



Pacific Northwest
NATIONAL LABORATORY

Proudly Operated by Battelle Since 1965

U.S. Department of Energy (DOE) Bioenergy Technologies Office (BETO) 2017 Project Peer Review

Advanced Supervisory Control and Data Acquisition (SCADA) for Biochemical Process Integration

3/8/2017

Biochemical Platform Review

Jim Collett

Pacific Northwest National Laboratory

Goal Statement

The Challenge: Fed-batch bioconversion of **highly variable biomass feedstocks** will require advanced supervisory control and data acquisition (SCADA) systems for **cost-competitive production of hydrocarbon biofuels and chemicals.**

The Goal: We will work with industrial partners on technology to market transfer of **Process Analytical Technologies (PAT) for biorefinery SCADA systems** to improve process control, reduce manual sampling, and increase product quality.

The Outcome: Bioenergy startups and established biorefineries will have access to **proven PAT equipment and methods that integrate seamlessly with SCADA systems** to enable profitable scale-up and informative scale-down of industrial bioprocesses.

Quad Chart Overview

Timeline

Project start date: 10/1/2015

Project end date: 9/30/2017

Budget

	FY 15 Costs	FY 16 Costs	Total Planned Funding (FY 17-Project End Date)
DOE Funded	\$300 K	\$300 K	\$300 K
Project Cost Share (Comp.)*	\$30 K		

Partners

- **Bend Research** (small business; bought by Capsugel during project)
- **Sartorius Stedim, N. A.**
- **Flownamics** (small business)

MYPP Barriers Addressed

Ct-A. Feedstock Variability

Achieved accurate, **real-time tracking of biocatalyst growth on multiple feedstock lots** with suspended lignin loadings up to 4.25% using dielectric spectroscopy (DS).

Ct-H. Efficient Upgrading of Sugars to Fuels & Chemicals

Demonstrated **automated, PID control of fed-batch bioconversion of hydrolysate to hydrocarbons** using online near-infrared spectroscopy (NIRS)

Ct-J. Conversion Process Integration

Worked with bioprocess manufacturers to develop a “super-stock” 30-liter bioreactor to demonstrate **OPC-networked PAT systems** that directly integrate with biorefinery SCADA systems to improve process control and reduce manual sampling.

1 - Project Overview

Critical Success Factors:

- Use **SCADA-compatible, commercial-off-the-shelf (COTS) equipment**.
- Achieve **real-time tracking and control of critical process parameters (CPPs)**.
- Ensure results are **generalizable for a variety of biocatalyst organisms**.

Dielectric spectroscopy (DS)

ABERTM
INSTRUMENTS



BEND RESEARCHTM
ACHIEVE VALUE THROUGH SCIENCE

 **sartorius**



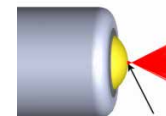
**Near-infrared
spectroscopy (NIRS)**



**30-liter
Bioreactor**

Raman spectroscopy

MARQMETRI 



 **FLOWNAMICS**[®]
Your Bioprocess Sampling Solution



**Automated
bioreactor
sampling**

1 - Project History

FY 14 (seed year)

- **DS validated** in high-solids, pretreated corn stover (PCS) hydrolysate.
- Bend Research evaluates **frequency scanning DS** for quantifying lipids.

FY 15

- Sartorius Stedim and PNNL create **preliminary NIRS calibration models** for glucose, xylose, ammonium, and cell mass.
- NIRS and DS **data integrated with industrial-class SCADA network** via OPC.
- 30 L bioreactor equipped with **Flownamics automated sampling system**.

FY 16

- **Go/No-Go milestone achieved** (50% reduction in manual sampling).
- **NIRS-based PID control of fed-batch bioconversion** of PCS hydrolysate.

FY 17 (ongoing)

- **Raman spectroscopy** for intracellular lipid quantification during bioconversion.
- **PAT outreach** - Fungal Partners Review Board, ABPDU, INL, NREL.

2 – Approach (Management)

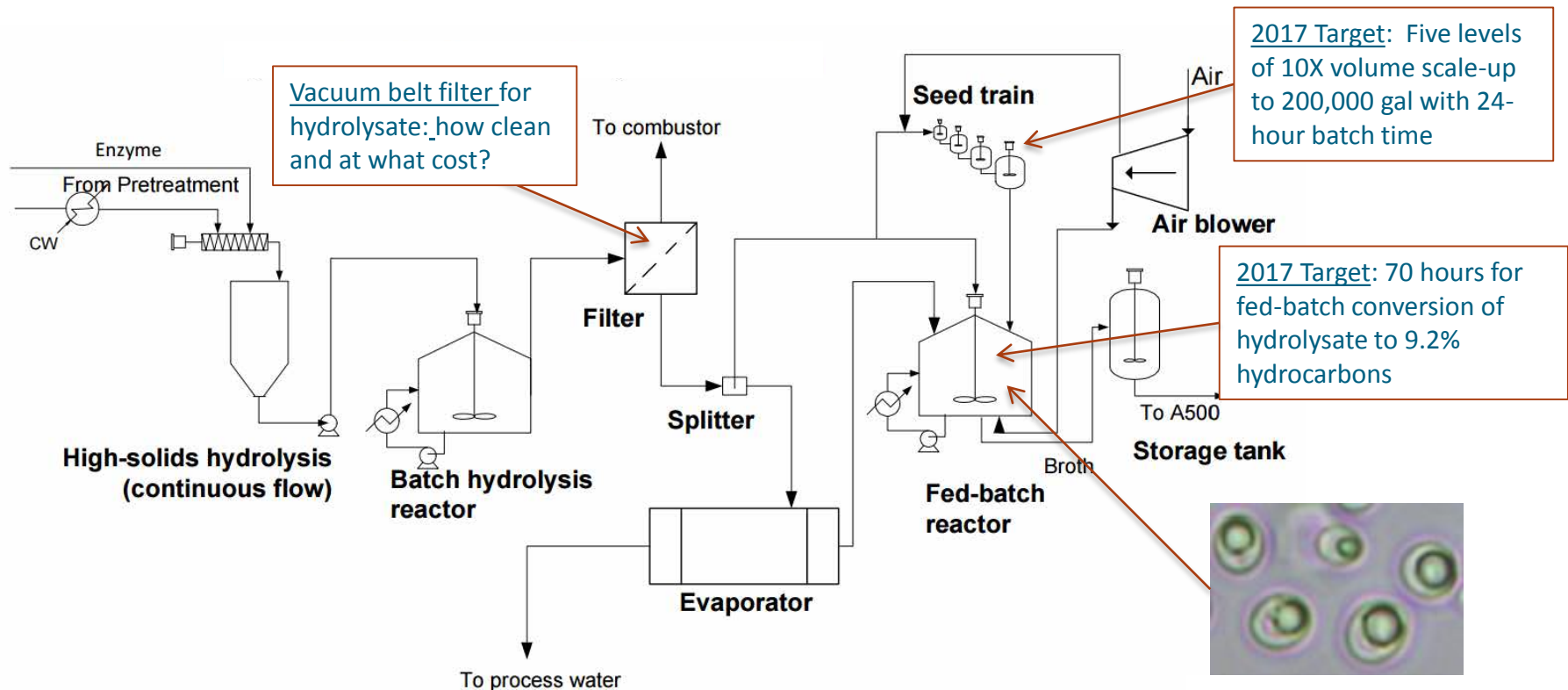
- Standard Project Management Good Practices
 - Statement of work and how it relates to DOE goals
 - AOP Project Management Plan
 - Quarterly milestones
 - Go/No Go decision point
- Frequent project communications
 - Teleconferences with BETO platform leads
 - Quarterly formal reporting to BETO
 - Site visits by industrial collaborators
- Draw on deep talent pool in analytical sciences, process control, machine learning, and chemical engineering at PNNL

2 – Approach (Technical)

Experimental Context:

Experiments designed for **synergy with Fungal Genomics and BC Analysis** AOPs.

Test case: *Lipomyces starkeyi* bioconversion of PCS to lipids.



Above – **Hydrolysate conditioning and bioconversion operations** in NREL sugars-to-hydrocarbons 2017 design case

Davis, R., et al., NREL/TP-5100-602232013

L. starkeyi oleaginous yeast accumulate up to 70% of dry cell mass as lipids during N limitation

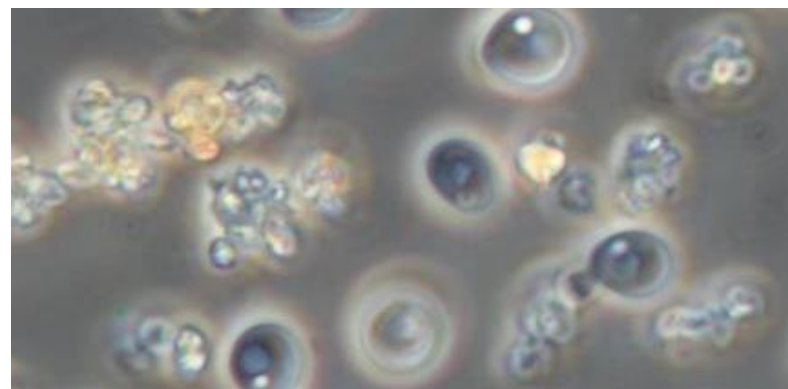
2 – Approach (Technical)

Bioconversion optimization challenges in fed-batch cultivation of oleaginous yeast

- **Nitrogen must be limited** to induce high-yield lipid synthesis.
- **Plant nitrogen in hydrolysate feed stream** may inhibit lipid synthesis.
- Microbial **consumption of glucose vs. xylose must be balanced**.
- Potential **accumulation of lignin solids and metabolic inhibitors** over time.
- **Lot-to-lot variation in feedstock composition and high spectral background** in hydrolysate may confound PAT approaches.

<i>Parameter</i>	<i>Minimum</i>	<i>Maximum</i>
Ash	0.8%	6.6%
Xylan	14.8%	22.7%
Lignin	11.2%	17.8%

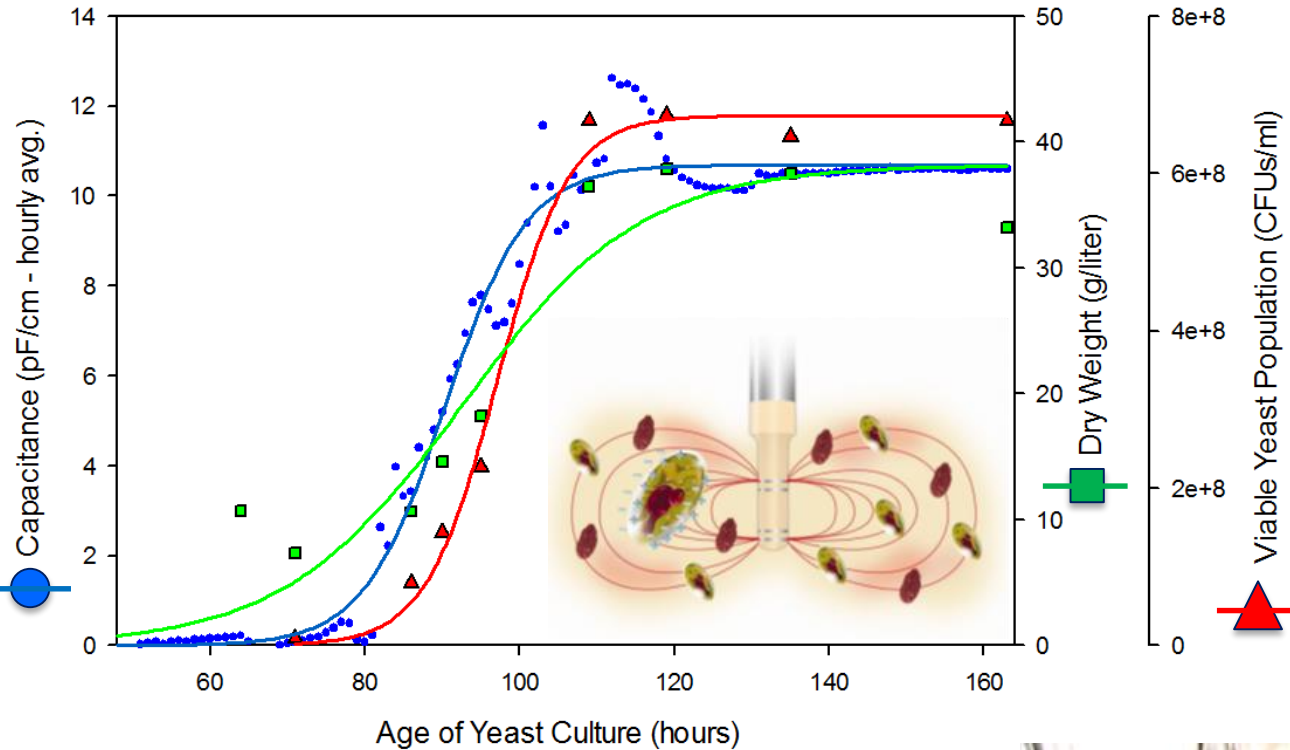
*500 samples were taken from 47 locations over three years
Cellulose, 2009. 16(4): p. 621-639



Lignin solids and *L. starkeyi* cells in in PCS hydrolysate

3 – Technical Progress

Dielectric Spectroscopy in high-solids hydrolysate

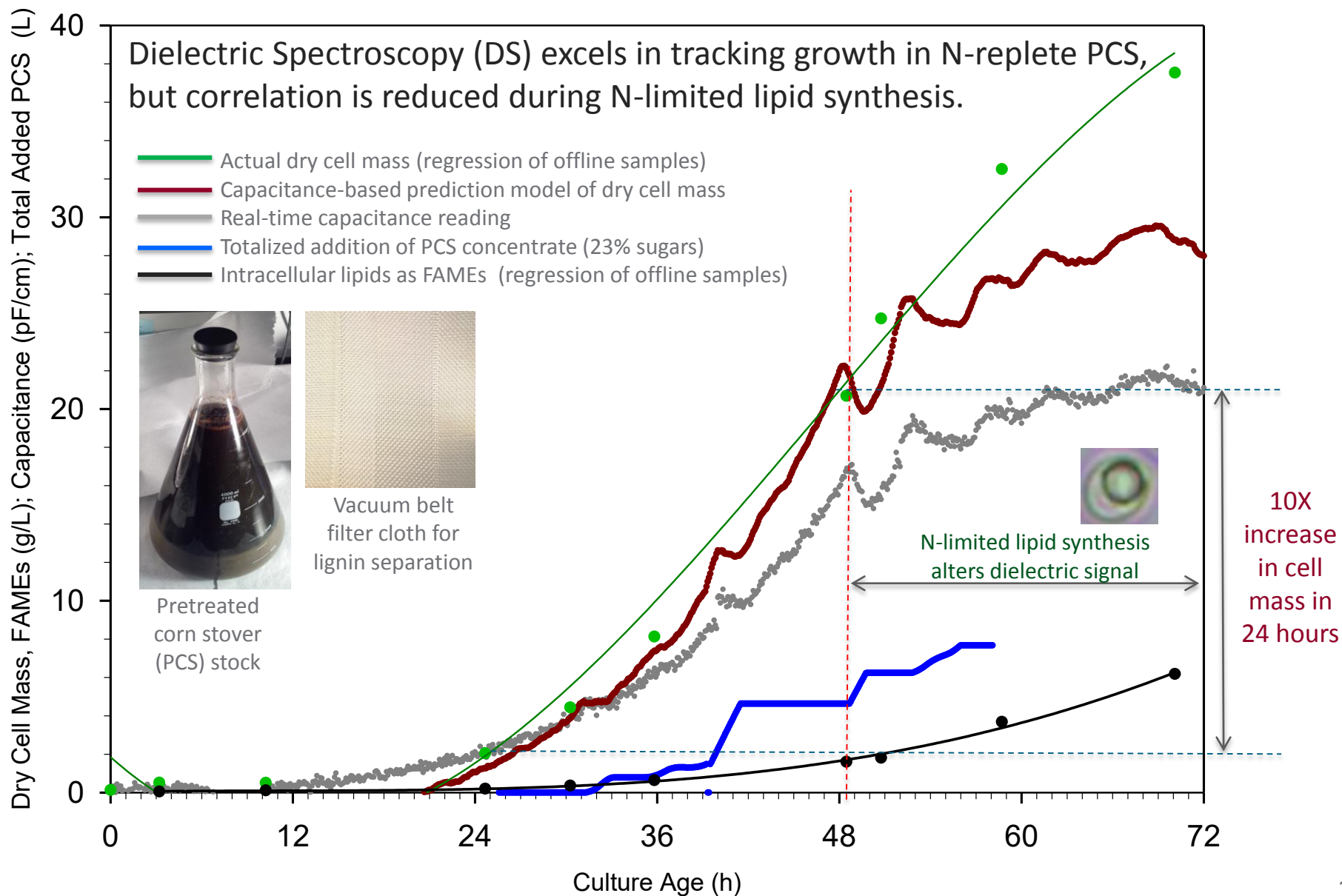


DS capacitance predicted increases in culture dry weight with >90% confidence in a bioreactor culture of *L. starkeyi* grown in the presence of 4.25% PCS insoluble solids.

Right – Samples from a carboy of harvested spent PCS media showing suspended lignin solids mixed with *L. starkeyi* cell mass.

DS also showed promise in tracking cell mass in cultivations of filamentous fungi on PCS.





Near-infrared spectroscopy (NIRS) in synthetic growth media

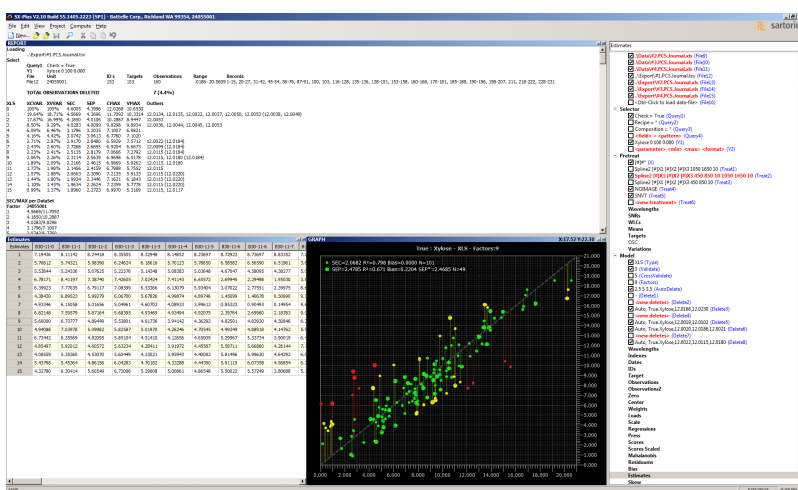
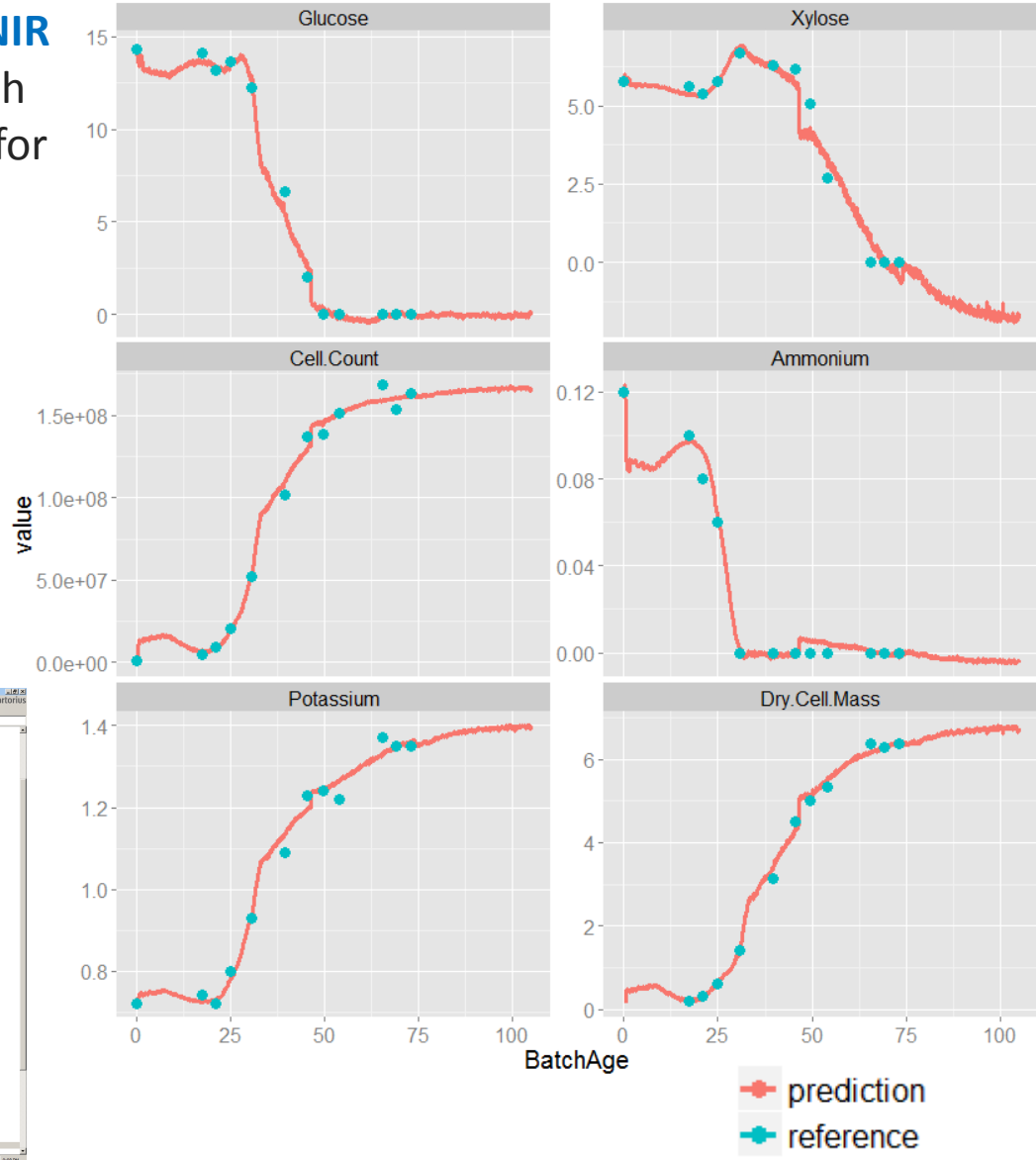


Sartorius *in situ* NIR Spectrometer with OPC data output for direct integration into biorefinery SCADA systems.



Initial NIRS calibration models

constructed in SX-Plus chemometrics software showed promise by tracking CPPs in cultivation of *L. starkeyi* in synthetic media.



Automated sampling enables rapid NIRS calibration model construction



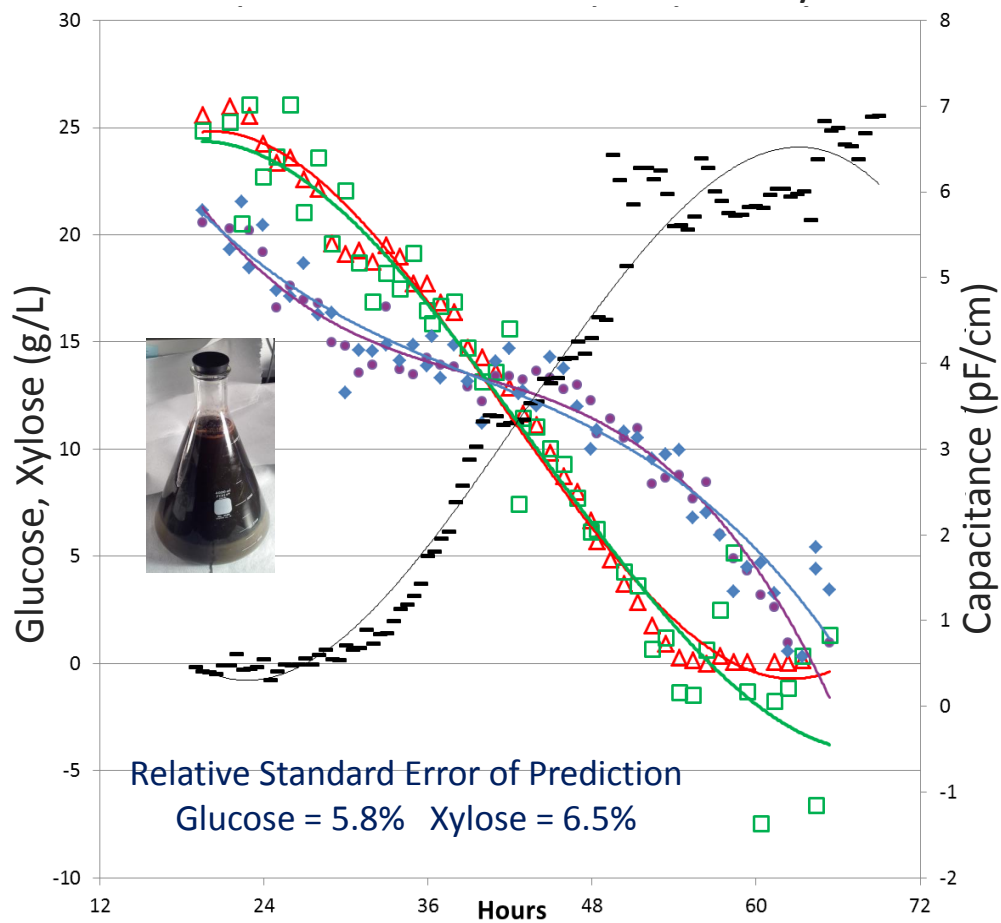
Flownamics 8000i system for high-frequency, 24-hour, cell-free sampling.



Flownamics was featured at the **2015 American Energy Manufacturing Competitiveness Summit**

Go/No-Go Milestone: "Can NIRS supplant 50% of manual sampling requirement?" achieved Q2 of FY16.

NIRS estimates of glucose and xylose during PCS bioconversion vs. offline assays

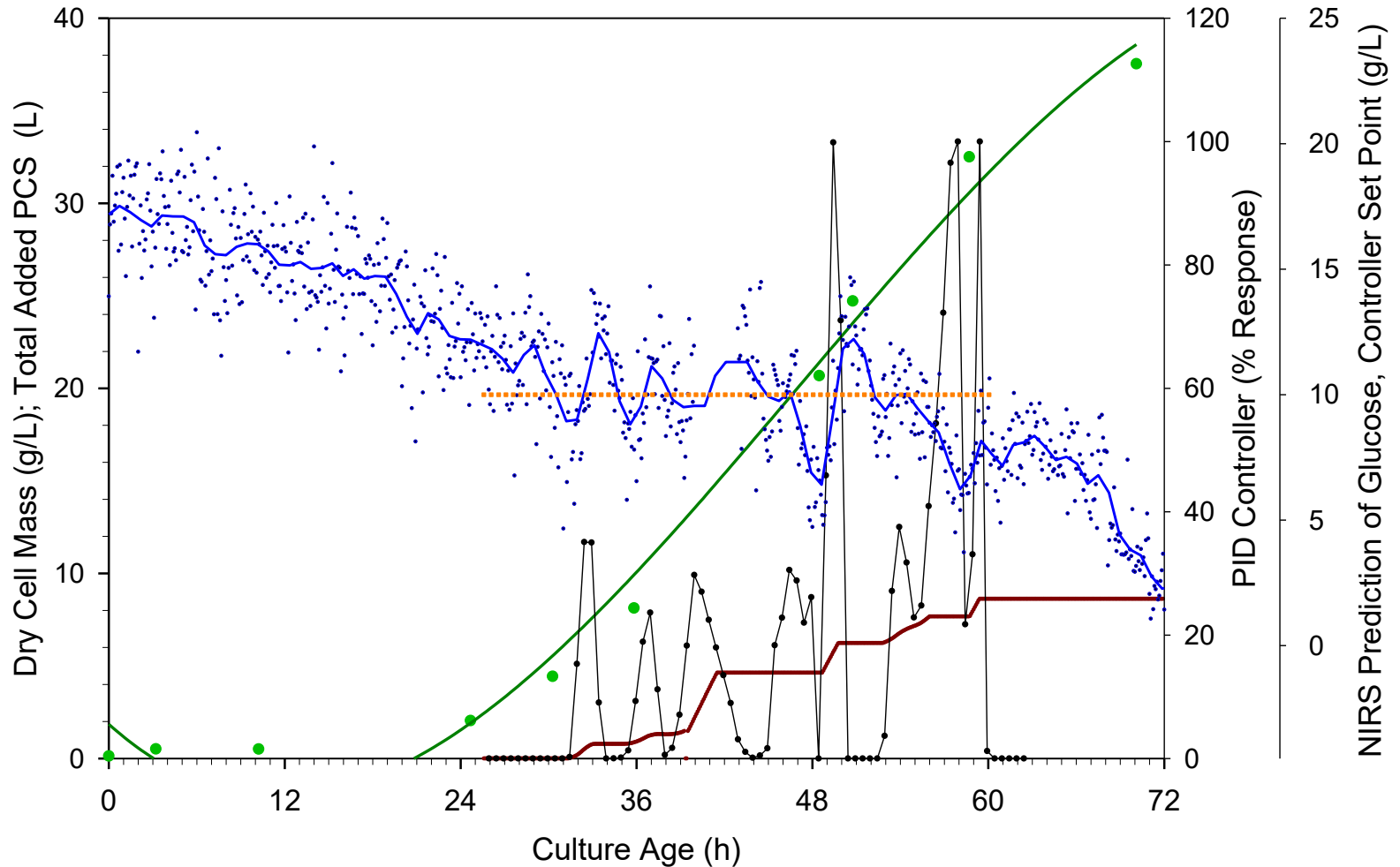


- △ Glucose Offline Sample (Flownamics)
- Xylose Offline Sample (Flownamics)
- Glucose NIR Estimate (Sartorius)
- ◆ Xylose NIR Estimate (Sartorius)
- Dielectric Capacitance (cells mass growth)

Cultivation of *L. starkeyi* on hydrolyzed PCS supplemented with minimal nutrients.

NIRS enables automated fed-batch control of bioconversion of PCS hydrolysate

20-liter bioreactor cultivation of *Lipomyces starkeyi* on pretreated corn stover hydrolysate.



- NIRS prediction of glucose concentration (moving average)
- Totalized addition of PCS concentrate (22.5% sugar content)
- Dry Cell Mass
- Set point of glucose PID control loop
- PID Controller Response (% of max output)

FY17 (ongoing) – Process Raman Spectroscopy for intracellular lipids quantification

We are working with Marqmetrix, a University of Washington spin-off company, to adapt their **Process Raman spectroscopy system to measure intracellular lipids** in oleaginous yeast during bioconversion.

Their **Raman BallProbe** uses a spherical sapphire lens that eliminates errors from focal length variability. The probe can continuously withstand pressures > 6000 psi and temperatures > 300°C.

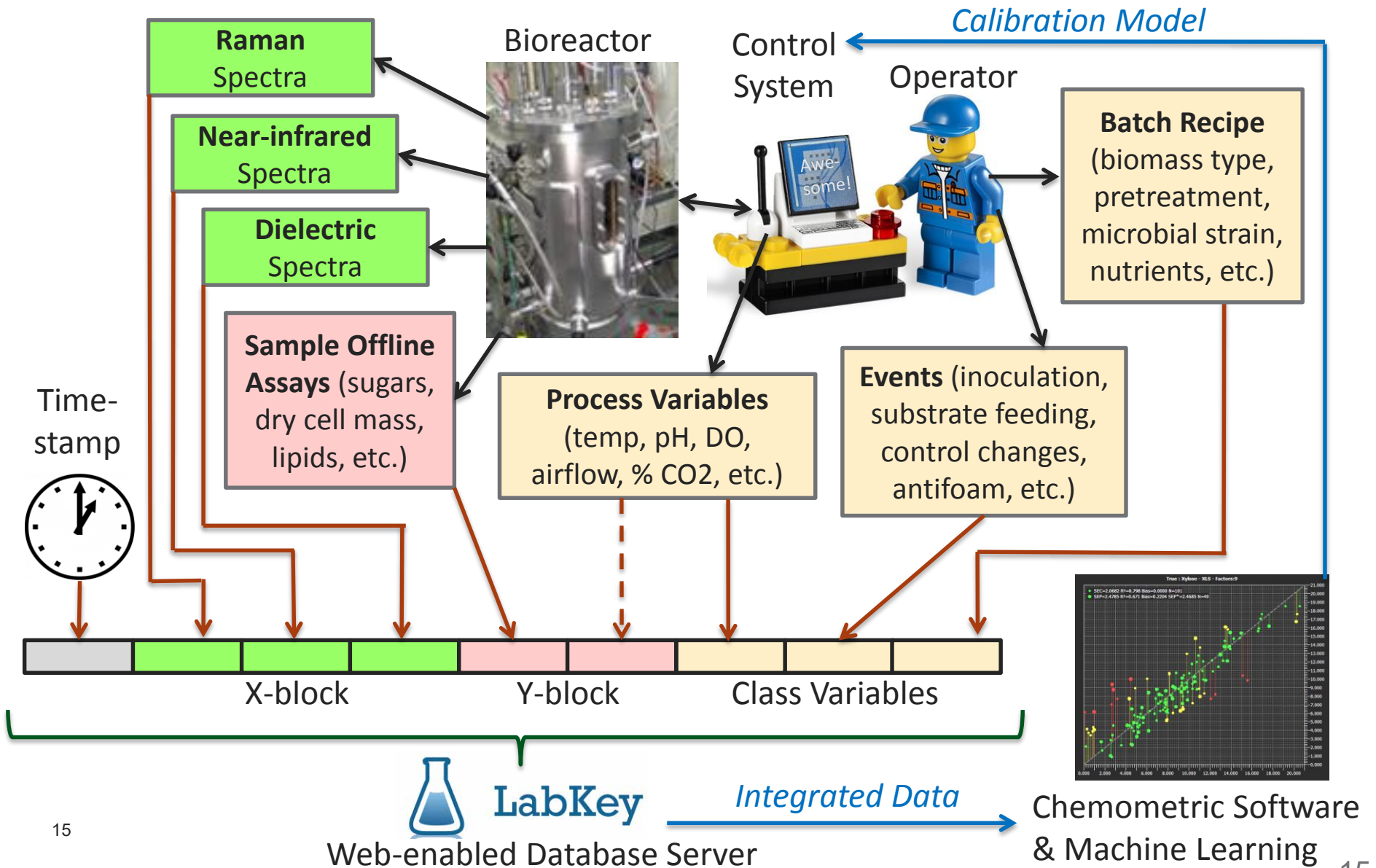


The BallProbe has been used to track glucose and ethanol during fermentative bioconversion of switchgrass hydrolysate. *Ewanick et al. Biotechnology for Biofuels 2013, 6:28*



L. Starkeyi cells with large, intracellular lipid droplets.

FY17 (ongoing) – Open-source database for fused chemometric data and machine learning



4 – Relevance



http://www.energy.gov/sites/prod/files/2014/12/f19/process_integration_workshop_report_dec_2014.pdf

This project directly addresses these **key recommendations made by industry stakeholders** at the 2014 PRINCE workshop hosted by BETO:

- Working with equipment manufacturers to develop **COTS equipment** for biomass conversion that is efficient and can be **predictably scaled up or down**.
- Determining specifications for process integration, including **development of online monitoring capabilities and analytical tools** that can be used throughout the biorefinery.
- **Tailoring technologies to expected particle sizes.**
- Optimization focused on **industrially relevant organisms.**

Overview: The goal of this project is to develop **Process Analytical Technologies** (PAT) that optimize bioconversion of biomass feedstocks with variable compositions and high levels of suspended solids.

Approach: **Dielectric, Near Infrared, and Raman Spectroscopy** are being adapted into a set of industrial PAT tools and methods using COTS equipment and software that will **directly integrate into biorefinery SCADA systems**.

Technical Accomplishments:

- **DS validate for tracking cell mass growth in high-solids PCS hydrolysate**
- Validated **NIRS calibration models of Critical Process Parameters** for bioconversion of PCS hydrolysate to hydrocarbons that could enable a 50% reduction in manual sampling and analysis
- Demonstrated **NIRS-enabled PID control of fed-batch bioconversion** of PCS hydrolysate to hydrocarbons

Acknowledgements



CAPSUGEL®

Brandon Downey

Jeff Breit

Lisa Graham



Dan Kopec

Kevin Filipiak



Paul Strand

Lucas Schimmelpfennig

Ashley Fisher-Kapp



Pacific Northwest
NATIONAL LABORATORY

Advanced SCADA: Jim Collett, Erik Hawley,
Richard Zheng, Brook Remington

Related Projects

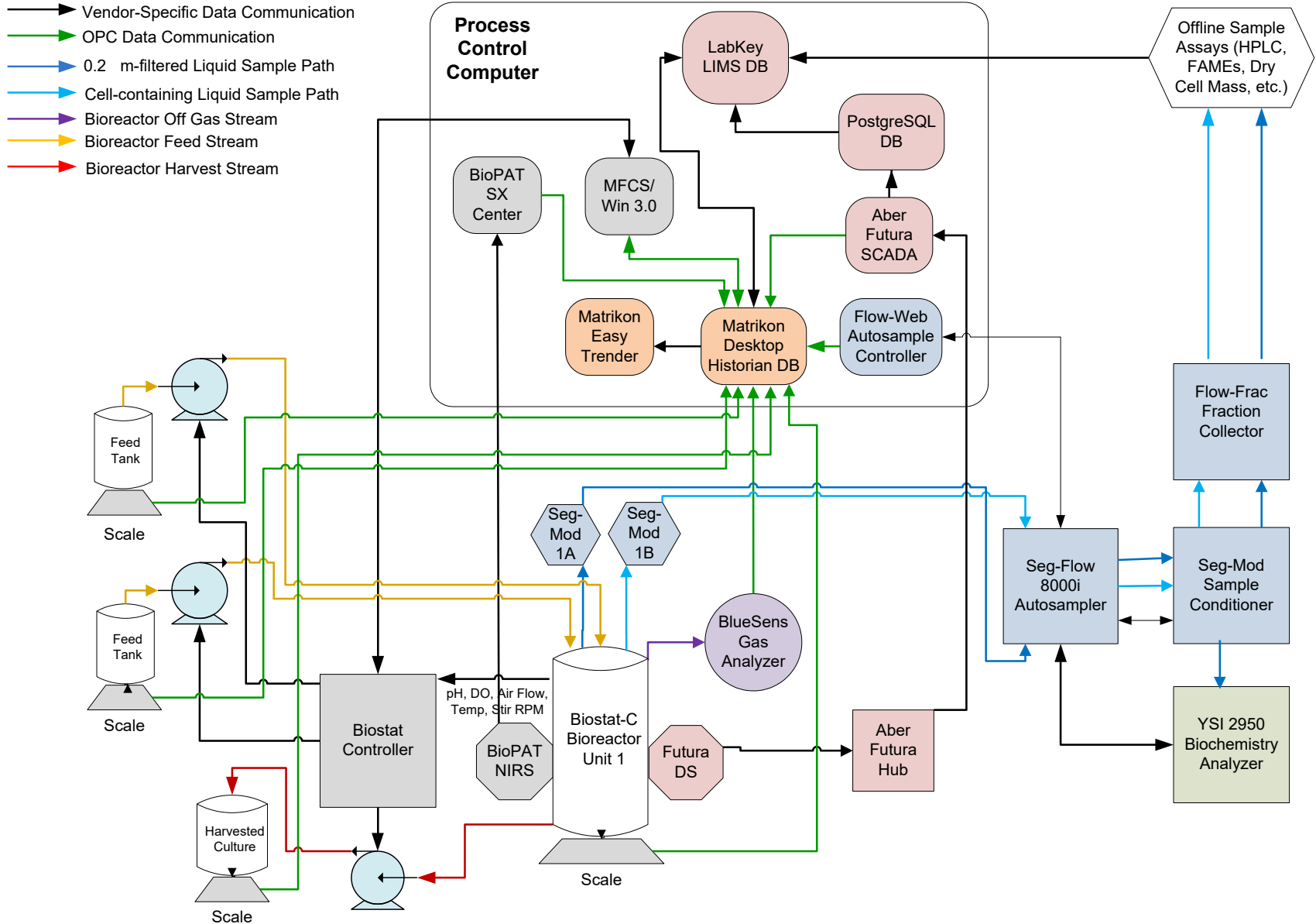
Fungal Genomics: Jon Magnuson, Mark Butcher,
Jim Collett, Dave Culley, Ziyu Dai, Shuang Deng,
Beth Hofstad, Ellen Panisko, Kyle Pomraning,
Swarnendu Tripathi

Biochemical Analysis: Sue Jones, Aye Meyer,
Yunhua Zhu, Jim Collett, Mark Butcher

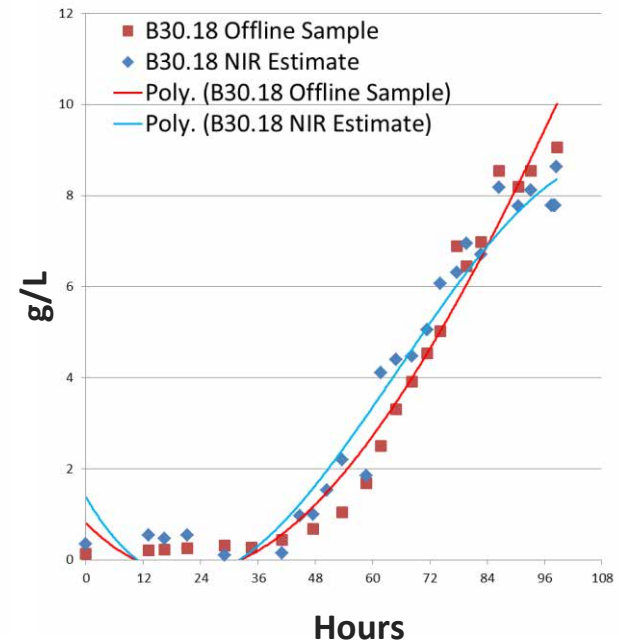
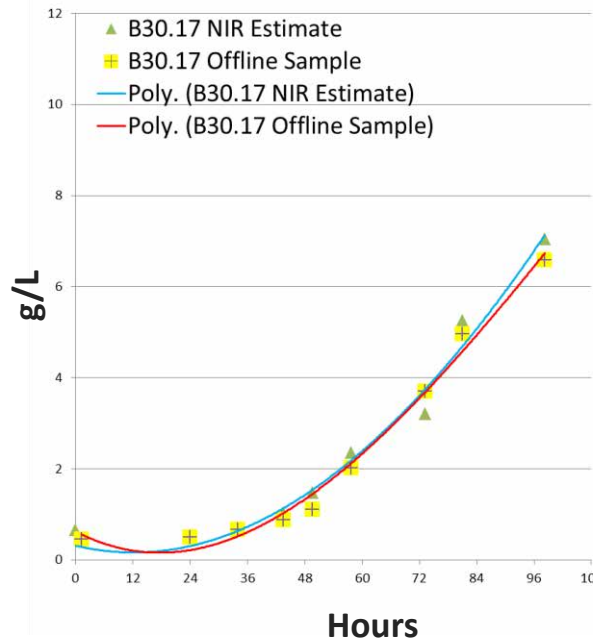
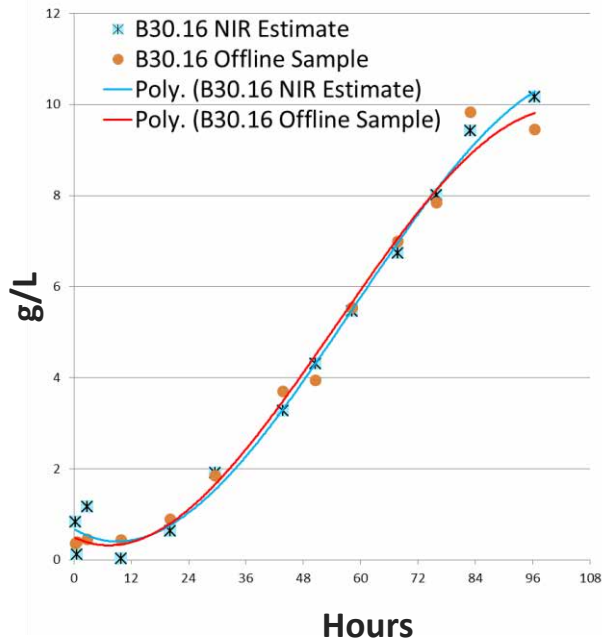


Additional Slides

Laboratory SCADA Integration Plan



NIRS tracks cell mass growth in PCS



Above: NIR estimates of dry cell mass in 3 cultivations of *L. starkeyi* grown on hydrolyzed, pretreated corn stover (PCS) containing 2-3% total sugars (blue line = NIR calibration model; red line = offline sample regression model).

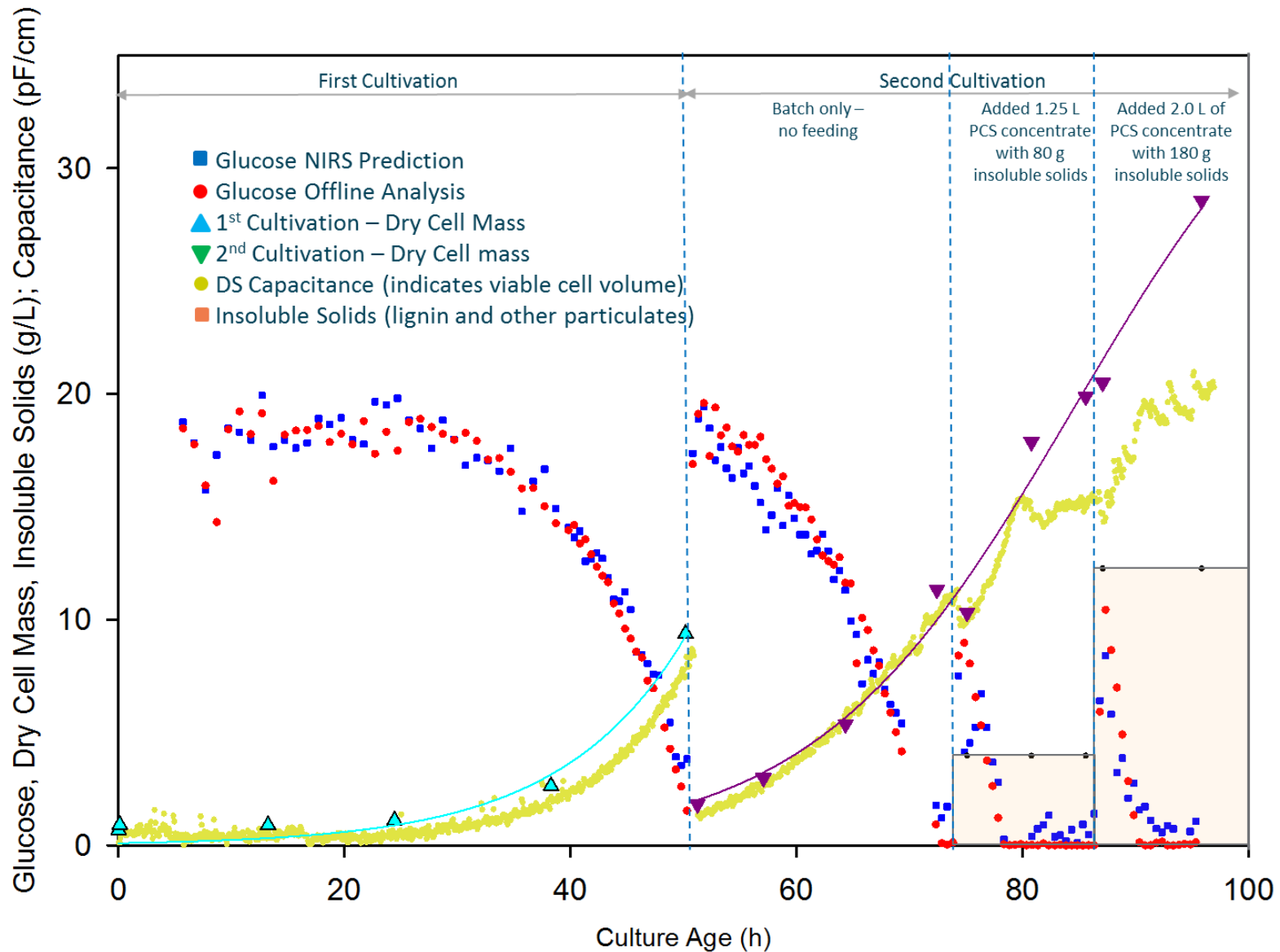
Preliminary NIR Performance in Pretreated Corn Stover Media



Parameter	Calibration Set Count	Prediction Set Count	Relative Standard Error of Prediction
Dry Cell Mass	32	15	3.25%
Glucose	49	21	8.49%
Xylose	70	34	10.25%
Ammonium	73	33	10.04%

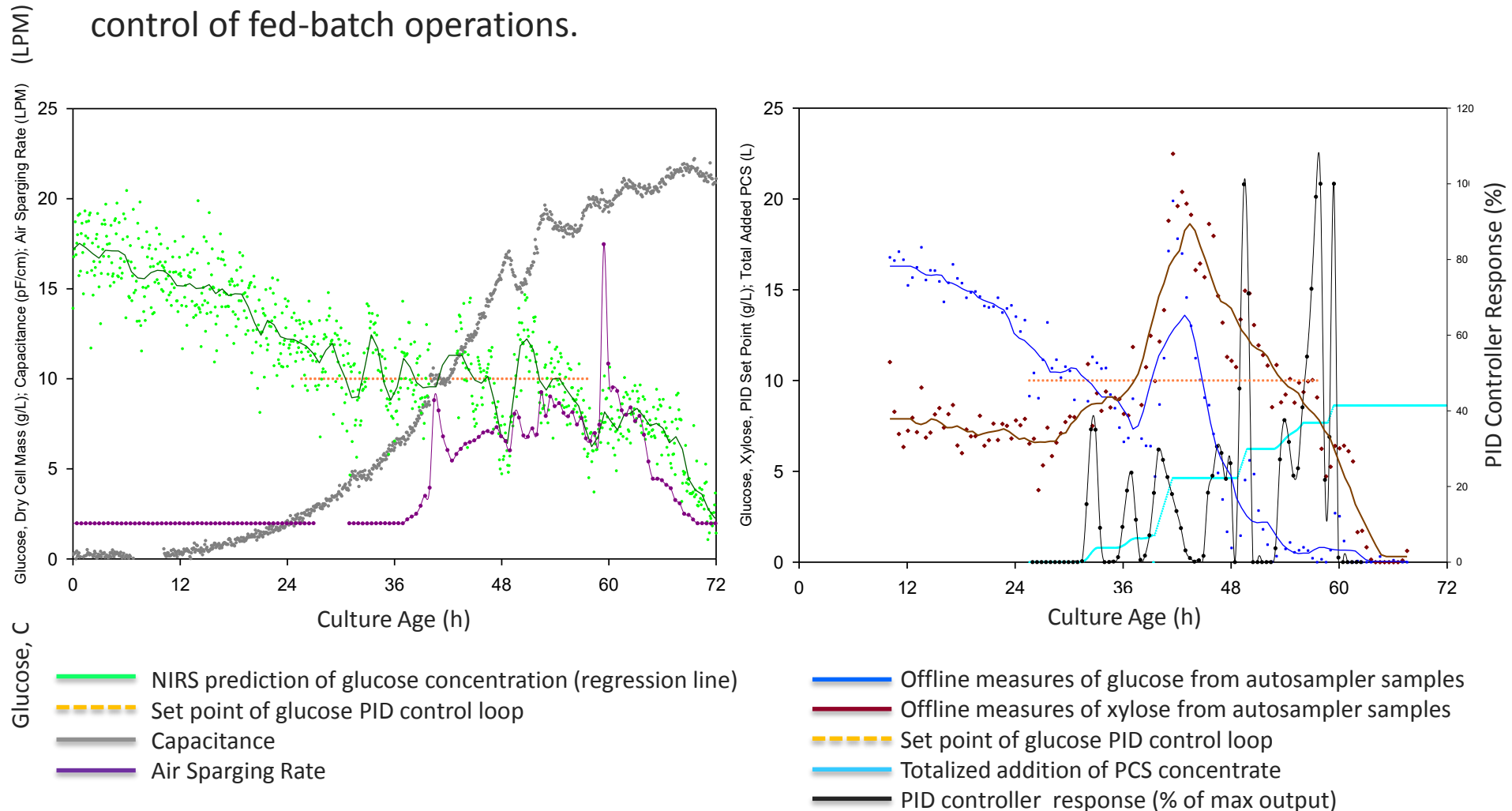


NIRS tracks glucose in PCS containing suspended lignin solids

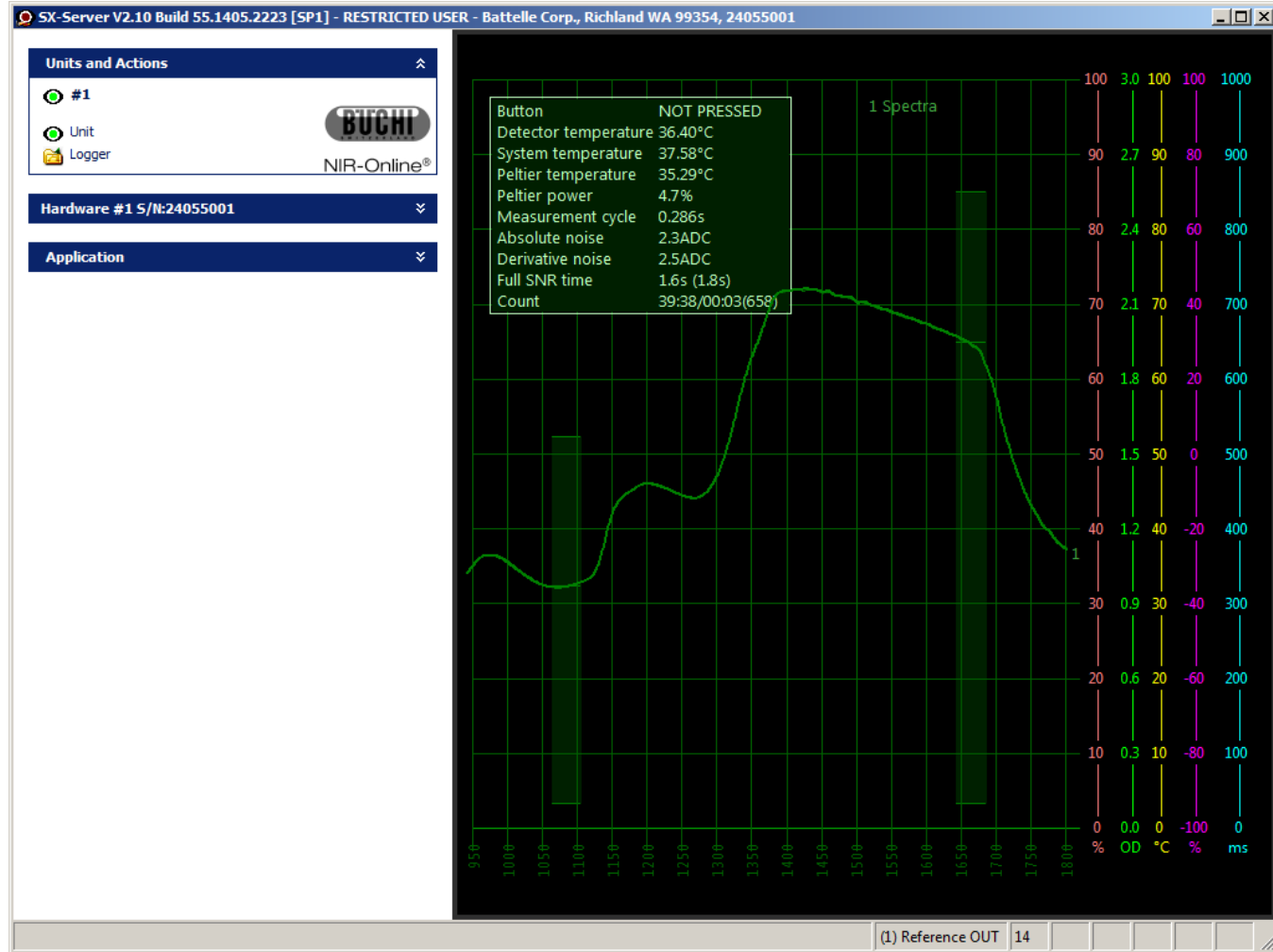
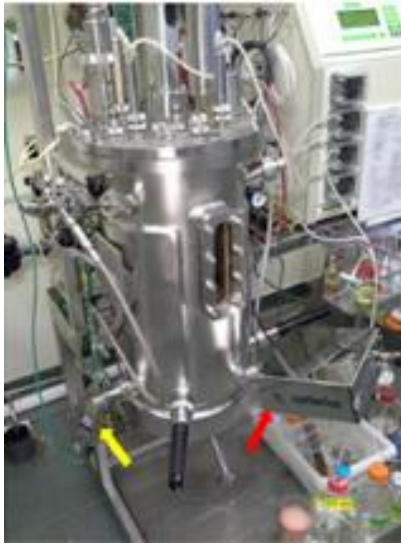


NIRS enables automated fed-batch control of bioconversion of PCS hydrolysate

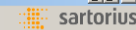
Near-infrared Spectroscopy (NIRS) enabled **PID control of automated, fed-batch cultivation** of *Lipomyces starkeyi* on PCS hydrolysate in a 30-liter bioreactor. Further refinement of chemometric models for glucose and xylose will facilitate tuning of PID control of fed-batch operations.



Sartorius SX-Server



SX-Plus for NIR Calibration Model Development



SX-Plus V2.10 Build 55.1405.2223 [SP1] - Battelle Corp., Richland WA 99354, 24055001

File Edit View Project Compute Help

REPORT Loading

..Export#1.PCS.Journal.tsv

Select

Query1 Check = True
Y1 Xylose 0 100 0.000
File Unit ID's Targets Observations Range Records
File2 24055001 153 153 160 .0186- 20.5609 1-15, 20-27, 31-42, 45-54, 56-76, 87-91, 100, 103, 116-128, 135-136, 138-151, 153-158, 160-168, 170-181, 185-188, 190-196, 198-207, 211, 218-222, 228-231

TOTAL OBSERVATIONS DELETED 7 (4.4%)

XLS	XCVAR	XVVAR	SEC	SEP	CMAX	VMAX	Outliers
0	100%	100%	4.6005	4.3996	12.0268	10.6520	
1	19.64%	18.71%	4.5669	4.3696	11.7092	10.5314	12.0134, 12.0135, 12.0022, 12.0037, 12.0050, 12.0053 (12.0038, 12.0049)
2	17.67%	16.99%	4.1850	4.0106	10.2887	8.9447	12.0053
3	8.50%	9.29%	4.0283	4.0099	9.8298	9.8934	12.0036, 12.0044, 12.0045, 12.0053
4	6.09%	6.46%	3.1796	3.2035	7.1007	6.9821	
5	4.16%	4.42%	3.0742	3.0613	6.7760	7.1020	
6	2.71%	2.87%	2.9170	2.8480	6.5929	7.5712	12.0022 (12.0184)
7	2.43%	2.60%	2.7268	2.6695	6.9204	6.6673	12.0099 (12.0184)
8	2.23%	2.41%	2.5135	2.8179	7.0666	7.2792	12.0115 (12.0184)
9	2.06%	2.26%	2.3114	2.5639	6.9698	6.5178	12.0115, 12.0180 (12.0184)
10	1.89%	2.09%	2.2166	2.4615	6.9869	5.9262	12.0115, 12.0180
11	1.73%	1.93%	2.1456	2.4159	6.7988	5.7552	12.0115
12	1.57%	1.88%	2.0663	2.3090	7.2135	5.9133	12.0115 (12.0220)
13	1.44%	1.80%	1.9934	2.3446	7.1621	6.1843	12.0115 (12.0220)
14	1.10%	1.43%	1.9634	2.2624	7.2359	5.7778	12.0115 (12.0220)
15	0.99%	1.37%	1.8960	2.2723	6.9970	5.3169	12.0115, 12.0117

SEC/MAX per DataSet
Factor 24055001

1	4.5669/11.7092
2	4.1850/10.2887
3	4.0283/9.8298
4	3.1796/7.1007
5	3.0742/6.7760

Estimates

Estimates	B30-11-0	B30-11-1	B30-11-2	B30-11-3	B30-11-3	B30-11-4	B30-11-5	B30-11-6	B30-11-6	B30-11-7	B30-11-7
1	7.19436	8.11142	8.24418	8.35505	8.32948	8.14852	8.23697	8.72923	8.73697	8.83352	7.62000
2	5.76812	5.74321	5.98390	6.24624	6.18616	5.70123	5.78650	6.58582	6.56590	6.51961	3.82000
3	5.53844	5.24336	5.07625	5.22376	5.14348	5.08383	5.03648	4.67047	4.58095	4.38277	5.02000
4	6.78171	8.41197	7.38740	7.42605	7.02424	7.41143	6.65572	2.69945	2.29498	1.95030	3.82000
5	6.39923	7.77035	6.79117	7.08399	6.53366	6.13079	5.93404	3.07022	2.77551	2.39975	8.62000
6	4.38430	6.89523	5.99279	6.06700	5.67826	4.99874	4.89746	1.45099	1.40678	0.50690	9.32000
7	4.93346	6.15058	5.01656	5.04961	4.60702	4.08933	3.99613	0.85323	0.90493	0.14954	9.62000
8	6.82148	7.59579	5.87164	5.68395	4.93469	4.93494	4.92075	2.39764	2.69960	2.18783	9.02000
9	5.60000	6.73777	4.89449	5.53801	4.61736	3.94142	4.36292	4.82501	4.83930	4.30846	6.62000
10	4.94088	7.03978	5.09982	5.82587	5.01970	4.26246	4.70545	4.99349	4.88918	4.14762	5.92000
11	6.73442	6.35569	4.82095	5.85104	4.51410	4.12656	4.65009	5.29967	5.33734	3.90019	6.42000
12	4.85497	5.92012	4.60572	5.63234	4.28411	3.91972	4.45587	5.58711	5.66860	4.26144	7.02000
13	4.08559	5.35360	4.53070	5.60449	4.33021	3.95943	4.40082	5.81496	5.99630	4.64292	6.22000
14	5.43768	5.45364	4.86156	6.04283	4.70182	4.33288	4.44390	5.91115	6.07358	4.56854	6.22000
15	4.32780	6.30414	5.60549	6.72096	5.29008	5.00861	4.86548	5.50022	5.57249	3.80688	5.72000

GRAPH X:17.52 Y:22.30

True : Xylose - XLS - Factors:9

SEC=2.0682 R²=0.798 Bias=0.0000 N=101
SEP=2.4785 R²=0.671 Bias=-0.2204 SEP*=2.4685 N=49

Estimates

- \Data\#2.PCS.Journal.xls (File9)
- \Data\#3.PCS.Journal.xls (File10)
- \Data\#4.PCS.Journal.xls (File11)
- ..Export#1.PCS.Journal.tsv (File12)
- ..Export#2.PCS.Journal.tsv (File13)
- ..Export#3.PCS.Journal.xls (File14)
- ..Export#4.PCS.Journal.xls (File15)
- <DbI-Click to load data-file> (File16)

Selector

- Check = True (Query1)
- Recipe = * (Query2)
- Composition = * (Query3)
- <fields> = <pattern> (Query4)
- Xylose 0 100 0.000 (Y1)
- <parameters> <min> <max> <format> (Y2)

Pre-treat

- #J#* (J)
- Spline2 [#]X1 [#]X2 [#]X3 1050 1650 10 (Treat1)
- Spline2 [#]X1 [#]X2 [#]X3 450 850 10 1050 1650 10 (Treat2)
- Spline2 [#]X1 [#]X2 [#]X3 450 850 10 (Treat3)
- NOIMAGE (Treat4)
- SNVT (Treat5)
- <new treatment> (Treat6)

Wavelengths

SNRs

WLs

Means

Targets

OSC

Variations

Model

- XLS (Type)
- 3 (Validate)
- 5 (Cross/Validate)
- 9 (Factors)
- 2.5 3.5 (AutoDelete)
- (Delete)
- <new deletes> (Delete2)
- Auto, True.Xylose,12.0166,12.0230 (Delete3)
- <new deletes> (Delete4)
- Auto, True.Xylose,12.0019,12.0102 (Delete5)
- Auto, True.Xylose,12.0020,12.0186,12.0021 (Delete6)
- <new deletes> (Delete7)
- Auto, True.Xylose,12.0022,12.0115,12.0180 (Delete8)

Wavelengths

Indexes

Dates

IDs

Target

Observations

ObservationsZ

Zero

Center

Weights

Loads

Scale

Regressions

Press

Scores

Scores Scaled

Mahalanobis

Residuals

Bias

Estimates

Skew

100%

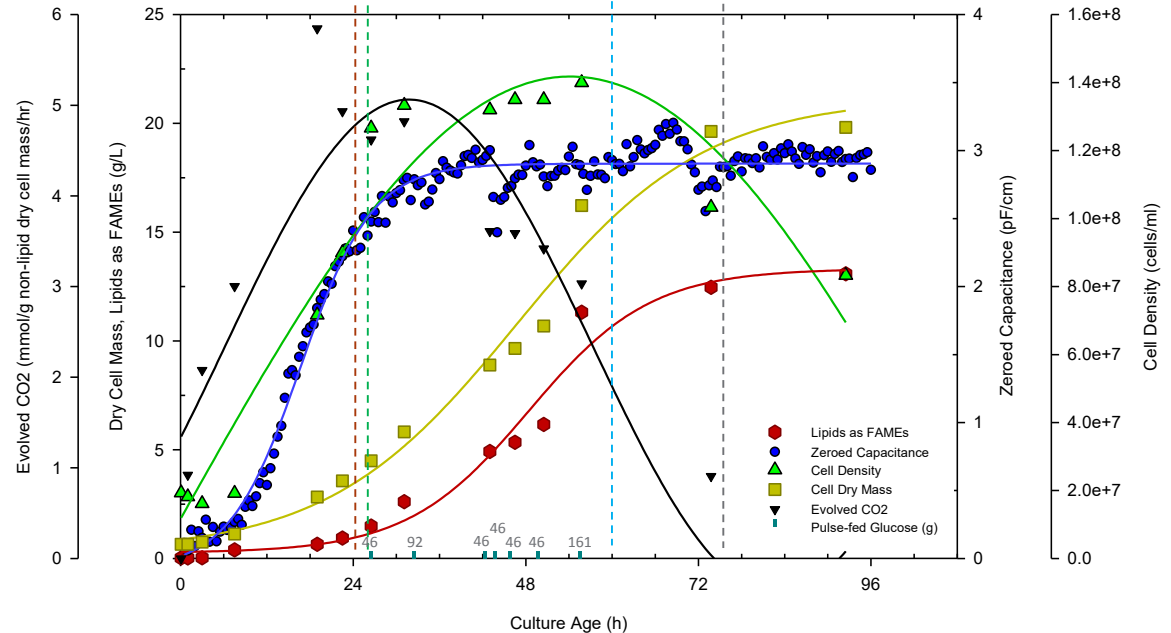
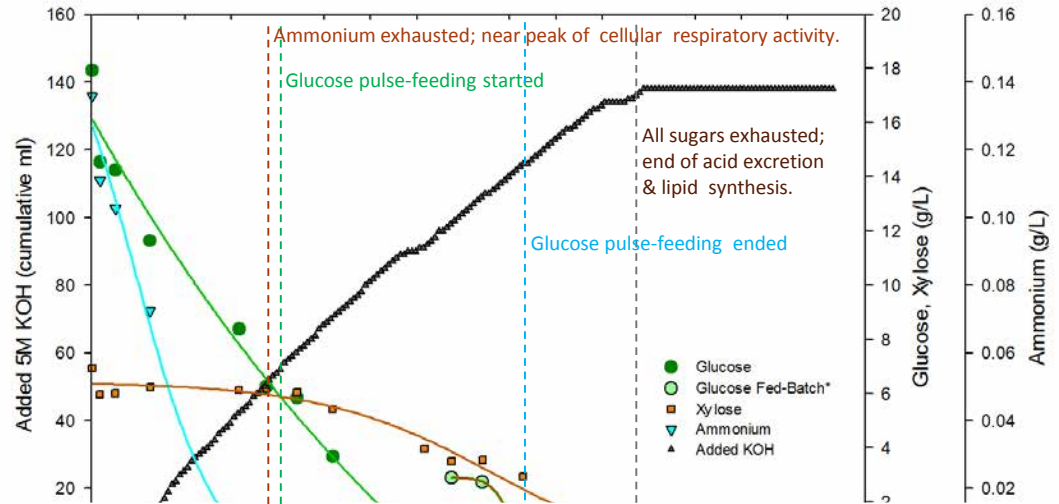
9/30/2015 9:08 PM

Lipomyces starkeyi bioreactor kinetics

Pulsed fed-batch cultivation of *L. starkeyi* on 2% sugar (2:1 glucose/xylose) minimal medium in a 20-liter stirred tank bioreactor. The agitation rate was set at 600 RPM, the pH controlled at 5.5 via addition of 5 M KOH, and the DO maintained at 50% via variable aeration at 0.2-2.5 vvm.

Additional glucose was pulse-fed into the culture after ammonia was exhausted from the medium:

Hours after inoculation	Glucose added (g)
26.50	46
32.50	92
42.25	46
43.75	46
45.75	46
49.75	46
55.50	161
Total	483

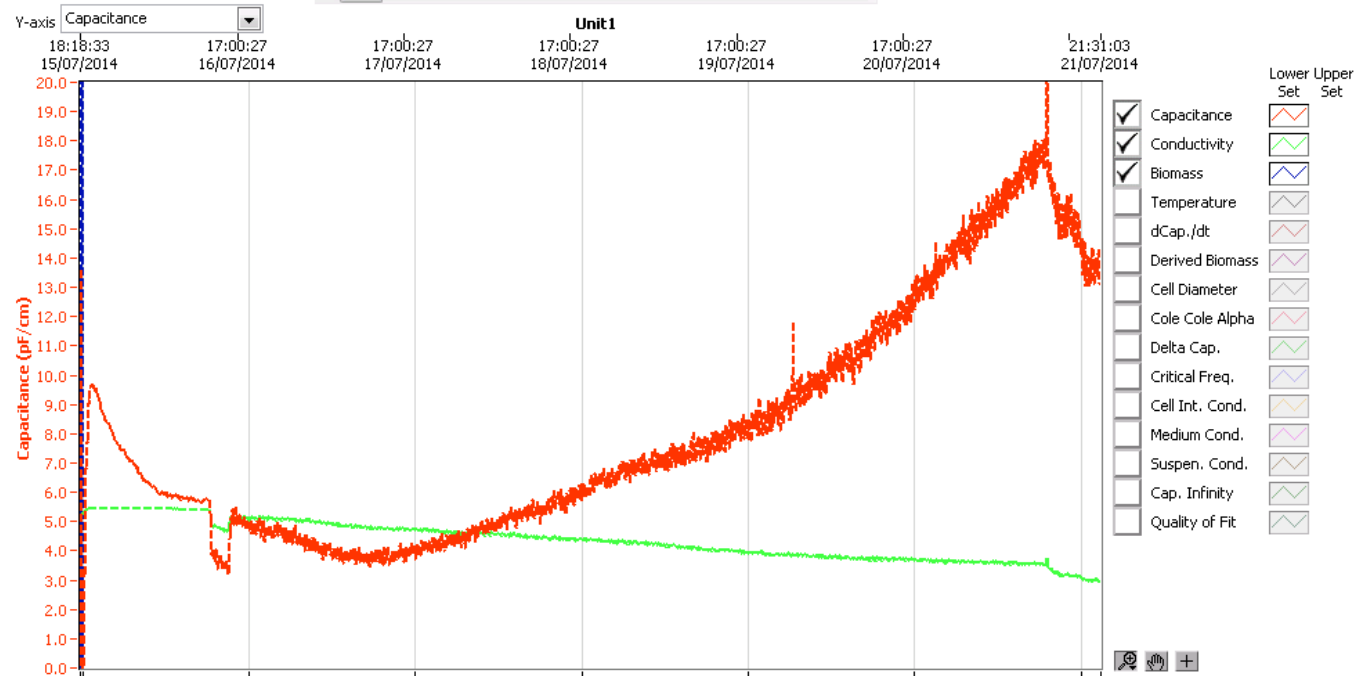


FY14-15 Synthetic Biology FOA Project

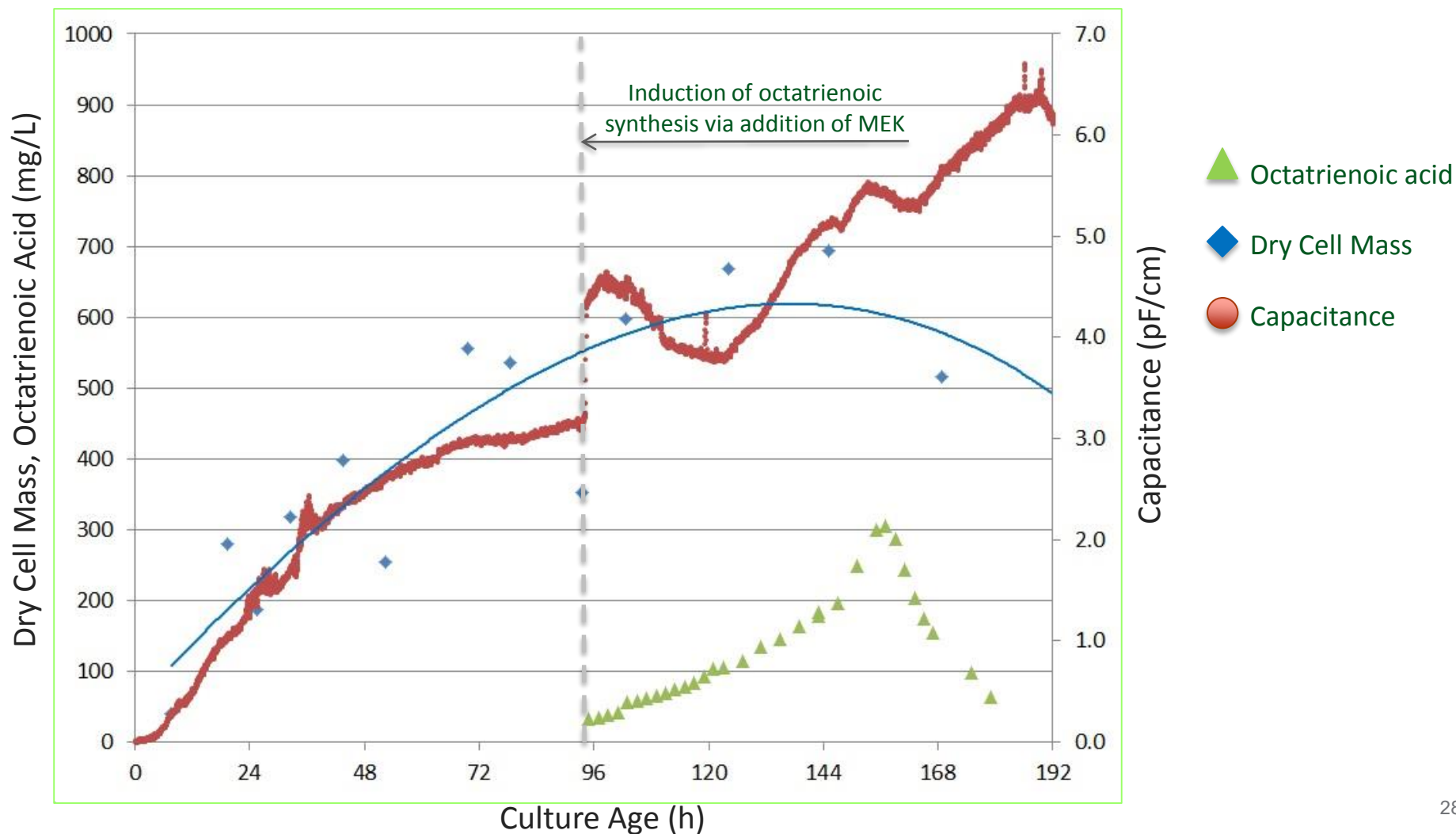
Dielectric spectroscopy tracking of *Aspergillus nidulans* filamentous fungi grown on lactose MM in a 30-liter bioreactor



February 17, 2017

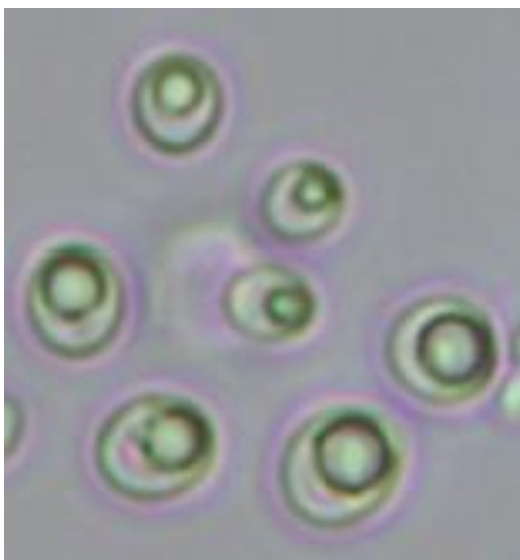


Dielectric spectroscopy tracking of *Aspergillus nidulans* during growth and synthesis of octatrienoic acid on hydrolyzed PCS in a 30-liter bioreactor

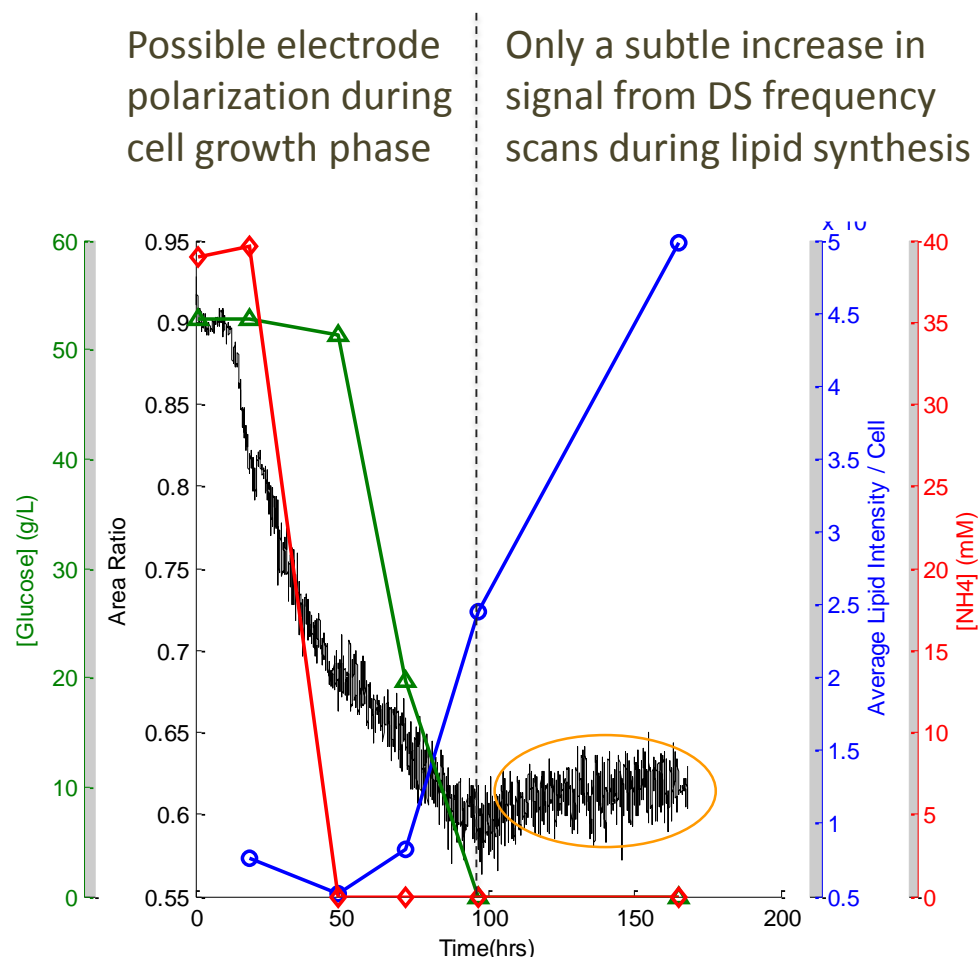


3 – Technical Accomplishments/ Progress/Results

FY15 Stretch Milestone: DS frequency scanning for tracking intracellular lipid content



L. starkeyi cells showing
internal lipid droplets.



Next Step: Compare samples of bioreactor medium with and without cells to **quantify electrode polarization effects** during bioconversion.

DS Frequency Scan Analysis

Total cell mass and its electrical properties are primary factors

Dielectric increment ($\Delta\epsilon$) relates to “amount” of charging

$$\Delta\epsilon = \frac{9PrC_m}{4\epsilon_0}$$

Where:

$\Delta\epsilon$ = Dielectric increment

P = Biomass volume fraction

r = Representative cell radius

C_m = Specific membrane capacitance

ϵ_0 = permittivity of free space (constant)

Critical frequency (f_c) relates to frequency dependence of charging
(stemming from electric characteristics of the population)

$$f_c = \frac{1}{2\pi r C_m (1/\sigma_i + 1/2\sigma_m)}$$

Where:

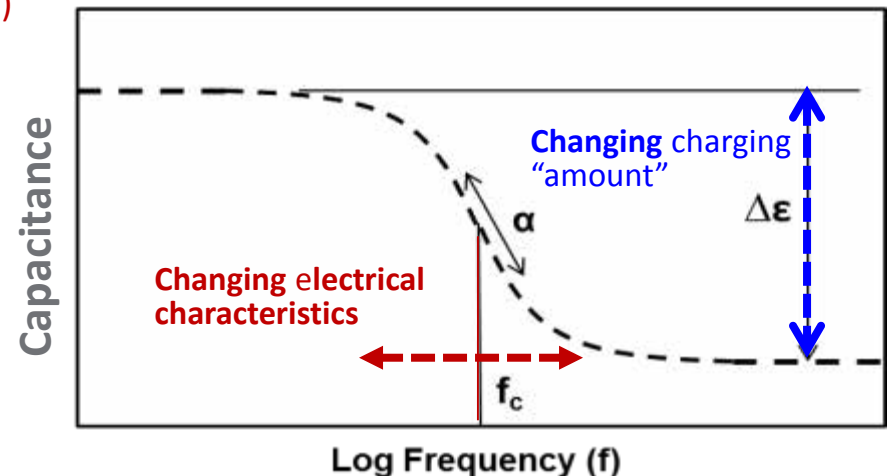
f_c = Critical frequency

σ_i = Intracellular conductivity

σ_m = Medium conductivity

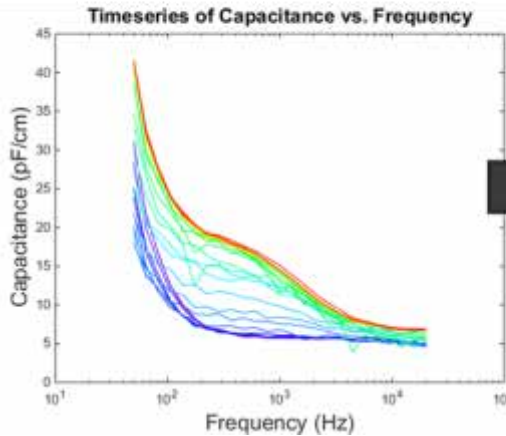
r = Representative cell radius

C_m = Specific membrane capacitance

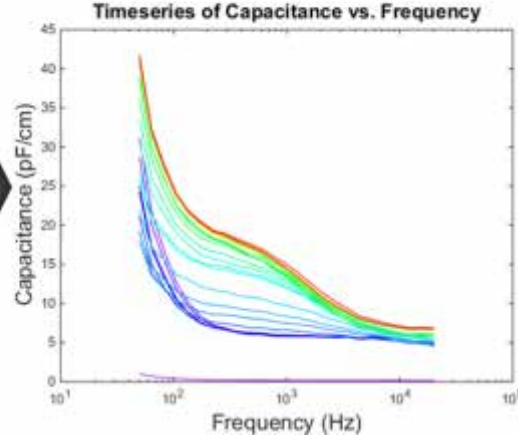


DS Frequency Scan Analysis

Raw Frequency Scans



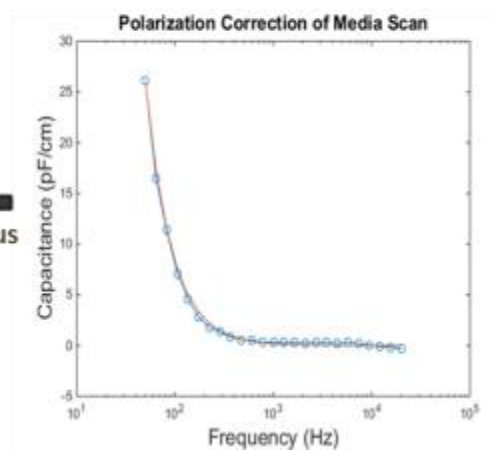
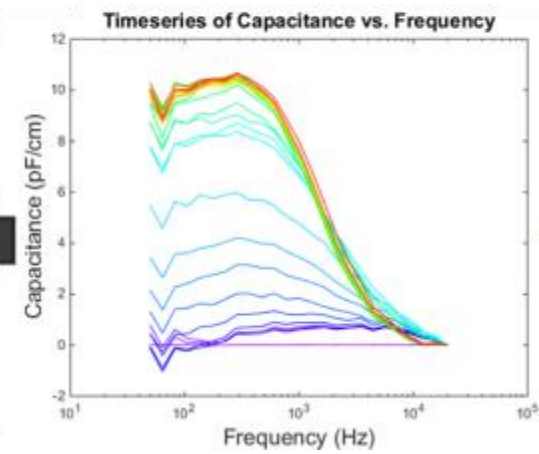
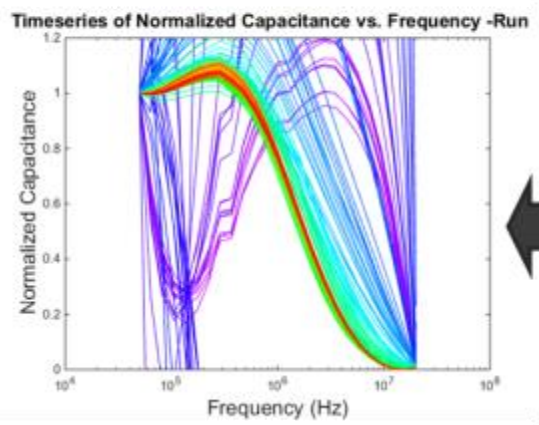
Moving Average Filter (window = 15min)



- Order=3
 - Window=13
- Sazitsky-Golay+ Normalization** • **Cole-Cole Model fits**

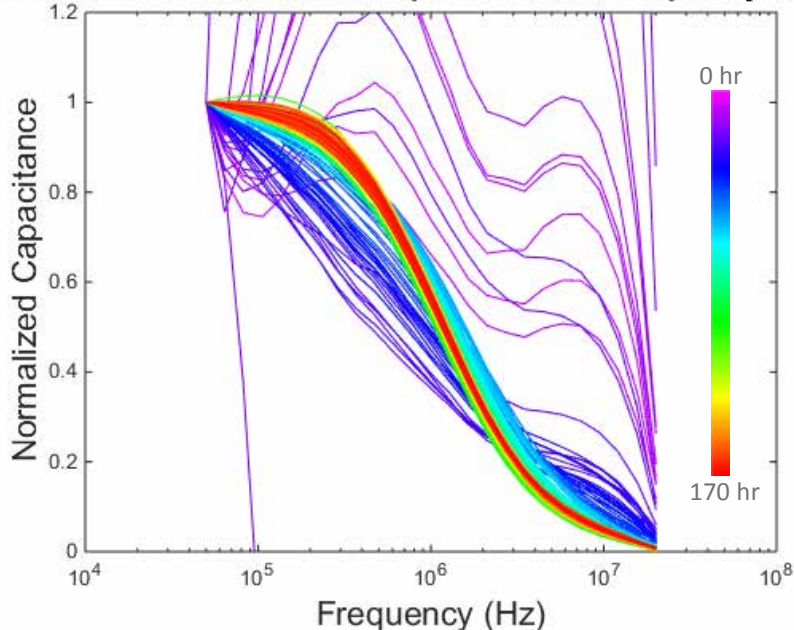
Polarization Correction

- Area Ratio



DS Frequency Scan Analysis

Timeseries of Normalized Capacitance vs. Frequency -Run 3

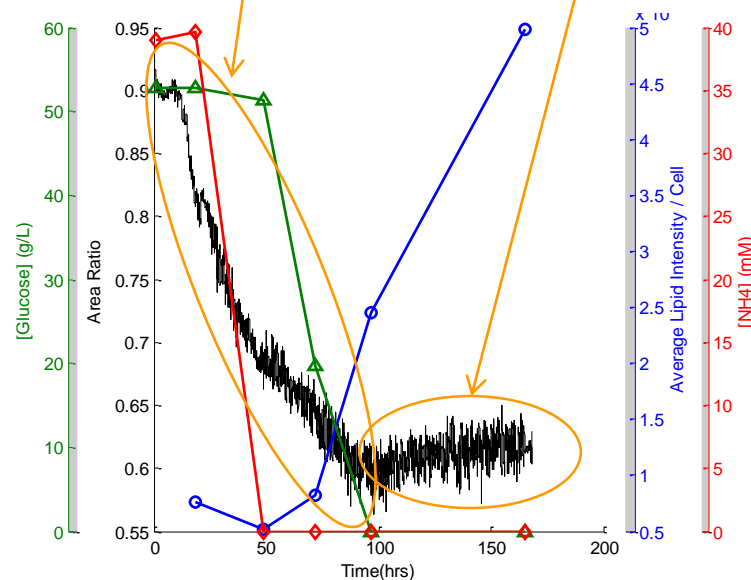


Apply Area
Ratio
Algorithm



Electrode polarization
effects

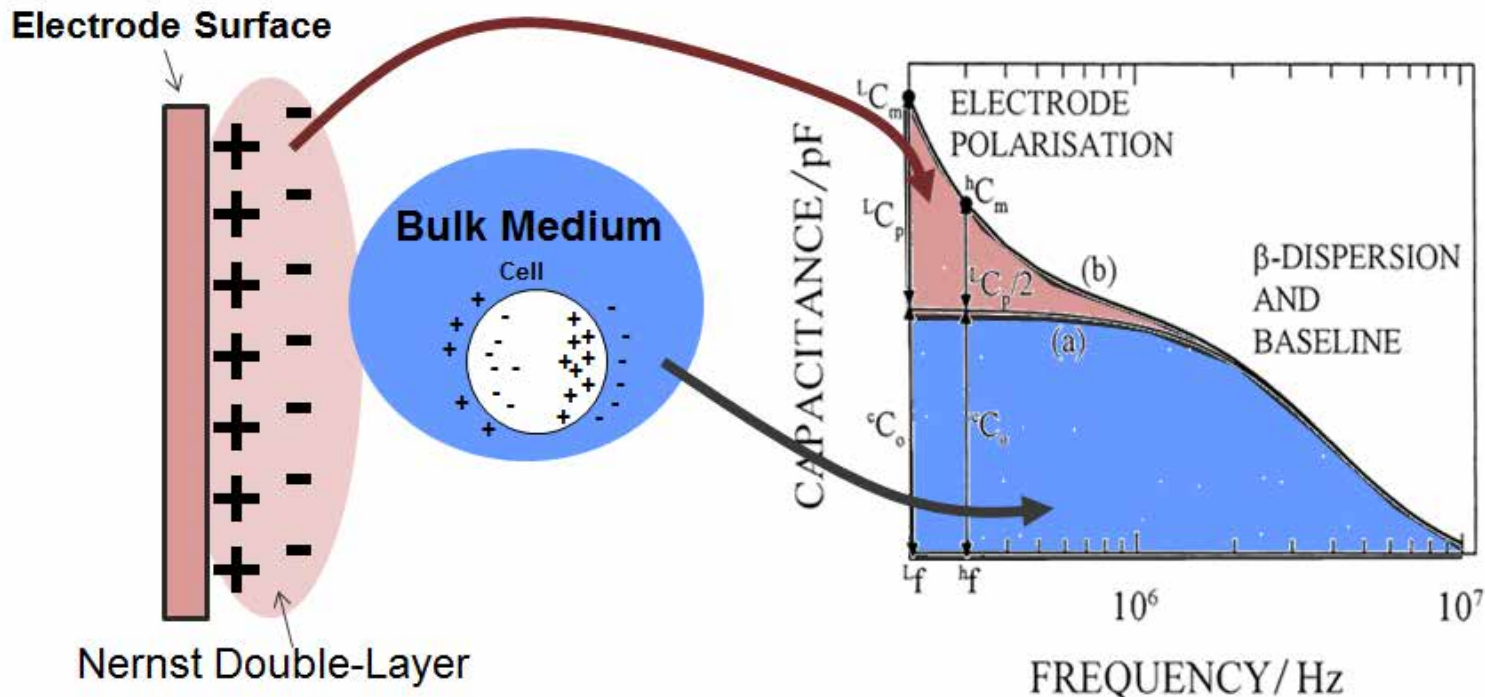
Subtle changes in DS
frequency scans during
lipid synthesis



DS Electrode Polarization Effects



- Electrode polarization stems from the same phenomena producing the dielectric signal for particles
- The effect must be corrected for in order to obtain a meaningful curve



Davey C: The influence of electrode polarisation on dielectric spectra, with special reference to capacitive biomass measurements: (II) Reduction in the contribution of electrode polarisation to dielectric spectra using a two-frequency method. *Bioelectrochemistry Bioenerg* 1998, 46:105–114.

Publications, Patents, Presentations, Awards, and Commercialization

Collett JR, PA Meyer, Y Zhu, ER Hawley, Z Dai, MG Butcher, and JK Magnuson. 2015. "Bioreactor performance data and preliminary biorefinery techno-economics for the production of distillate fuels via bioconversion of pretreated corn stover by *Lipomyces starkeyi*." Accepted for poster presentation at the 37th Symposium on Biotechnology for Fuels and Chemicals, San Diego, CA on April 27, 2015. PNNL-SA-107273.

Collett JR. 2015. "Process Analytical Technologies in the New Bioeconomy for Fuels and Chemicals." Presented by James R. Collett (Invited Speaker) at the Sartorius Upstream/Downstream Technology Forum, Foster City, CA on October 6, 2015. PNNL-SA-113501. Online Near Infrared and

Collett JR, AB Remington, Z Dai, BA Hofstad, MG Butcher, EA Panisko and JK Magnuson. 2017. "Online Near Infrared and Dielectric Spectroscopy for Real-Time Tracking of Critical Process Parameters for Bioconversion of Pretreated Corn Stover." Accepted for oral presentation at the 39th Symposium on Biotechnology for Fuels and Chemicals, San Francisco, CA, CA on May 2, 2017. PNNL-SA-123014.