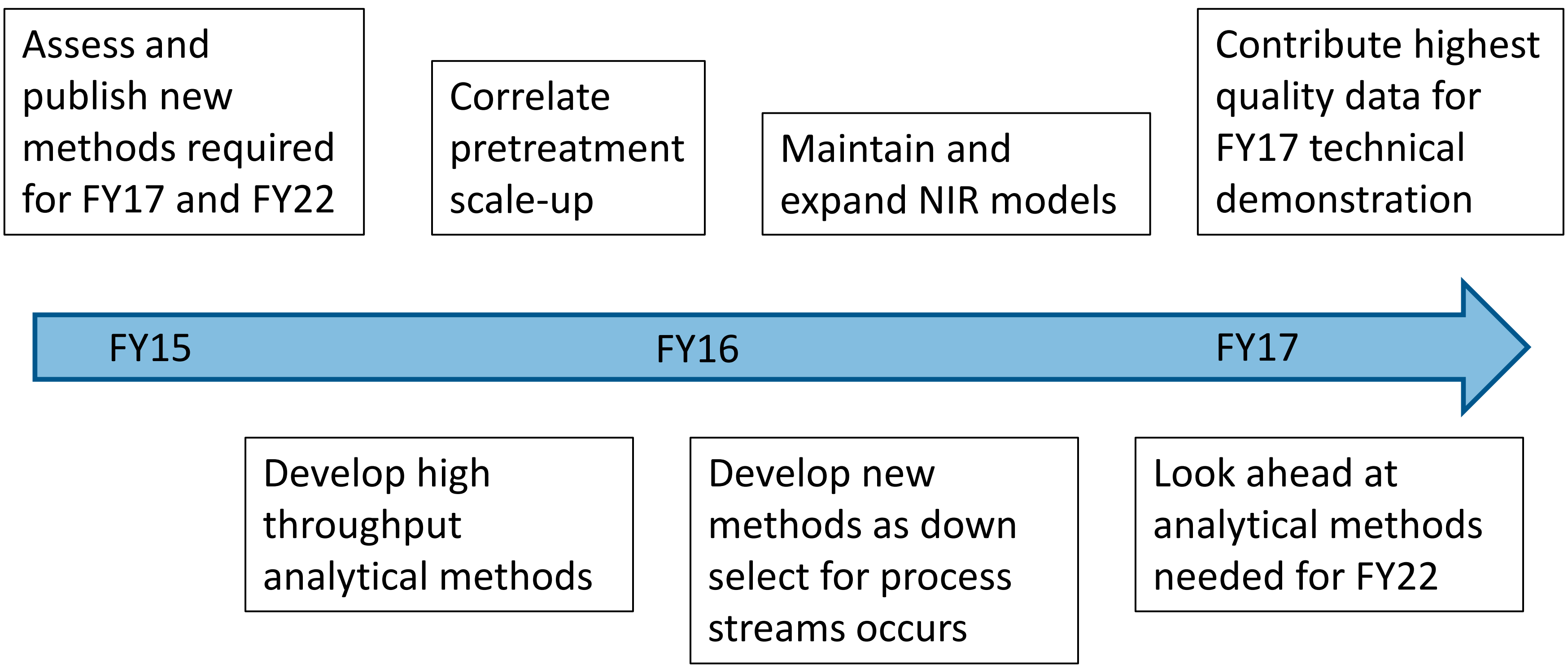
- *We respond to scientists and engineers with new methods to support new processes and products*
- *We respond to external partners with development ideas as well*
- *We make methods publicly available on our website*

Relevance - Technology Transfer

- ***We regularly work with external partners; these relationships are based on our reputation for excellence in cellulosic biofuels in general, and Biomass Compositional Analysis in particular***
  - *NIR licensing and prediction model collaborations with*
    - *Abengoa*
    - *Chromatin*
    - *INL*
    - *Colorado State University*
    - *University of Illinois at UC*
    - *Washington State University*
  - *Compositional Analysis Projects with*
    - *Shell*
    - *Toyota*
    - *DuPont*
    - *Abengoa*

Future Work

- ***Develop analytical methods for <u>all</u> process streams for FY17/22 demos***
- ***Continue high-throughput composition research***
- ***Remain agile to respond to new processes and technical developments***
- ***Continue to maintain world class capabilities at NREL Laboratories***



Summary

Overview

- Provide DOE/BETO tasks with high quality analytical results
- Develop new methods in response to program goals
- Provide ongoing stewardship to analytical laboratories used by multiple researchers at NREL

Approach

- Develop and maintain relationships with collaborators to determine analytical needs
- Work closely with collaborators to develop analytical methods for new processes
- Optimize methods to utilize funding efficiently

Accomplishments

- Coordinated thousands of discrete biomass compositional analyses to BETO-funded work
- Identified (in collaboration with colleagues) key analytical challenges and provided solutions for FY17 and FY22 technical goals.
- Developed high throughput analysis procedures

Relevance

- Quality analytical data essential to evaluate processes and products
- Support the biofuels and bioproducts community with common procedures

Future work

- Develop and publish methods to address future technical goals
- Continue to support colleagues working on BETO-funded projects and tasks
- Expand and maintain NIR models for internal and external use

Acknowledgements

- Amie Sluiter
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- Kelsey Ramirez
- Michelle Reed
- Justin Sluiter
- David Templeton
- Jeff Wolfe

Additional Slides

Responses to Previous Reviewers' Comments

This project was part of the earlier Biochemical Process Integration (BPI) Project; our work was only mentioned tangentially in the 2013 Peer Review…

- Analytical methods are applicable to future work….
- Part of the work (e.g., development of laboratory analytical procedure's, process integration, and pilot-scale verification) is very important to the Office.

Publications, Patents, Presentations, Awards, and Commercialization

1. Wolfrum, Edward J., et al. "A laboratory-scale pretreatment and hydrolysis assay for determination of reactivity in cellulosic biomass feedstocks."*Biotechnol Biofuels* 6 (2013): 162.
2. Sluiter, Amie, and Ed Wolfrum. "Near infrared calibration models for pretreated corn stover slurry solids, isolated and in situ." *Journal of Near Infrared Spectroscopy* 21.4 (2013): 249-257.
3. Payne, Courtney, et al. “Rapid Analysis of Composition and Reactivity in Cellulosic Biomass Feedstocks with Near-Infrared Spectroscopy” submitted to Biotechnology for Biofuels
4. Sluiter, Justin, et al. “Evaluation of Brazilian Sugarcane Bagasse Characterization: Inter-laboratory Comparison” submitted to the Journal of Agriculture and Food Chemistry
5. Emerson, Rachel, et al. "Drought effects on composition and yield for corn stover, mixed grasses, and Miscanthus as bioenergy feedstocks." *Biofuels* 5.3 (2014): 275-291.
6. Kristin J. Vicari, Sai Sandeep Tallam, Tatyana Shatova, Koh Kang Joo, Christopher J. Scarlata, David Humbird, Edward J. Wolfrum, Gregg T. Beckham, “Uncertainty in Techno-Economic Estimates of Cellulosic Ethanol Production due to Experimental Measurement Uncertainty”, Biotechnology for Biofuels 5:23-27 (2012).
7. A. Sluiter, J. Sluiter, E. Wolfrum, "Methods for Biomass Compositional Analysis", in “Catalysis for the Conversion of Biomass and Its Derivatives”, Book Chapter, Max Planck Research Library for the History and Development of Knowledge, Proceedings 2.” Berlin: Edition Open Access (2013), ISBN 978-3-8442-4282-9
8. A. Sluiter, J. Sluiter, E. Wolfrum, M. Reed, R. Ness, C. Scarlata, J. Henry, “Improved Methods for the Determination Drying Conditions and Fraction Insoluble Solids (FIS) in Biomass Pretreatment Slurries”, ms. in preparation
9. A. Sluiter, E. Wolfrum, S. Maletich, “Near Infrared Calibration Models to Predict Fraction Insoluble Solids in Acidic and Neutral Pretreatment Slurry”, ms. in preparation
10. Licensed NIR spectra and associated wet chemistry to Abengoa
11. Licensed NIR spectra and associated wet chemistry to Chromatin

Acronyms

- **FIS- fraction insoluble solids**
- **DMR – deacetylated & mechanically refined**
- **NIR- near infrared**