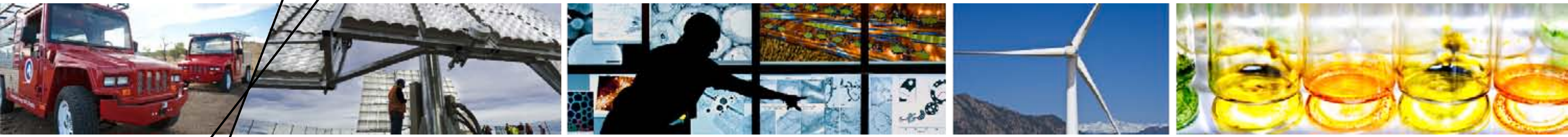
DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

Pilot Scale Integration Project



March 25, 2015
Biochemical Conversion Area

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NREL

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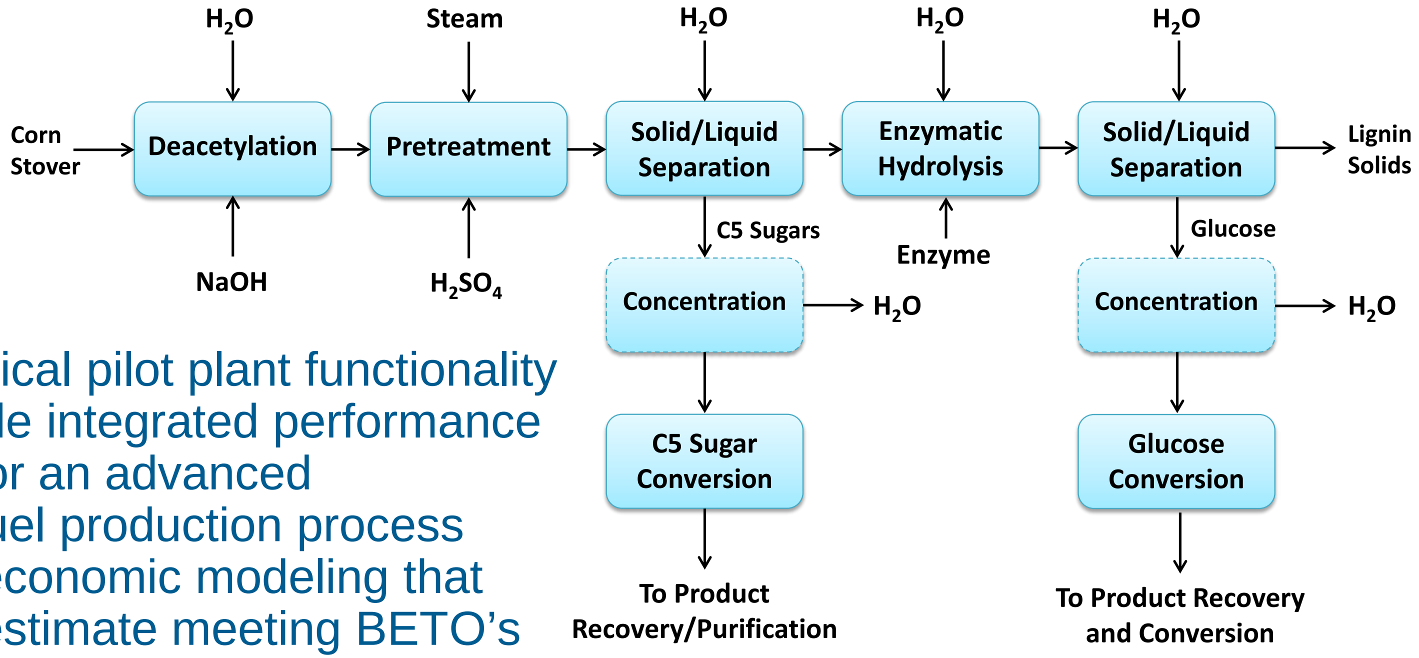
High-Level Project Goal



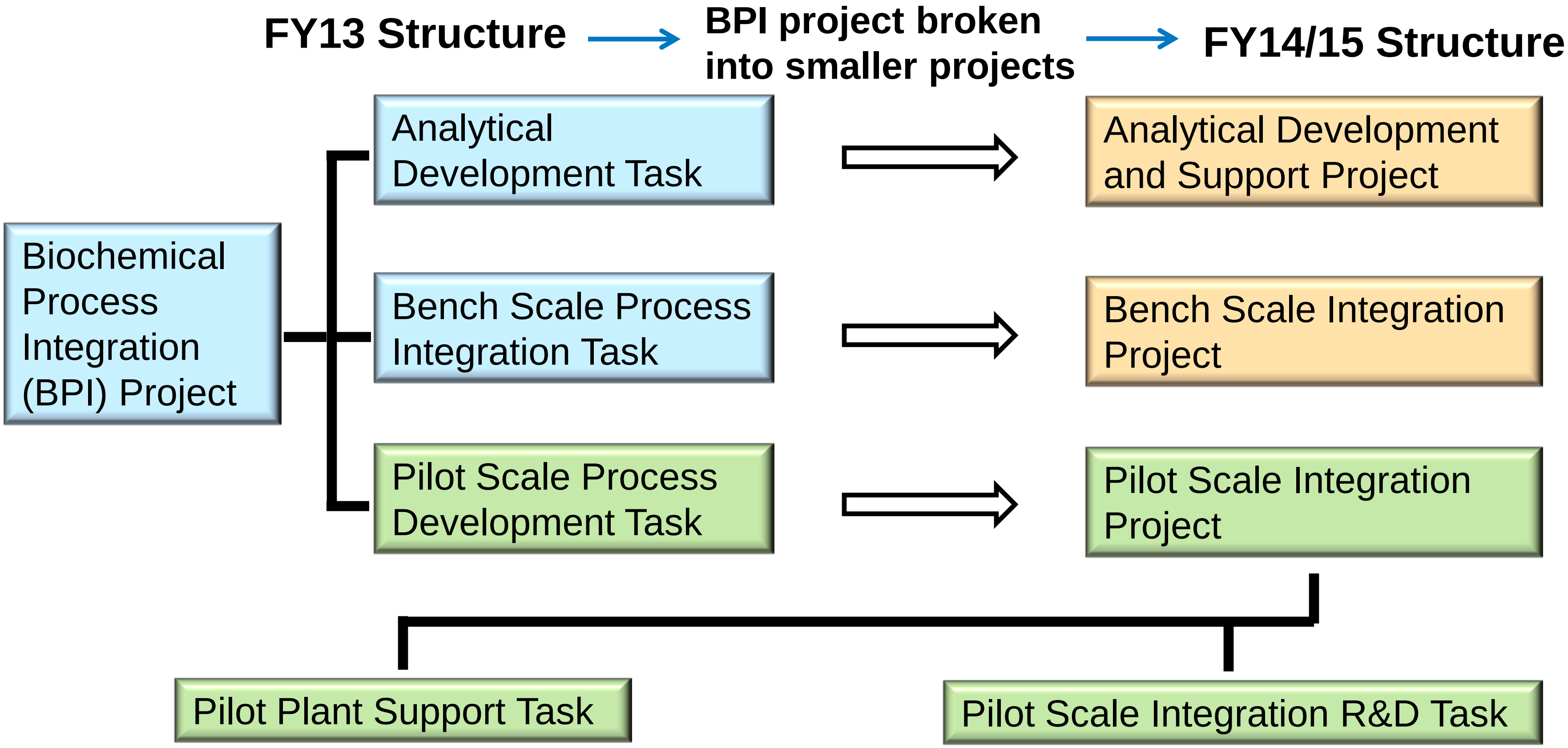
Goals

- Maintain biochemical pilot plant functionality
- Produce pilot-scale integrated performance data as needed for an advanced hydrocarbon biofuel production process
- Provide data for economic modeling that produces a cost estimate meeting BETO's cost targets.

Support BETO's and the Biochemical Conversion Technology Area's mission to deploy cost-effective biofuels production technology.

- Maintains pilot scale facility that is available for BETO and industry use
- Generates process-relevant integrated performance data
- Facilitates knowledge transfer to industry

Project Overview



FY13 Structure → **BPI project broken into smaller projects** → **FY14/15 Structure**

Biochemical Process Integration (BPI) Project

- Analytical Development Task → Analytical Development and Support Project
- Bench Scale Process Integration Task → Bench Scale Integration Project
- Pilot Scale Process Development Task → Pilot Scale Integration Project
  - Pilot Plant Support Task
  - Pilot Scale Integration R&D Task

Maintain pilot plant's functionality and operational readiness to support BETO and industrial stakeholder work, and evolve its capability to support process relevant work on hydrocarbon-based fuel production technologies.

Perform applied research using pilot plant capabilities to explore key issues impacting process performance (pretreatment, enzymatic hydrolysis and fermentation) or equipment/scale-up issues with significant uncertainties.

Quad Chart Overview

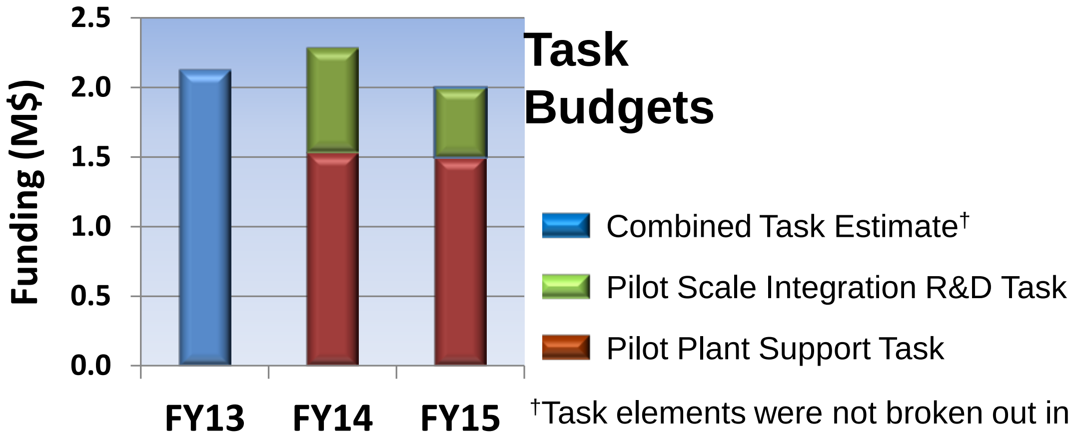
Timeline

- Project start date: FY13
- Project end date: FY17
- Percent complete: 40%

Overall Project Budget (M$)*

	Total FY10–12 Costs	FY13 Costs	FY14 Costs	Total Planned Funding FY15–End
Current Project	$7.16	$2.13	$2.29	$7.29
Old BPI Project	$20.45	$4.85		

*No project cost share



†Task elements were not broken out in FY13

Barriers

- Biochemical conversion process integration
- Cleanup/Separations

Subcontracts

- Benz Technology and Katzen International, aeration studies
- Pilot plant maintenance and upgrades

Other collaborations

- MAST Center, membranes

Biomass Research Facility/Pilot Plant

Project highly focused on the pilot plant.

South High Bay

Baghouse

Laboratories/Offices

Boiler Building

Cooling Tower

Outdoor Product Storage Tank

North High Bay

Distillation Building

Pilot Plant

North High Bay (1994)

- Integrated 1 ton/d process train
- Feed handling through product separation
- Houses utilities systems

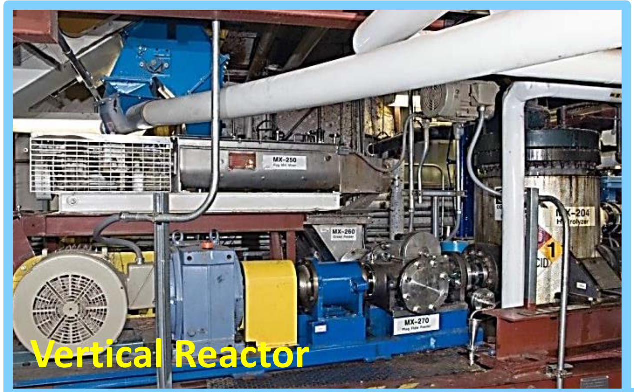
South High Bay (2010)

- Two integrated 0.5 –1.0 ton/d process trains
- Feed handling through high solids enzymatic hydrolysis
- Space for expansion

North High Bay Equipment

Pretreatment

- 1.0 ton/d vertical reactor
- 0.2 ton/d horizontal screw reactor
- 160-L batch reactor
- 1-L and 4-L batch reactors


Vertical Reactor

Fermentation

- 30-L seed vessel
- Two 160-L vessels
- Two 1500-L vessels
- Four 9000-L vessels


1500-L Vessels


9000-L Vessels

Separations

- Distillation column (19-sieve trays)
- Perforated 100-L basket centrifuge
- Forced recirculation evaporator


Evaporator

South High Bay Equipment

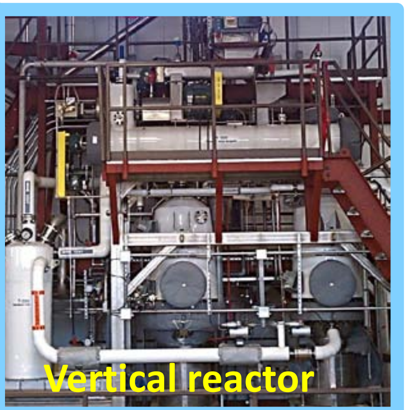
Feed Handling
- Two knife mills
- Continuous conveyance systems
- Multiple hoppers and weigh belts

Pretreatment
- 1.0 ton/d vertical reactor
- 0.5 ton/d horizontal screw reactor

Enzymatic Hydrolysis
- 1900-L paddle reactor
- Four 4000-L paddle reactors

Separations
- Screw presses
- Perforated 450-L basket centrifuge


Baghouse


Vertical reactor


4000-L Paddle Reactor

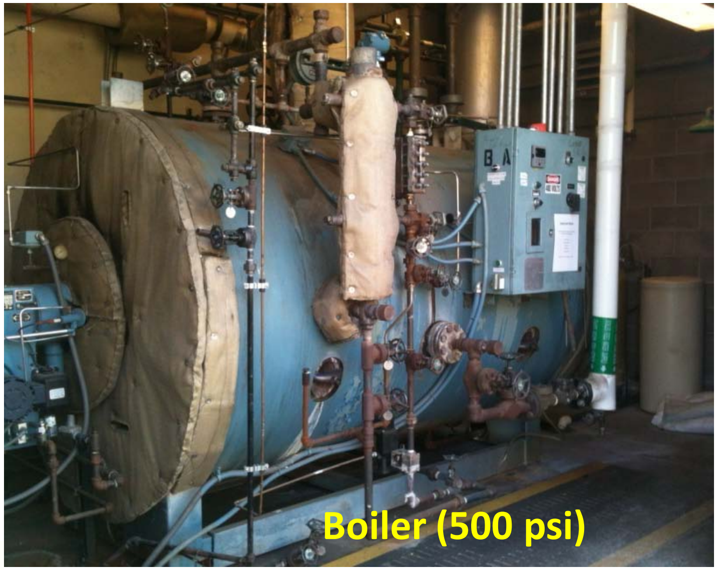

Horizontal Reactor


1900-L Paddle Reactor


Centrifuge

Pilot Plant Utility Systems

- Steam
  - 500 psi, 3400 lb/h boiler
  - 300 psi, 1200 lb/h backup boiler
  - Distributed in high (up to boiler pressure) and low pressure (35 psi) headers
- Cooling water
- Process water
- Chilled water
- Deionized water
- Hot process water
- Plant compressed air



Boiler (500 psi)

Cooling Tower

Boiler (300 psi)

Technical Objectives/Approach

Pilot Plant Support Task

Maintain or improve pilot plant safety

- Routine process hazard analysis (PHA) and management of change
- Monitor and repair/replace key safety devices and systems

Maintain pilot plant operability/functionality to meet programmatic and industrial stakeholder needs

- Routine and emergency maintenance/repair of equipment and utility systems
- Monitor maintenance activities and parts inventory
- Maintain and update documents (P&IDs, SOPs, etc.)

Add new capabilities as needed to meet programmatic needs

- Interface with other NREL programmatic projects to understand process evolution and associated new equipment/processing requirements
- Implement key decision points or milestones to acquire needed capabilities
- Ensure capabilities are available to meet future needs

Technical Objectives/Approach/Challenges

Pilot Scale Integration R&D Task

Explore cost and performance impacts of aeration in large-scale vessels

- Aeration cost/performance information needed for large-scale tanks
- Review literature and solicit information from industry and consultants familiar with large-scale aeration processes in stirred tank reactors and alternative bioreactors to develop better cost/performance information
- Limited information available on tank sizes anticipated for biofuels production and it has proven difficult to engage larger engineering firms/fermentation vendors

Measure on-line residence time distribution (RTD) in pretreatment reactors

- Facilitates kinetic/fluid dynamic model development, pretreatment optimization studies and better reactor design
- Develop and implement on-line measurement technique and determine impact of reactor operating conditions on RTD
- Difficult application; go/no-go decision for further development by end of Mar 2015

More information on rational and approach in additional slides section

Technical Objectives/Approach/Challenges

Pilot Scale Integration R&D Task

Explore alkaline-based pretreatment process

- Develop pilot scale performance data on this process option on corn stover
- Acquire performance information and assess economics
- Determine future activities in this area by end of Sept 2015 (go/no-go)

Determine feasibility of acid preimpregnating biomass prior to pretreatment

- Determine if preimpregnation provides cost/performance advantages
- Test two options in pretreatment reactor at same operating conditions
- Difficult to accurately match reaction acid concentration

Pre-impregnated Corn Stover **Corn Stover**

Technical Objectives/Approach/Challenges

Pilot Scale Integration R&D Task

50-mL Batch

1-L Batch

Compare pretreatment performance across reactor scales (FY15)

- Pretreatment performance in large-scale reactors may be more quickly and efficiently assessed at bench scale
- Correlate performance from 50-mL batch reactors to 1 ton/d continuous reactor
- Not clear if a valid relationship can be found

1 ton/d Continuous

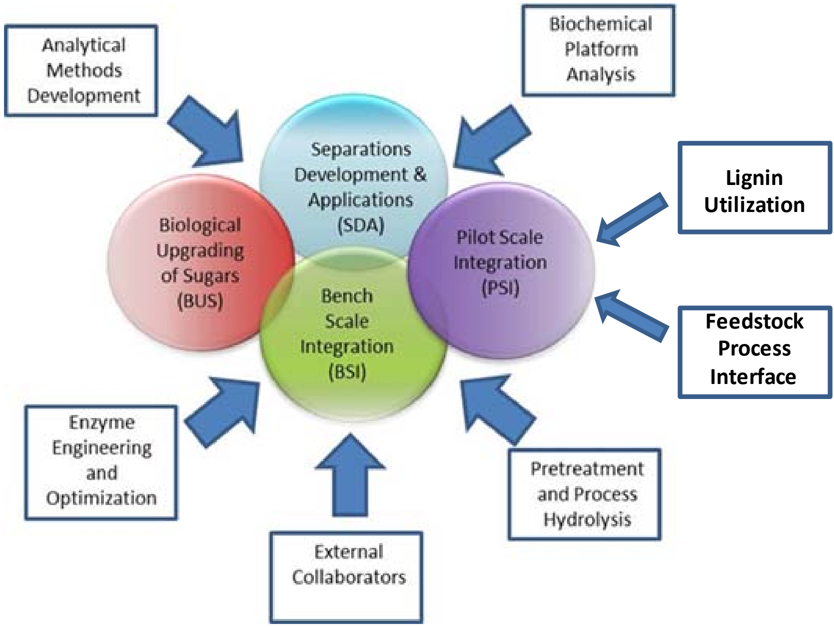
Continue membership in the Membrane Engineering, Science and Technology (MAST) Center a NSF Industry/University Cooperative Research Center

- Opportunity to keep abreast of and participate in new membrane technology with potential applications to biorefineries
- Several projects have been performed directly relevant to our mission

Management Approach

Project Management

- Three-year work plan (reviewed during BETO FY15 AOP Merit review process) with defined milestones and Go/No-Go decisions points aligned with BETO's 2017 objectives
- Plan annually updated to incorporate latest research results or new R&D directions
- Highly interactive with other NREL projects to defined best technical path

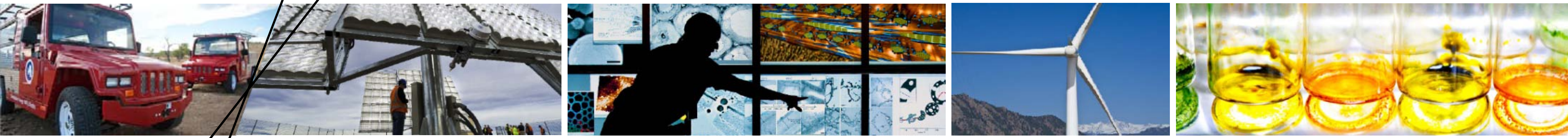


Challenges (Pilot Plant)

- Overcome biomass handling and equipment operation difficulties
- Maintain focus on safe plant operations and updating documentation during heavy pilot plant use

Critical Success Factors

- Successfully implement and demonstrate pilot plant capabilities needed to support BETO's 2017 goals and beyond
- Advance understanding of integrated process performance for biochemical-based advanced hydrocarbon fuel production processes in collaboration with other NREL-led BETO projects to achieve BETO cost targets



Technical Accomplishments

Pilot Plant Support Task

Pilot Plant Support

- Maintenance and repair activities occurred year round
- PHAs performed on four systems in 2014
- New feed systems installed that improved process control and reliability
  - Integrated feed hopper and weigh belt installed down stream of the North High Bay pretreatment reactors
  - Poorly functioning feed screw conveyers replaced with drag tube conveyor
  - Hopper added for feeding wet biomass
- Installed backup boiler
- 450-L basket centrifuge made operational

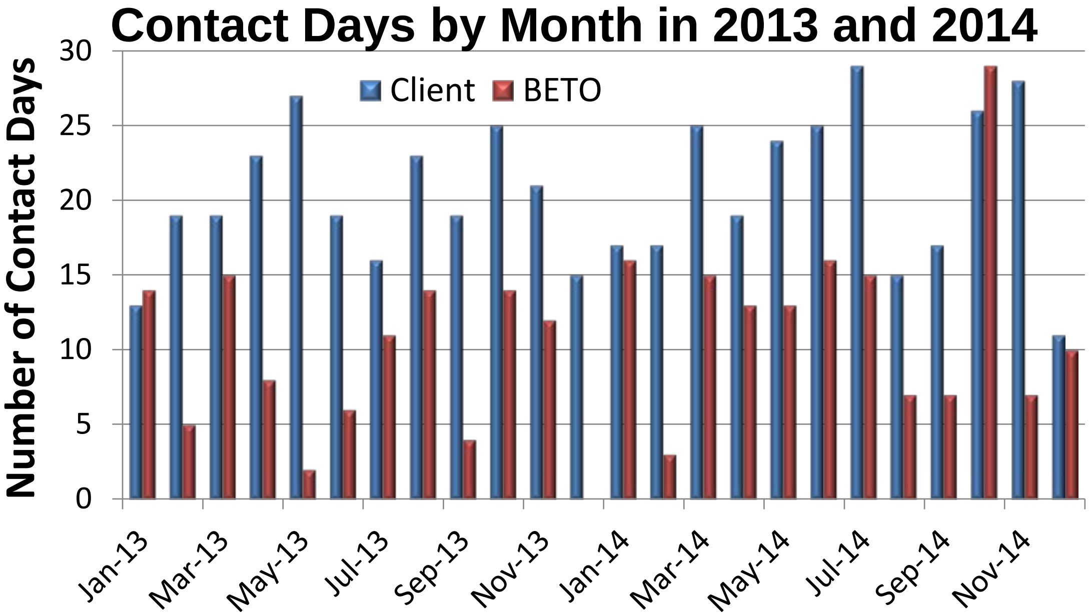
Integrated feed hopper and weigh belt

Wet feed hopper and drag tube conveyor

Pilot Plant Usage

Clients (Projects)

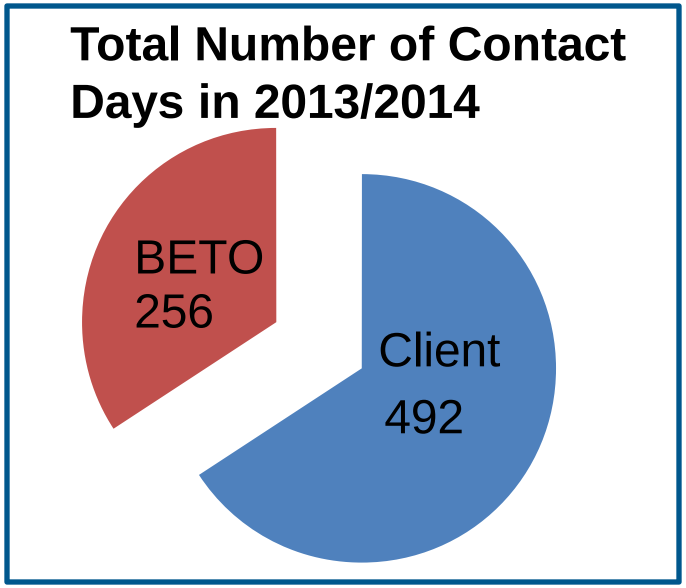
- Work for others arrangements fully funded by the client
- Joint Industry/NREL projects from competitive solicitation awards (partially funded by DOE)



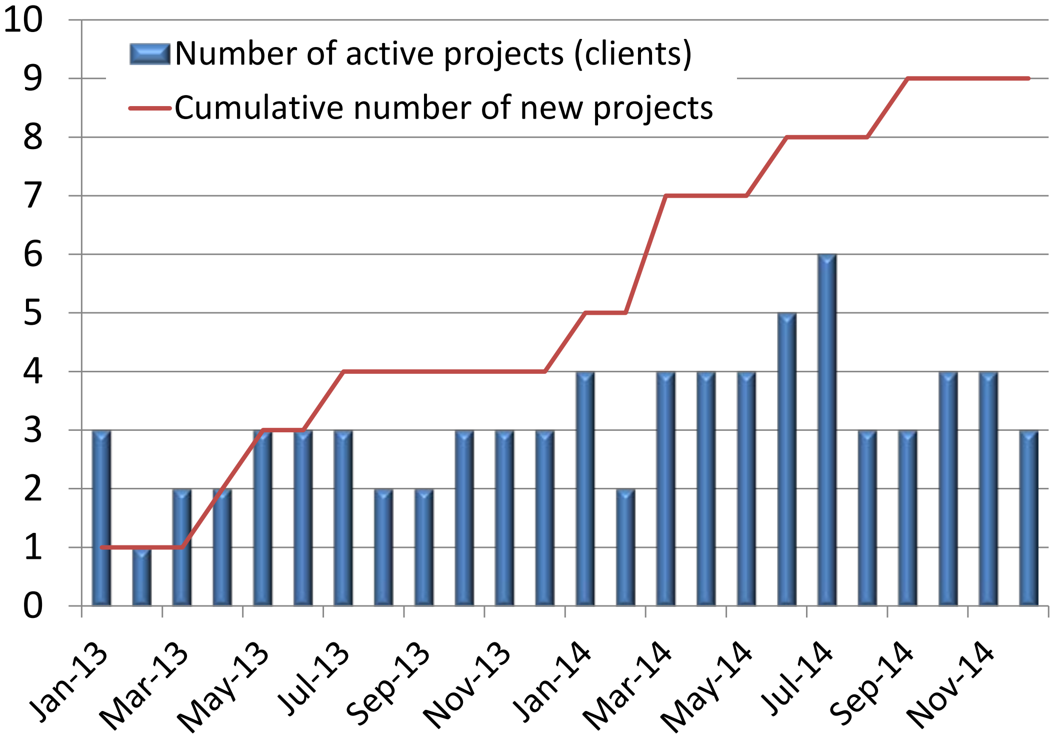
Contact day: A day in which work was performed for one or more clients using any pilot plant unit operation

Unit operations

- Feed handling/preparation
- Large scale pretreatment
- Enzymatic hydrolysis
- Fermentation
- Separations/Product recovery
- Small scale pretreatment

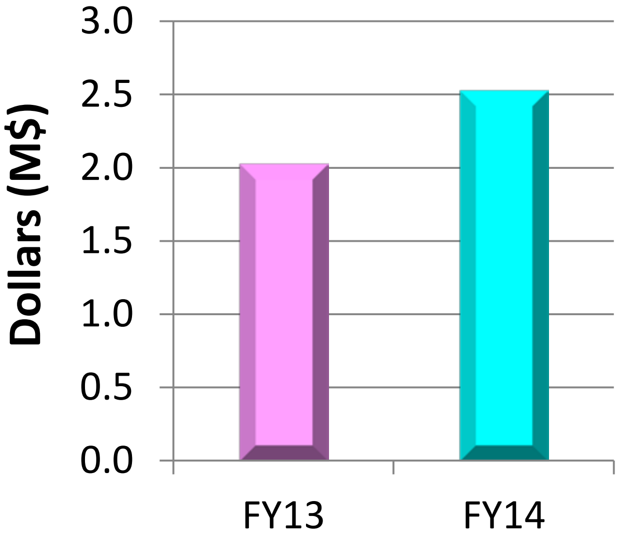


Pilot Plant Usage

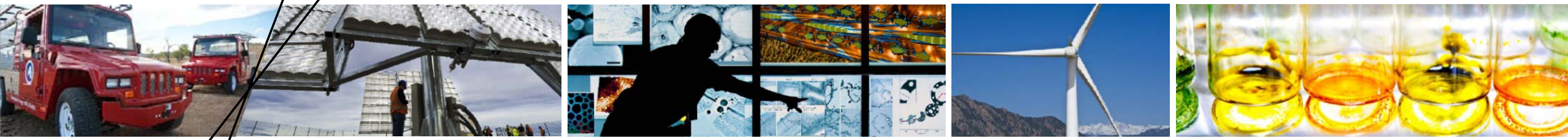


- Average of three client-based projects use the pilot plant every month
- Four to five new projects added every year

Dollar Value of Work Performed for Clients



Pilot plant is highly used by industry to generate materials and process-relevant performance information.



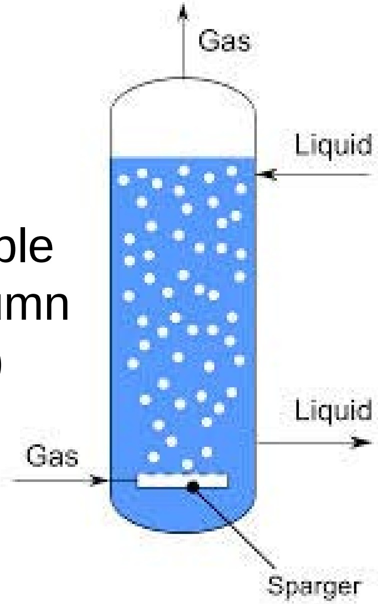
Technical Accomplishments

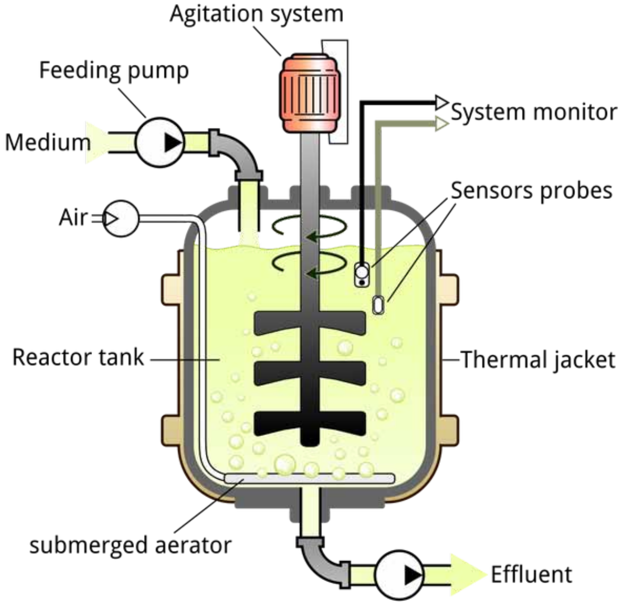
Pilot Scale Integration R&D Task

Aeration Studies

- **Aeration** is essential to achieve biological sugars-to-hydrocarbon production targets, for both enzyme and lipid production.
- **Technical issue:** Molecular oxygen (O_2) is only sparing soluble in aqueous media (~7 ppm in H_2O using air)
- **Consequence:** Biological productivity (e.g., product formation rate) limited by rate of gas-liquid oxygen mass transfer (Oxygen Transfer Rate (OTR, mMol/L-h))
- **To improve confidence in design, need to understand:**
  - What OTR levels are economically achievable at large scales
  - How aeration and power inputs (and associated capital and operating costs) vary with bioreactor scale and type

Bubble Column (BC)





Stirred Tank Reactor (STR)

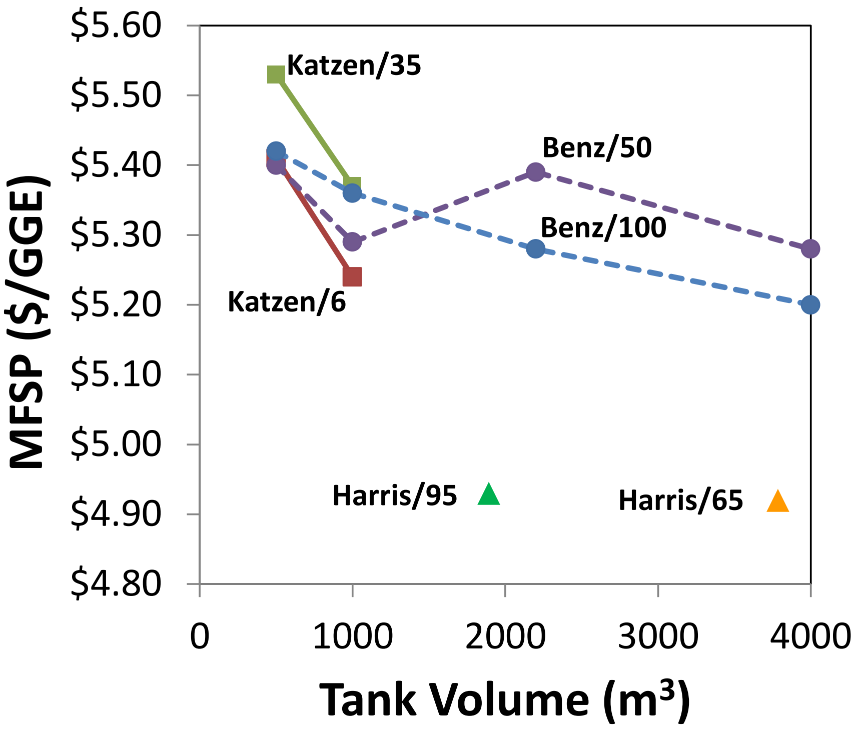
Aeration Results

Literature review

- Results confirm that new information resides in private domain: Large knowledge gaps remain in the public literature regarding "optimal" aerobic reactor designs and operating conditions for large-scale systems to produce biofuels.
  - o Difficult to achieve high (OTR ≥ 100 mMol O_2/L-h) at large scales
  - o Pneumatically agitated bioreactors (bubble columns, airlift, etc.) may enable lower cost

Subcontracted Studies

- Obtained aerated system cost estimates from engineering consultants (Benz Technology and Katzen International), in addition to existing information from engineering firm Harris Group
- Results assessed using NREL TEA model
- Identified inconsistencies in base case aerobic STR design



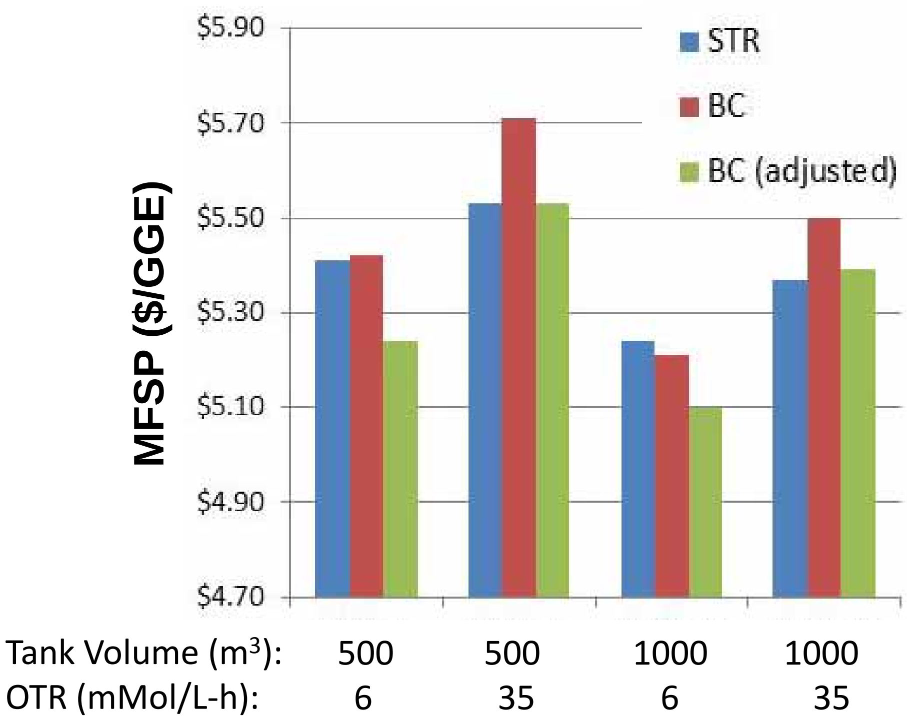
Legend: Data Source/OTR Value (mMol/L-h)

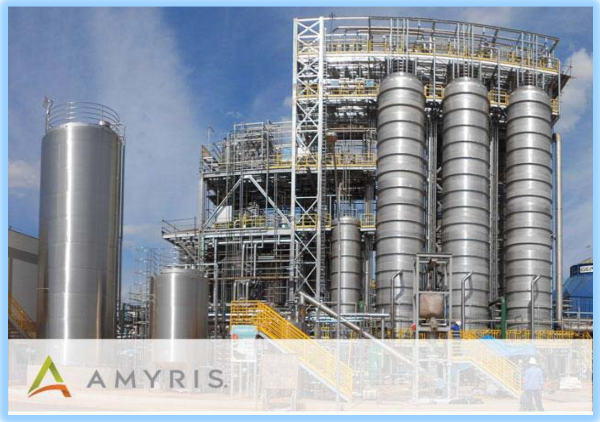
Abbreviations:
Minimum Fuel Selling Price-MFSP
Gasoline Gallon Equivalent-GGE

Aeration Results

Bubble Column

- Katzen provided preliminary design data for a bubble column
- BC values were also adjusted to same volume as STR; Katzen assumed large gas holdup (higher volume)





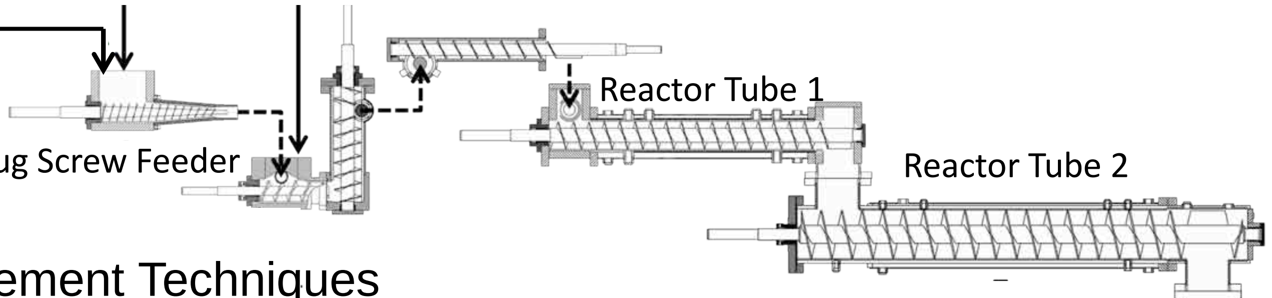
Amyris Bubble Column System

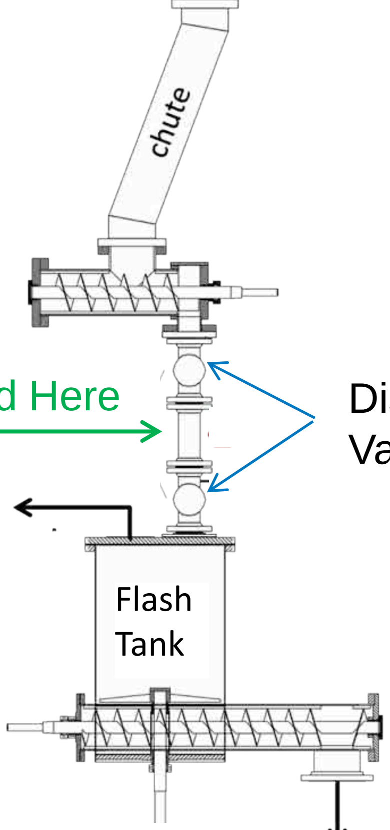
http://www.amyris.com/Multimedia-Resources/134/Amyris-Biofene-Production-Facility-Brazil

Conclusion

- Maximum scale for aerobic systems is unknown and will depend on microbe and cultivation protocol
  - Still unclear if 1 M L tank is viable (this is assumed in the 2013 design case)
  - Important to specify microbe and process to understand specific productivity and OTR requirements
- Airlift, bubble column, deep jet, and other innovative bioreactor designs may have lower costs

RTD Measurement in the Horizontal Reactor



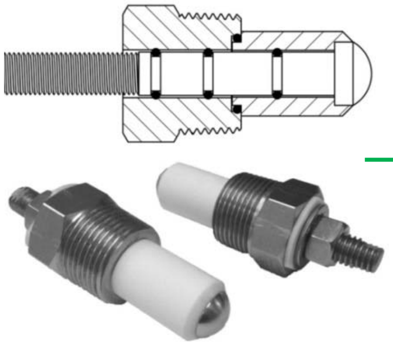


RTD Measurement Techniques

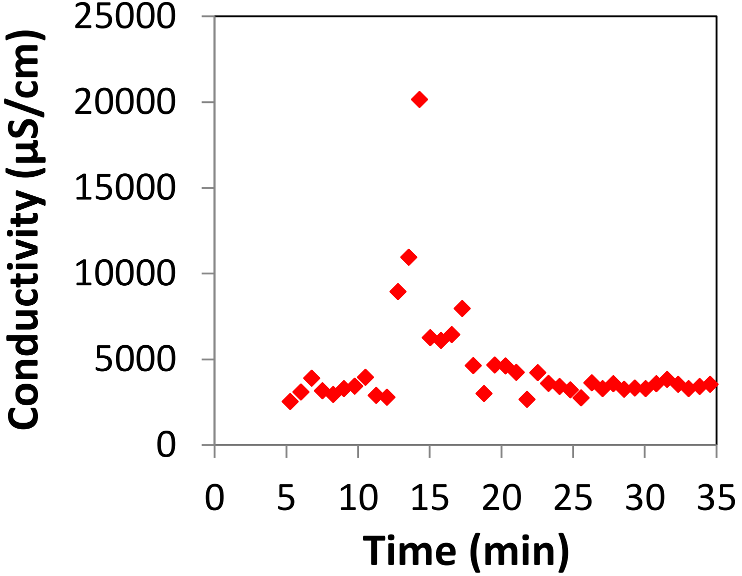
On-line: NaCl detected by conductivity

Off-line: NaCl or TiO_2 detected by ash and NIR spectroscopy measurements

Conductivity Probes



Typical Profile for One Measurement



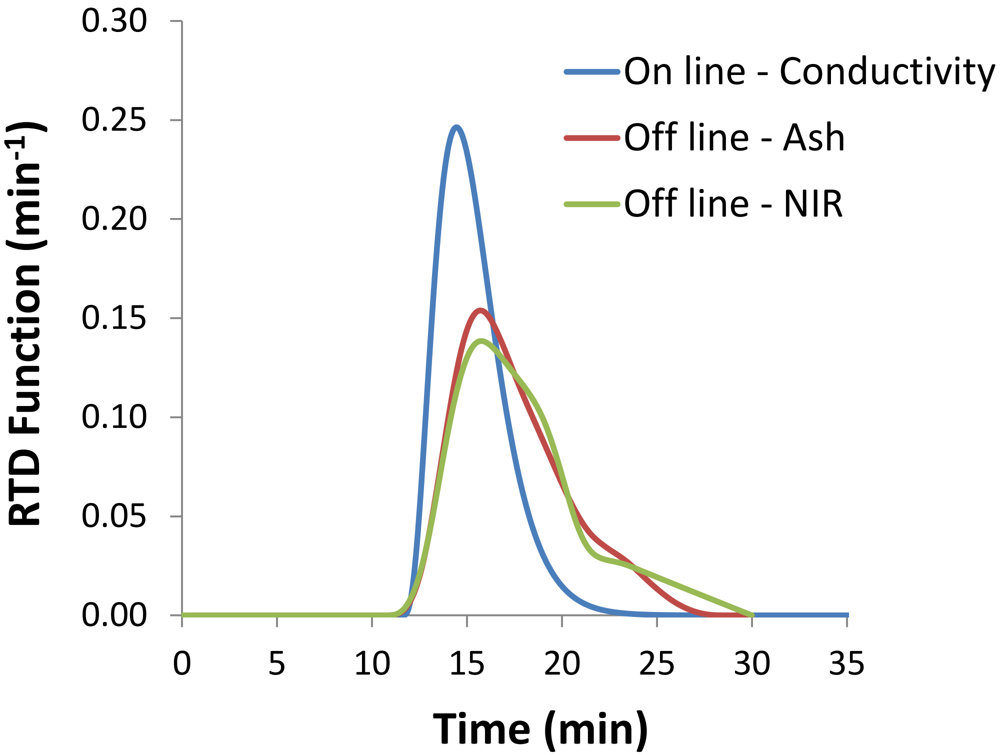
Validating On-Line RTD Measurements

Method

- NaCl and TiO_2 added simultaneously
- Samples collected at the off-line sample point every 15 sec

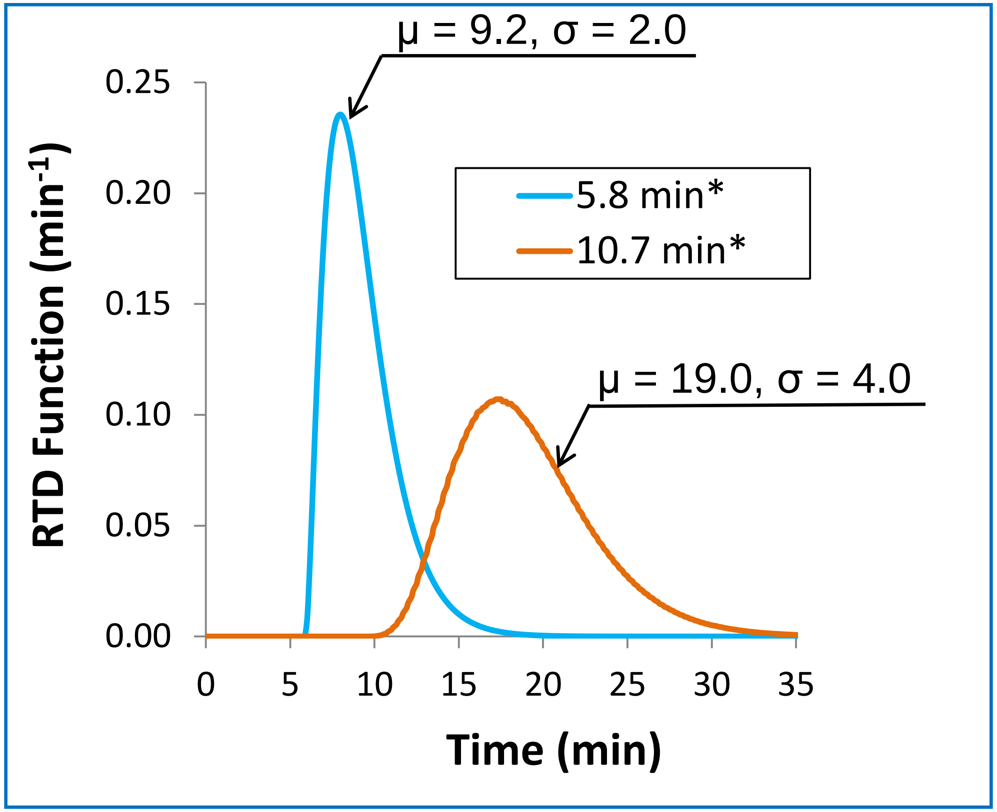
Results

- Ash and TiO_2 measurements gave consistent results
- Slightly longer residence time for off-line samples consistent with spatial separation of the two sampling points
- Results successfully validated the on-line method



Impact of Changing Residence Time on RTD

RTD at two different residence times at the same tube fill fraction (different feed rates)



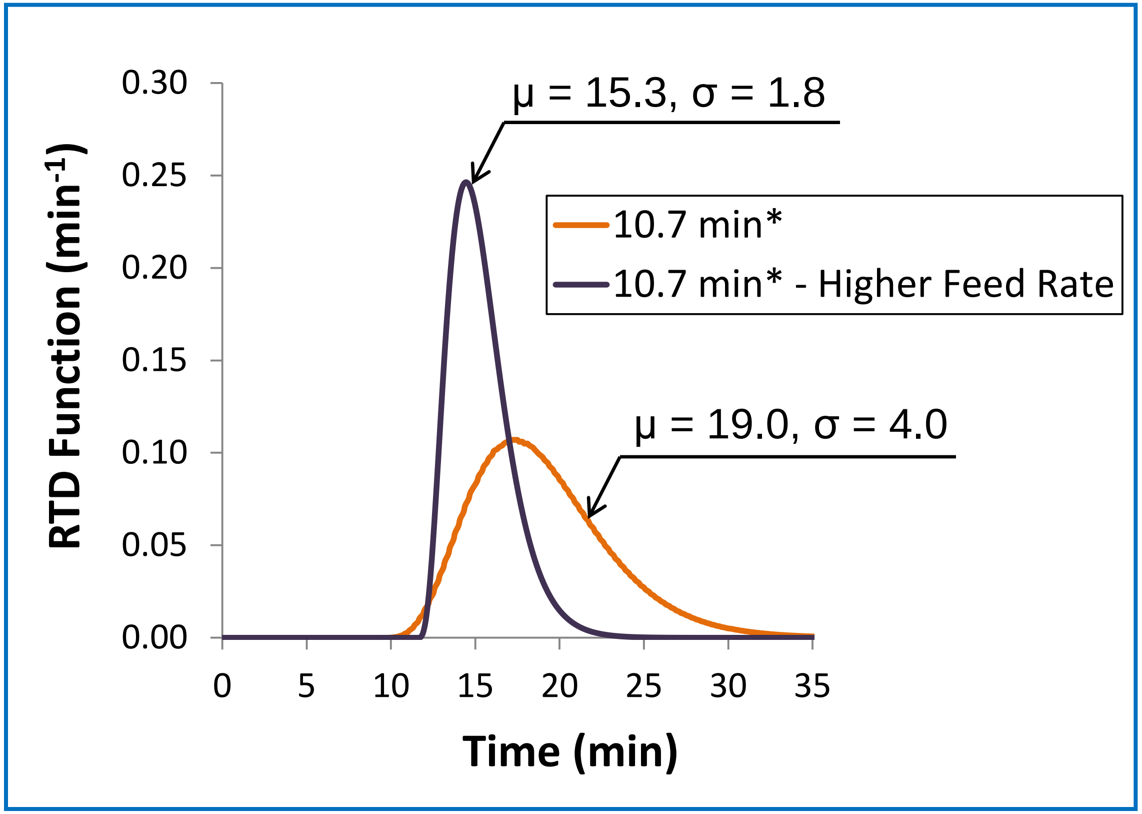
Preliminary Findings

- The mean residence time (μ) is significantly longer than the theoretical minimum
- The tracer is more dispersed (larger standard deviation, σ) at longer residence times
- Both of these findings are expected, but the shift in the mean residence from the theoretical minimum is informative and needed to better understand reaction kinetics

* Theoretical minimum residence time (breakout time) based on screw speeds and no material slippage

Impact of Changing Feed Rate on RTD

RTD at two different feed rates (different tube fill fractions) at the same residence time



Preliminary Findings

- Mean and standard deviation decrease at a higher tube fill fraction
- Less back mixing and material slippage occurs with more material in the reactor tubes

Conclusions

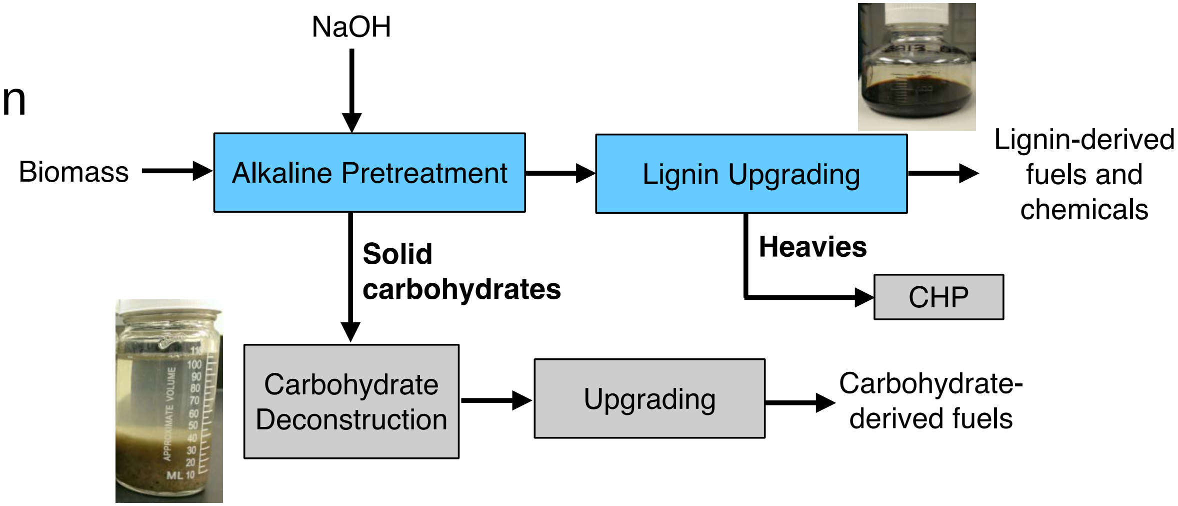
- A rapid on-line RTD measurement technique has been developed
- Provides a better estimate of actual pretreatment residence times and provides better insight into reaction kinetics, fluid dynamics and reactor design

* Theoretical minimum residence time (breakout time) based on screw speeds and no material slippage

Alkaline Pretreatment Background

- Previous work[1] on corn stover in small batch reactors (100 mL tube, 50 mL working volume) suggested effective lignin extraction (soluble lignin yield) was possible

- Process tested in larger batch 160-L reactor (40 L working volume) with the following changes/additions:
    - o Non-extracted corn stover
    - o Lower NaOH loadings (30-80 mg/g) used
    - o Mixing employed
    - o Anthraquinone loading varied instead of fixed
    - o Enzymatic hydrolysis performed on residual solids



NaOH

Biomass

Alkaline Pretreatment

Lignin Upgrading

Lignin-derived fuels and chemicals

Heavies

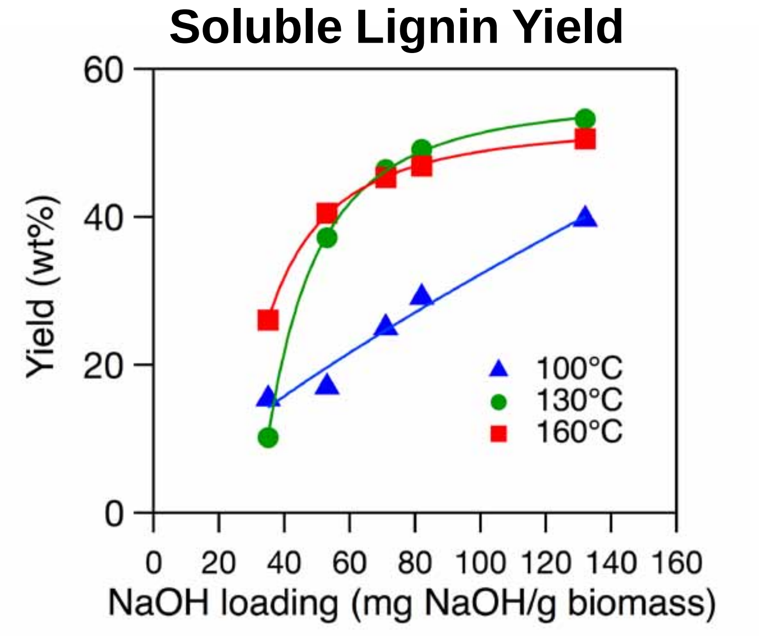
CHP

Solid carbohydrates

Carbohydrate Deconstruction

Upgrading

Carbohydrate-derived fuels



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[1] Karp et al., Sus. Chem. Eng. 2014, 2, 1481-1491.

Pilot Scale Alkaline Pretreatment

- Pretreatment conditions followed a central composite experimental design (3 factors)
  - Temperature: Factorial values 100–140 C (entire range: 86–154 C)
  - NaOH loading: 40–70 mg/g dry stover (30–80 mg/g)
  - Anthraquinone loading: 0.05–0.20% (w/w, based on dry stover (0–0.25%))
  - Constant factors:
    - Total solids fixed at 10% (w/w)
    - Residence time fixed at 30 min

- Enzymatic hydrolysis (EH) testing
  - Cellulose concentration: 2% (w/w)
  - Enzyme loading: 20 mg protein per g cellulose; cellullase:hemicellulase ratio 4:1
  - Temperature: 48 C
  - Time: 5 days

Batch 160-L Reactor

- Paddle mixing
- Indirect steam heating (jacket)
- Vented and cooled with water

Alkaline Pretreatment Results

Solubilized Lignin Yield **Xylan Loss*** **EH Glucose Yield** **EH Xylose Yield**

NaOH Loading (mg/g)

95%

Temperature, ^{o}C

mperature, ^{o}C

Major Findings

- Anthraquinone loading was not a significant factor
- Generally increasing treatment severity increased solubilized lignin yields and enzymatic digestibility, but also increased xylose losses

Conclusions

- Results are promising and further testing in continuous reactors is warranted
- Remains a 2022 process option for utilizing lignin for fuels/chemicals production

*Cellulose loss constant at 1–2%

Relevance – Pilot Plant

Project maintains the pilot plant to support BETO and industry-led/industry-funded projects

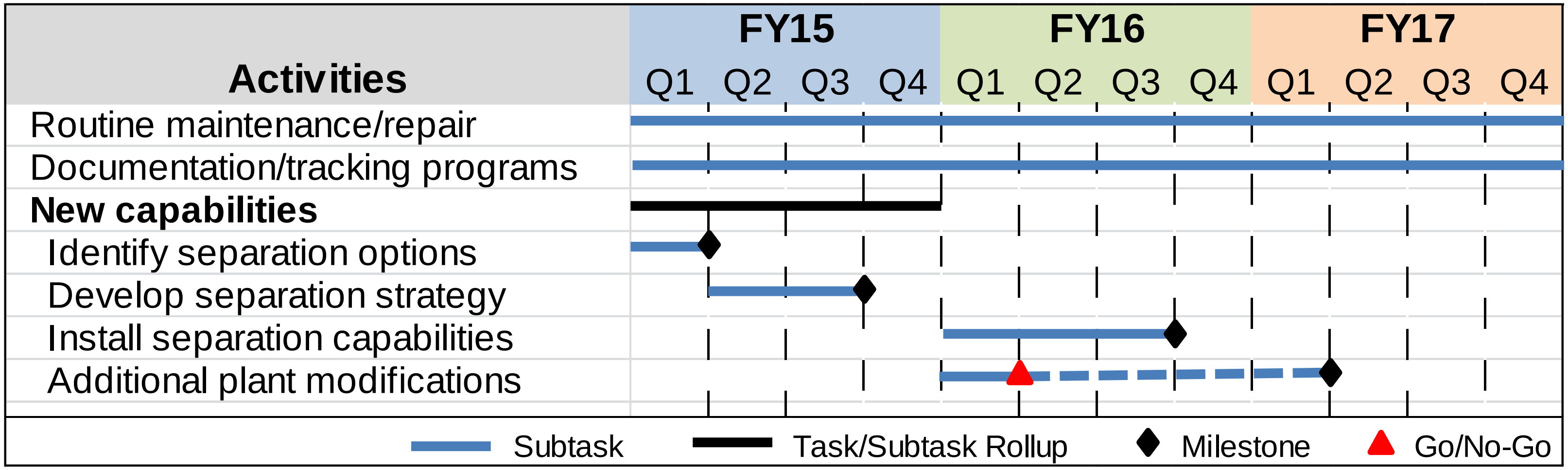
- Process relevant materials (pretreated biomass) and performance information is more effectively produced in pilot scale equipment
- Small quantities of materials are produced and freely supplied to many companies and universities (19 shipments made in FY13/14)
- Facility highly used by industry to test technology and generate process materials, thus reducing commercialization risk
- Results from both BETO-funded R&D projects and industry-led projects directly support MYPP goals to develop cost-effective advanced biofuels

Relevance – R&D Activities

Project performs applied R&D addressing process integration issues with significant uncertainties or with large or unknown cost impacts

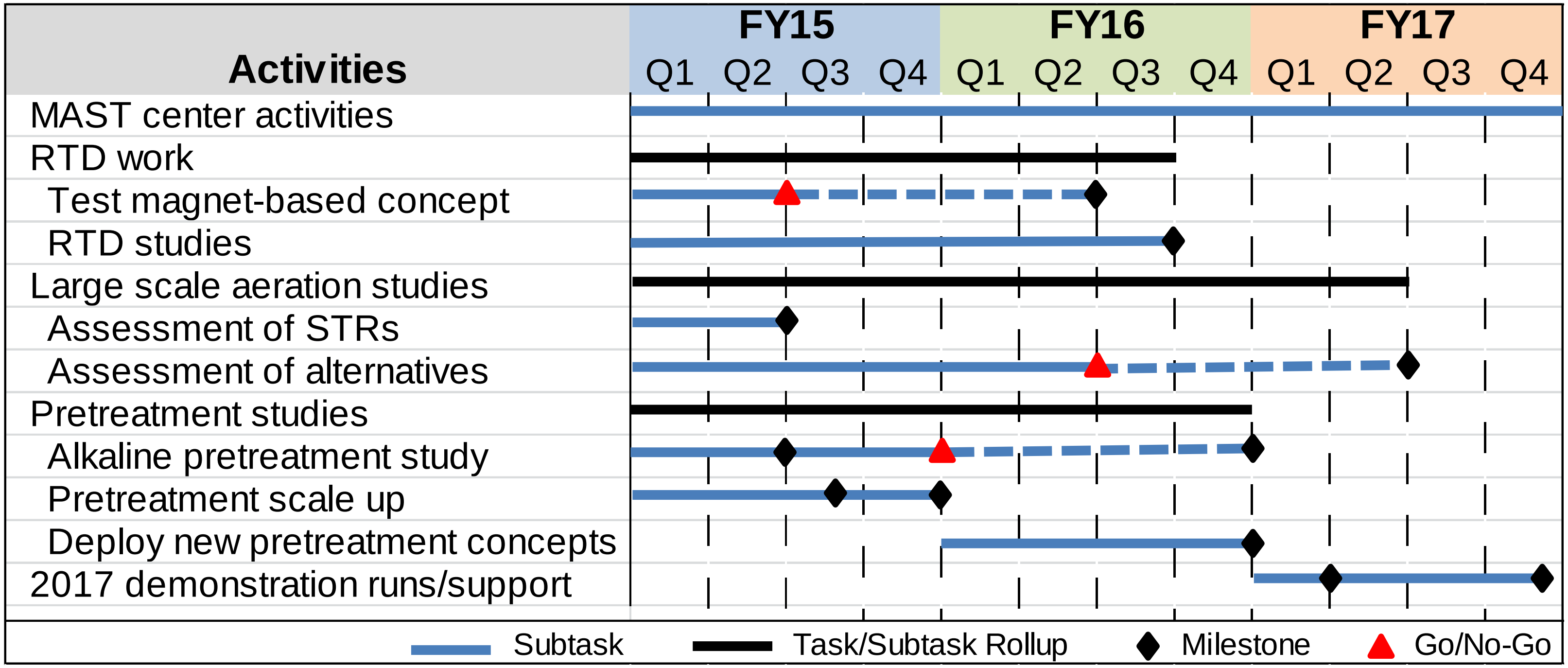
- Current R&D activities directly support MYPP goals by resolving process uncertainties (aeration of STRs), reducing cost (alternative aeration strategies), improving process performance (RTD studies), or investigating 2022 process options (alkaline pretreatment)
- Supports near-term 2017 cost goals by providing integrated performance data and materials (pretreated slurries and enzymatic hydrolysates) to either NREL- or industry-led projects demonstrating technology for production of advanced biofuels

Future Work-Pilot Plant Support Task



- Ongoing pilot plant maintenance/support activities
- Major effort to identify and install separations and sugar concentrating equipment supporting 2017 processing requirements
- Additional pilot plant requirements for 2017 demonstration work to be identified by the end of 2015 with a decision point (Go/No-Go) to categorize additional needs and path forward

Future Work-Pilot Scale Integration R&D Task



- MAST: Center membership ongoing
- RTD studies: Decide (Go/No-Go) if a new magnetic-based technique is better and continue to assess process impacts on RTD using one or both methods
- Aeration studies: Finish assessment of stirred tank reactors (STRs), analyze alternative designs and decide (mid-FY16, Go/No-Go) if further work is needed
- Pretreatment studies: Perform continuous, high-solids alkaline pretreatment and determine path forward (Go/No-Go); complete scale up studies and determine future pretreatment needs to support 2017 goals
- 2017 demonstration runs/support: Provide support for 2017 goals as needed

Summary

- R&D efforts address near-term issues/questions that have impacts on process cost and performance
- Major objective is to maintain a functional pilot plant supporting BETO and industrial projects needing this capability
    - Pilot plant has been heavily and effectively used by industry to further their process development goals
    - The largest fraction of the budget is used to maintain pilot plant functionality
- Ability of the pilot plant to produce process relevant materials and to perform integrated operation will contribute to achieving BETO's 2017 goals and beyond

Team Members

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Other Contributors, Partners

- MAST Center

Biocatalyst Developers

- Novozymes

Questions?

Additional Slides

Previous Review Comments

- Comment: Project relevance is not compelling – most of this kind of work is done by industry. Need to understand process fundamentals – not just optimize process by trial and error.

- Response: Progress over the last five years has built upon accumulated research results from many sources both internal and external to NREL; our own experimental work employs experimental design techniques and statistical analysis. Unfortunately, it is difficult with the time given to adequately convey the magnitude of experimental work that went into this effort. Because of the various elements of this project, the funding is spread across multiple efforts including analytical method development and support, development of an arabinose-utilizing *Zymomonas*, pilot plant maintenance and support, in addition to bench and pilot-plant integrated process development. Also, funding levels were increased in the last few years to support continuous pilot plant runs, which require significantly more resources and time to execute than bench scale work.

Previous Review Comments

- Comment: The stated objective of integrated process performance and translating performance from bench to pilot was not adequately addressed. Data were provided for only 6 pilot scale runs during the last year for this project, two of which had severe contamination issues. Rather than performing a pilot scale dose response curve for enzyme loading, it would have been more beneficial to select the enzyme loading at the bench scale and then perform replicate pilot scale runs using a single, well-defined process.
- Response: Pilot plant runs are expensive and time consuming, so pilot-scale work was only planned for the last year of the project after sufficient performance information was produced at bench scale. The presentation did not convey the large amount of experimental performance testing and optimization that occurred at the bench scale, prior to pilot plant runs, to identify the best operating conditions and strategies. In FY12, our bench scale work identified the best conditions (e.g, pretreatment operating conditions, enzymatic hydrolysis solids loadings, etc.) that minimized cost, except for enzyme loading. While enzyme dosage at the bench scale was still too high to be economic, there was accumulating evidence that cellulose conversion yield would be better in the new high-solids enzymatic reactors being used pilot plant because these reactor systems promote better mixing, but pilot scale work was the only way to test this hypothesis. The first run was performed at a lower enzyme loading (32 mg protein/g cellulose) than employed in the bench work (40 mg/g). We were hoping to meet the cost target in early runs so that additional replication could be performed, but cost was too high and so enzyme loading was further reduced to better understand enzyme loading and yield tradeoffs and associated cost impacts, which could only be adequately understood from pilot scale testing. But it also takes about two months to execute and fully analyze data from a single pilot plant. We needed to execute runs quickly and could not wait for complete results to perform the next run. We choose to stepped down enzyme loading in two increments (to 26- and 19-mg/g) and achieved overall conversion at the 19 mg/g enzyme loading that met the cost target and this condition was replicated.

  We believe dealing with contamination was a valuable learning experience, which we hope will be helpful to the industry. The problem which occurred during the early runs led us to develop a more robust inoculum production protocol and operating procedures that overcame the problem of non-sterile hydrolysates. It took pilot scale operations for this issue to become apparent and better understood.

Previous Review Comments

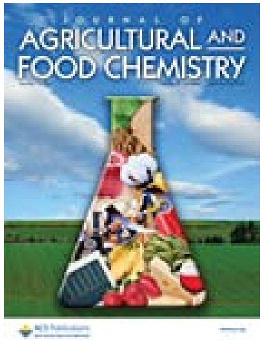
- Comment: This project will transition to hydrocarbon production; better understand aeration and cost; TEA; are there basic TEA models for HC production from sugars and the cost of sugar required to hit the ~$3/gal and technical feasibility.

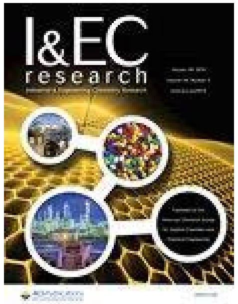
- Response: As with all of the NREL projects, we are transitioning from ethanol to a hydrocarbon, which has become a BETO priority and has created more uncertainty than normal in developing future work plans. This project, in particular, is highly guided by technoeconomic analysis and except for obvious efforts to further improve sugar production from biomass, we are waiting on development of final technoeconomic models and new multi-year technical target to better define our future work plans. However, we are pursuing work in the future to better understand aeration cost and performance for stirred tank vessels and alternative systems.

Publications

- Borole, A.P., Hamilton, C.Y., Schell, D.J. 2013. “Conversion of residual organics in corn stover-derived biorefinery stream to bioenergy via a microbial fuel cell.” Env. Sci. Technol. 47(1), 642-648.
- Katahira, R., Sluiter, J., Schell, D., Davis, M. 2013. "Degradation of carbohydrates during dilute sulfuric acid pretreatment can interfere with lignin measurements in solid residues." J. of Agricul. Food Chem. 61(13), 3286-3292.
- Datta, S., Lin, Y., Schell, D., Millard, C., Ahmad, S., Henry, M., Gillenwater, P., Fracaro, A., Moradia, A., Gwarnicki, Z., Snyder, S. 2013. “Removal of Acidic Impurities from Corn Stover Hydrolysate Liquor by Resin Wafer based Electrodeionization (RW-EDI).” Ind. Eng. Chem. Res. http://pubs.acs.org/doi/abs/10.1021/ie4017754
- Shekiro III, J., Kuhn, E., Nagle, N., Tucker, T., Elander, R., Schell, D. 2014 “Characterization of pilot-scale dilute acid pretreatment performance using deacetylated corn stover.” Biotechnol. Biofuels, 7:23.
- Sievers, D., Schell, D.J. 2014 “Performance and techno-economic assessment of several solid–liquid separation technologies for processing dilute-acid pretreated corn stover” Bioresourc. Technol. 167, 291-296.

Environmental Science & Technology


JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

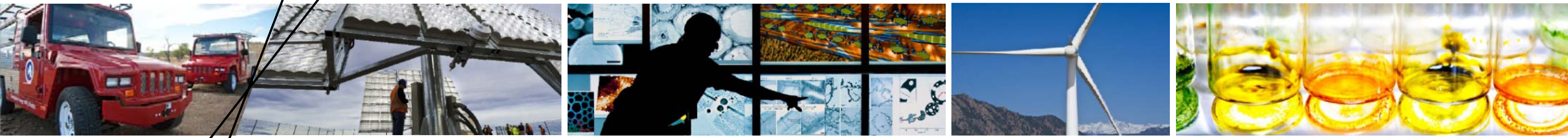

I&EC research

Biotechnology for Biofuels


BIORESOURCE TECHNOLOGY

Presentations

- Sievers, D., Kuhn, E., Tucker, M., Stickel, J., Wolfrum, E. “Residence Time Distribution Measurement and Analysis of Pilot-Scale Pretreatment Reactors for Biofuels Production” AIChE 2013 Annual Meeting, Nov 3-8, 2013, San Francisco, CA.
- Borole, A, Pannell, C., Hamilton, C. ,Schell, D., “Wastewater treatment in cellulosic biorefineries via bioelectrochemical systems” 35th Symposium on Biotechnology for Fuels and Chemicals, April 29th, 2013, Portland, OR.
- Schell, D., “Integrated cellulosic ethanol production—Historical progress and recent pilot scale demonstrations,” 35th Symposium on Biotechnology for Fuels and Chemicals, April 29th, 2013, Portland, OR
- Schell, D. “NREL and Biomass Research Overview.” MAST Center IAB Fall Meeting. September 23, 2013. Boulder, CO.



Additional Technical Details

Project Specific Objectives/Approach

Pilot Scale Integration R&D Task (FY13/14)

Explore impact of aeration in large-scale vessels

- Rational: Understand aeration requirements (cost and performance) and the biological basis for lipid production to achieve high productivity hydrocarbon production in vessels significantly larger than currently being used in commercial aerated processes (collaboration with Biochemical Platform Analysis Project)
- Approach:
  - Review literature and solicit information from industry, consultants, and engineering companies engaged in large-scale aerated processes to improve understanding of oxygen transfer rates (OTR) as a function of vessel size for conventional stirred tanks and alternatives (e.g., bubble column)
  - Analyze results and update economic models as this information becomes available

Oxygen Transfer – Key Equations

Oxygen Transfer Rate: $\mathbf{OTR = k_L a\,(C^* - C_L)}$

where:

OTR = oxygen transfer rate (mMol O_2/L-h)
k_L = liquid boundary layer mass transfer coefficient (m/h)
a = specific gas-liquid interfacial area (m^2/m^3)
C^* = concentration of oxygen at liquid interface (at saturation) (mMol O_2L)
C_L = concentration of oxygen in bulk liquid (mMol O_2/L)

Metabolic Oxygen Demand: $\mathbf{OUR = \mu X / Y_{X/O2}}$
(Oxygen Uptake Rate or OUR)
where:

OUR = oxygen update rate (mMol O_2/L-h)
μ = exponential cell growth rate (at cultivation conditions) (1/h)
X = cell mass concentration (g dry cell mass/L)
$Y_{x/O2}$ = microbial cell mass yield on oxygen (for carbon source) (g DCM/g O_2)

Under pseudo steady state conditions $OUR = OTR = \mu X / Y_{X/O2}$ and bioreactor productivity is roughly proportional to OTR

Putting the Design Report on a Biological Basis

- **Palmitate free fatty acid production**

  Stoichiometry (net) of C_{16} FFA production from C6 & C5:

  $$4\ \text{Glucose} + O_2 \rightarrow 1\ \text{Palmitate (FFA)} + 8\ CO_2 + 8\ H_2$$
  $$4.8\ \text{Xylose} + O_2 \rightarrow 1\ \text{Palmitate (FFA)} + 8\ CO_2 + 8\ H_2O$$

- **Maximum palmitate yield is 0.36 g FFA/g sugar (G or X)**

- **Assume Q_P proportional to OTR and:**

  Cell mass concentration in reactor, X = 10 g/L
  Cell mass exponential growth rate, μ = 0.2 h^{-1}
  Cell mass growth yield on sugar, $Y_{X/S}$ = 0.5 g dry cell mass/g sugar
  Cell mass growth yield on O_2, $Y_{X/O2}$ = 1 g dry cell mass/g O_2
  Palmitate (C_{16} FFA) yield on sugar, $Y_{P/S}$ = 0.28-0.36 g palmitate/g sugar

➔ *Under these conditions, an OTR (OUR) of 62.5 mMol O_2/L-h is needed to enable a volumetric productivity of 2 g palmitate/L-h*

Oxygen Transfer in Submerged Cultivation

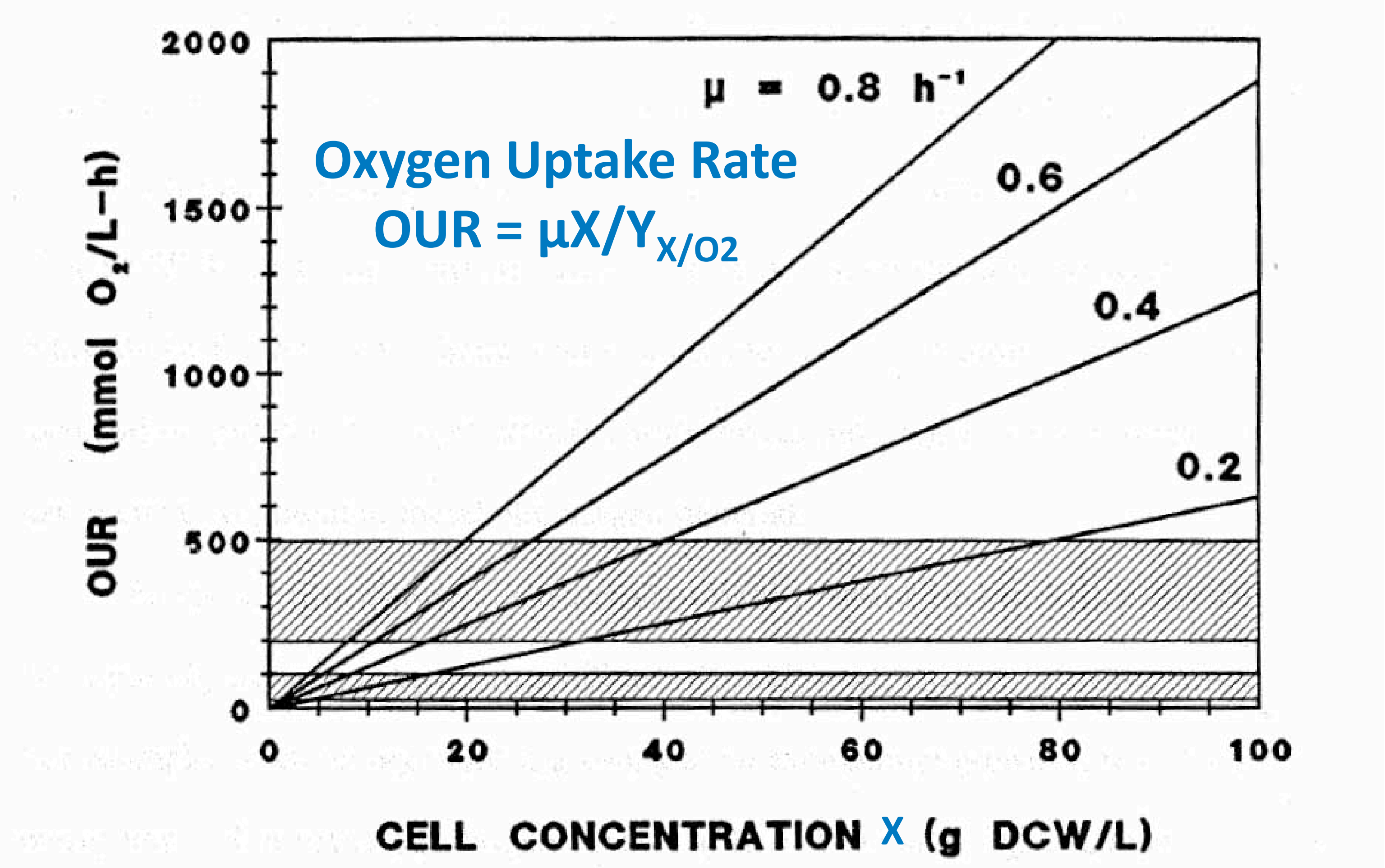


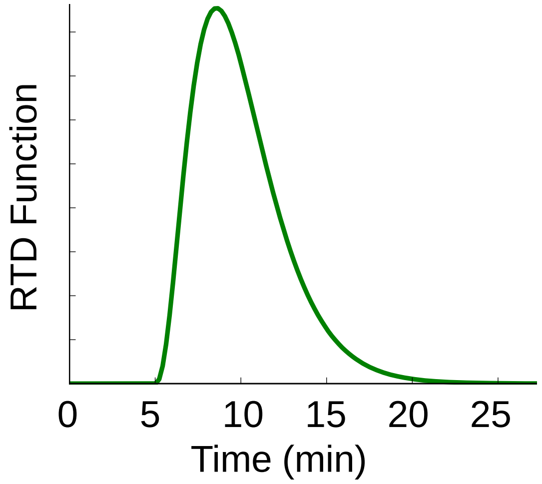
Figure 2.3 Metabolic oxygen demand as a function of cell concentration. Oxygen demand is calculated by Equation (21) using Y_{O_2} = 1 g DCW/g O_2. Shaded regions indicate ranges of maximum oxygen transfer rates reported for bench and pilot scale (upper region) and large scale (lower region) agitated bioreactors.

Project Specific Objectives/Approach

Pilot Scale Integration R&D Task (FY13/14)

Develop and implement an on-line technique to measure residence time distributions (RTD) in pilot-scale pretreatment reactors

- Rational: Allows measurement of actual residence times and facilitates development of applicable fluid dynamic and kinetic models that could be used to optimize pretreatment performance and reduce cost
- Approach:
  - Review literature to understand applicable techniques
  - Select and test technique(s) and implement in one or more of the pilot scale pretreatment reactors
  - Determine impact of reactor operating conditions on RTD and subsequently on pretreatment performance



RTD Analysis Procedure

1. **Raw data is parsed and the mean of the last 3-5 seconds prior to airlock valve cycling is taken as a discrete data point, when there should be material within the airlock and contacting the probes. In most cases, this is close to the peak of each 'square' wave in the raw data.**
2. **Baseline is determined and subtracted from data points. Data are also divided by tracer dose to normalize responses between different runs (if different tracer doses were used).**
3. **The above steps were repeated for each separate run, then the normalized data points combined into a composite.**
4. **A regularization-based signal smoothing method generated an average response curve and outlier data points were rejected based on Chauvanet's criterion.**
5. **A second regularization-based smoothed curve is generated using constraints. First and last points forced to zero and signal can only increase before the peak and decrease after the peak. The mean, variance, and skew are calculated numerically from this curve and used as estimates.**
6. **An optimization function is used to fit the Gamma distribution to the existing data points by manipulating the distribution's input parameters: mean, variance, skew, scale. The standard deviations of these final parameters are also reported.**
7. **Final variables calculated. Scale parameter is used to scale the data points for display purposes. Uncertainty analysis and error propagation calculated for parameters.**

- **Peaks of pseudo-square waves taken as conductivity data**
- **Baseline subtracted and normalized for tracer dose**
- **Gamma distribution function fit to the data**
- **Mean, variance, skew calculated from the Gamma distribution parameters**

Project Specific Objectives/Approach

Pilot Scale Integration R&D Task (FY14)

Explore alkaline-based pretreatment process

- Rational: Develop pilot scale performance information on this process as a possible option for utilizing the solubilized lignin fraction for producing fuels and chemicals (collaboration with Lignin Utilization (LU) Project)
- Approach:
  - Based on information generated from bench-scale studies (from LU project) perform alkaline pretreatment in larger batch and continuous reactors
  - Collect pretreatment performance (yield and mass balance) data and test enzymatic digestibility of the residual solids
  - Assess economics and future possibilities for this process (FY15 and beyond)

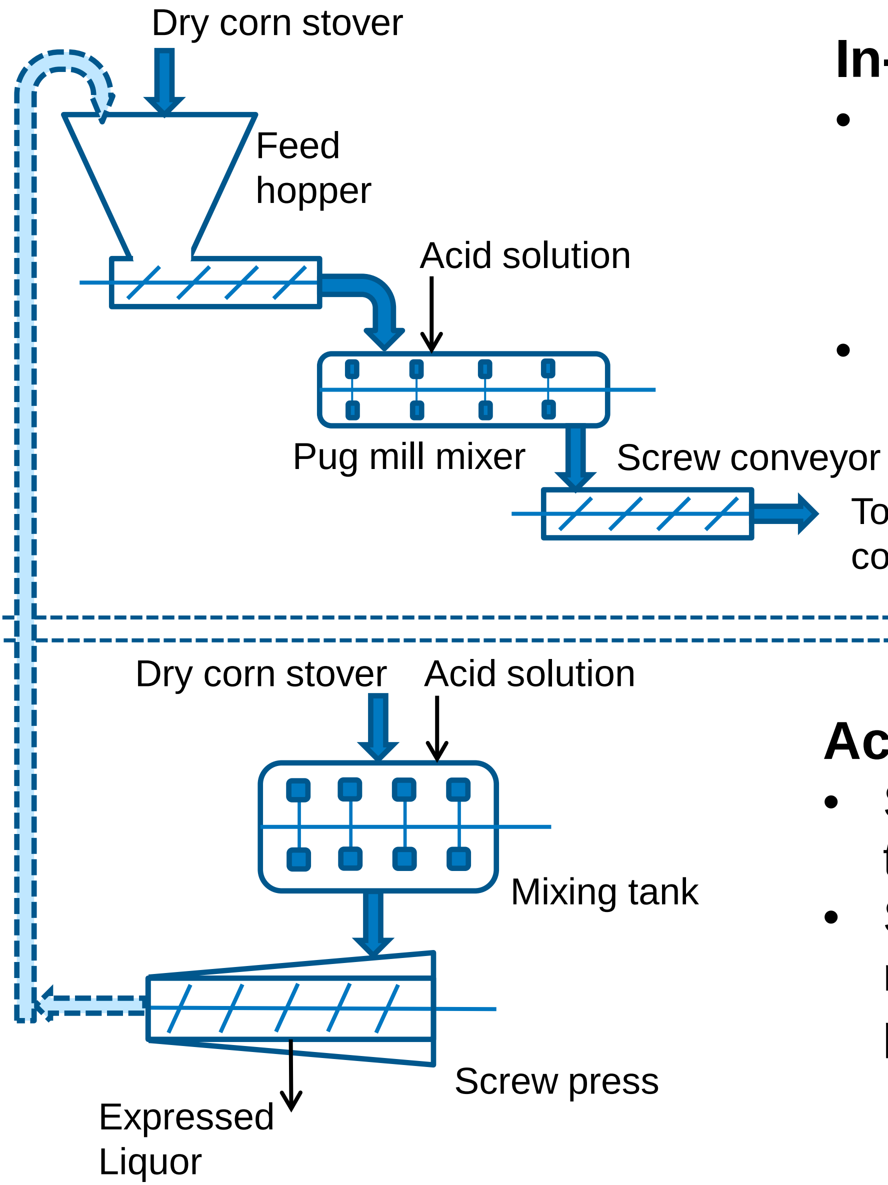
Project Specific Objectives/Approach

Pilot Scale Integration R&D Task (FY13/14)

Determine impact of pre-impregnating biomass with acid prior to pretreatment

- Rational: Determine if an acid pre-impregnation process enhances pretreatment performance enough to justify the additional process cost and complexity compared to a simpler in-line impregnation process
- Approach:
    - Test the two options in a pilot scale pretreatment reactor at the same operating conditions
    - Compare pretreatment yields

Acid Pre-impregnation Study



In-line acid impregnation

- Dry stover and acid solution mixed continuously in a pug mill mixer with a contact time of one minute at ~ 30%–40% (w/w) total solids
- Slurry immediately pretreated

Acid pre-impregnation

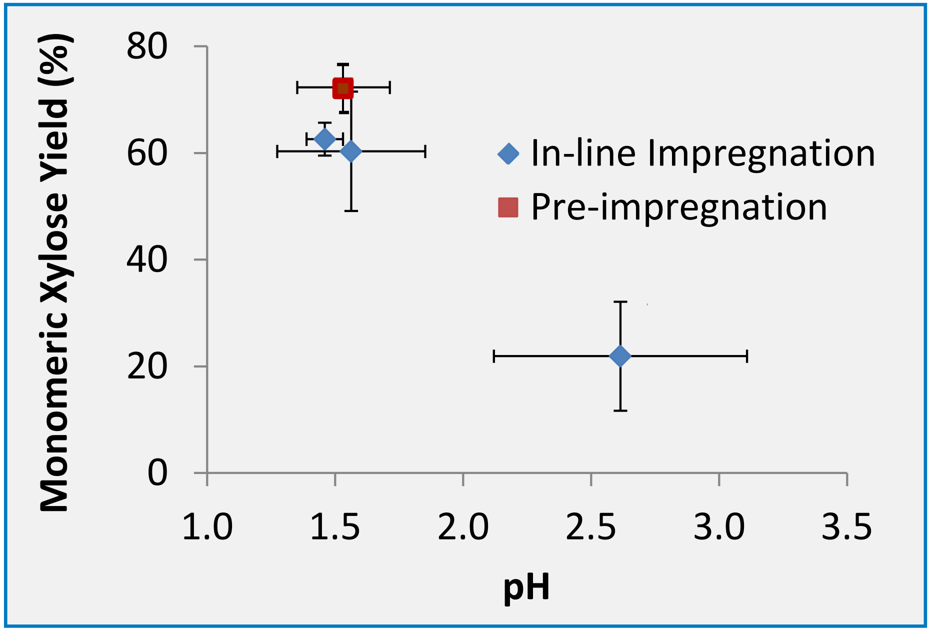
- Stover and acid solution mixed in batch tank for two hours at ~10% total solids
- Slurry dewatered by a screw press and material allow to sit for at least one day prior to pretreatment

Acid Pre-impregnation Study Results

Methodology

- Compared the two impregnation methods at the same operating conditions, but targeting the same effective acid concentration (pH) during reaction is difficult
- Varied acid loading during in-line operation in an attempt to bracket the acid concentration for a single pre-impregnation condition
- Replicated each operating conditions three times (one standard deviation error bars shown)

Monomeric Xylose Yield as a Function of Reaction pH



Other operating conditions: 160 C, 10 min

Results

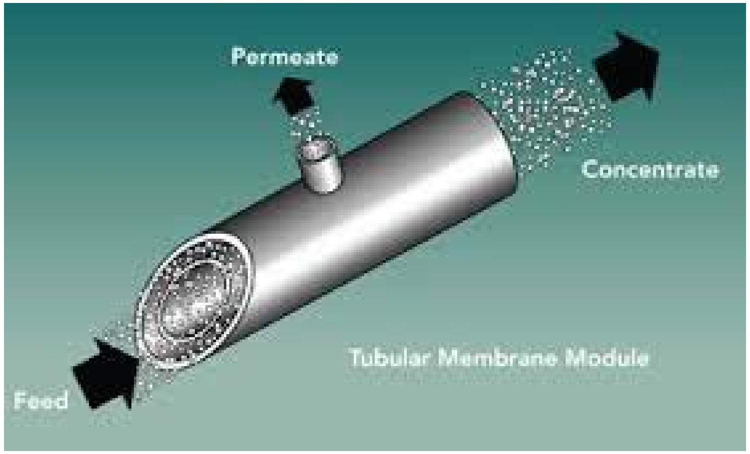
- This process is highly variable as reflected by the large error bars
- Results “suggest” that pre-impregnation is more effective
- No further work planned until new pretreatment processes evolve and there is a need to understand this issue

Project Specific Objectives/Approach

Pilot Scale Integration R&D Task (FY13/14)

Continue membership in the Membrane Science, Engineering, and Technology Center (MAST, www.mastcenter.org) an NSF Industry/University Collaborative Research Center

- Rational:
  - Provides a unique opportunity to interact with both university and industrial researchers on projects supporting many aspects of membrane development and use
  - Participation allows us to keep abreast of and participate in development of new membrane technologies having potential applications to biorefineries
  - Specific projects directly applicable to our mission have been performed by university researchers (most recent is development of a new lyotropic (surfactant) liquid crystal membranes for low cost separation of bio-molecules from aqueous solutions)



Project Specific Objectives/Approach

Pilot Scale Integration R&D Task (FY15)

Compare pretreatment performance in bench to pilot scale reactor systems and develop correlation function

- Rational: If such a relationship exist, optimization and screening studies more quickly and efficiently performed in small reactors can be used to predict performance in large scale reactors and will decrease process development time (collaboration with Analytical Development, Feedstock Process Interface, and Pretreatment and Process Hydrolysis Projects)
- Approach:
  - Generate pretreatment performance information in small (50 mL) batch reactors, bench-scale (1 L and 4 L) steam-injected reactors, and the continuous (25 kg/h dry feed) pilot scale reactor
  - Use pretreatment performance and enzymatic digestibility information to develop correlations