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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

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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

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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)

ABER™ INSTRUMENTS

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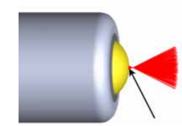
Raman spectroscopy

MARQMETRIX



30-liter Bioreactor

sartorius

Near-infrared spectroscopy (NIRS)

FLOWNAMICS® *Your Bioprocess Sampling Solution*

Automated bioreactor sampling

1 - Project History

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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.

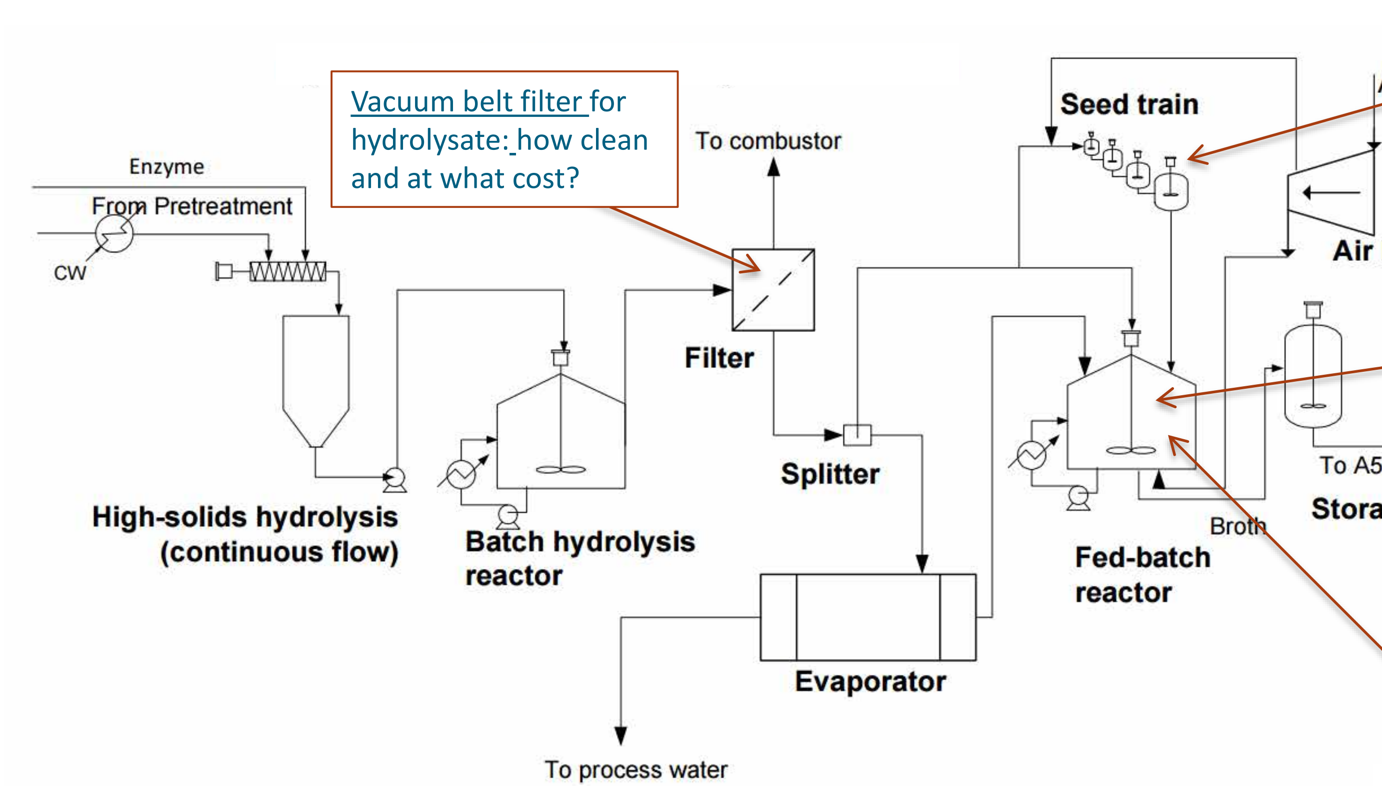
2 – Approach (Management)

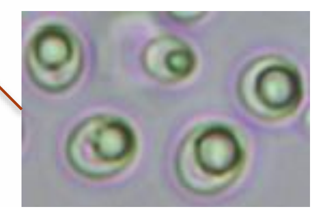
- Standard Project Management Good Practices
  - Statement of work and how it relates to DOE goals
  - AOP Project Management Plan
  - Quarterly milestones
  - Go/No Go decision point
- Frequent project communications
  - Teleconferences with BETO platform leads
  - Quarterly formal reporting to BETO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

Experimental Context:

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

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





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

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

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

2 – Approach (Technical)

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Bioconversion optimization challenges in fed-batch cultivation of oleaginous yeast

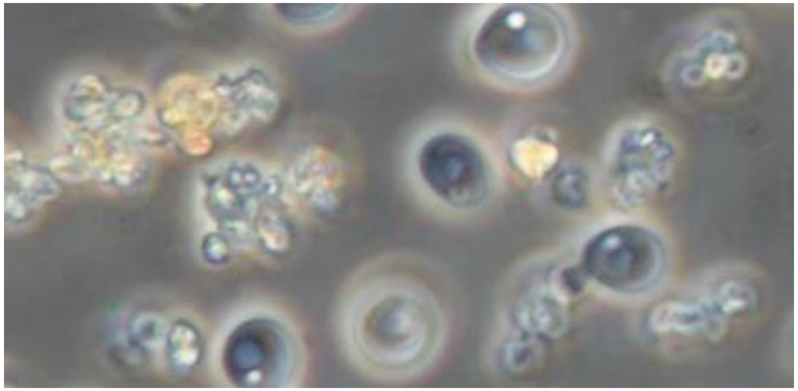
- **Nitrogen must be limited** to induce high-yield lipid synthesis.
- **Plant nitrogen in hydrolysate feed stream** may inhibit lipid synthesis.
- Microbial **consumption of glucose vs. xylose must be balanced**.
- Potential **accumulation of lignin solids and metabolic inhibitors** over time.
- **Lot-to-lot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

Table 1. Corn Stover Compositional Variation*

Parameter	*Minimum*	*Maximum*
Ash	0.8%	6.6%
Xylan	14.8%	22.7%
Lignin	11.2%	17.8%

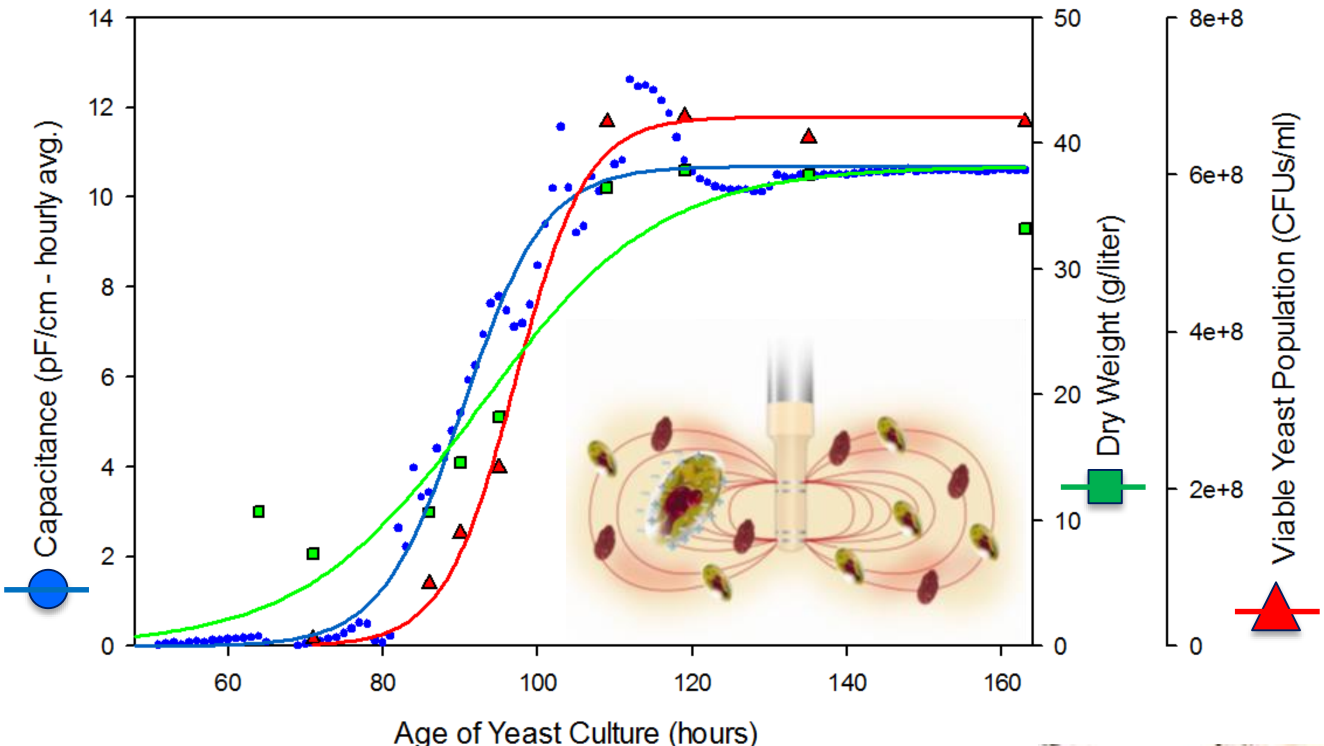
*500 samples were taken from 47 locations over three years

***Cellulose*, 2009. 16(4): p. 621-639**



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

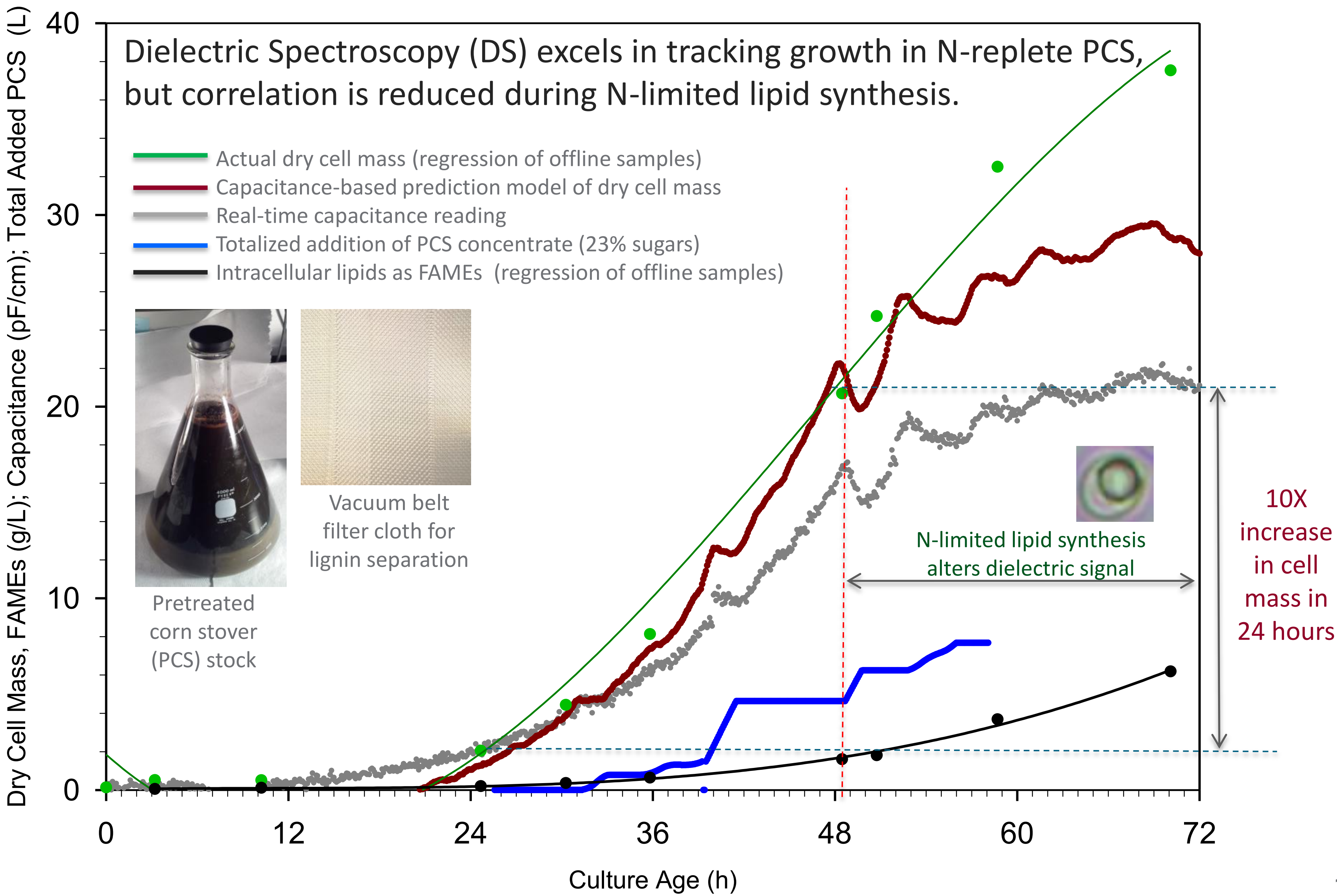
3 – Technical Progress
Dielectric Spectroscopy in high-solids hydrolysate



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.

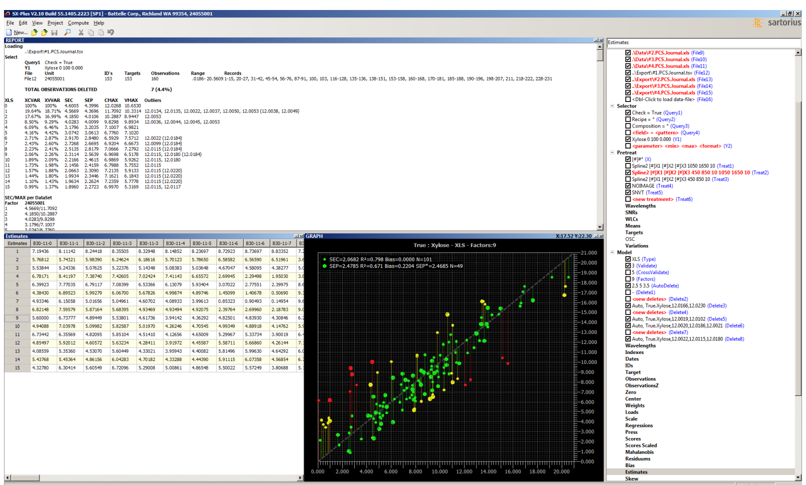
DS also showed promise in tracking cell mass in cultivations of filamentous fungi on PCS.

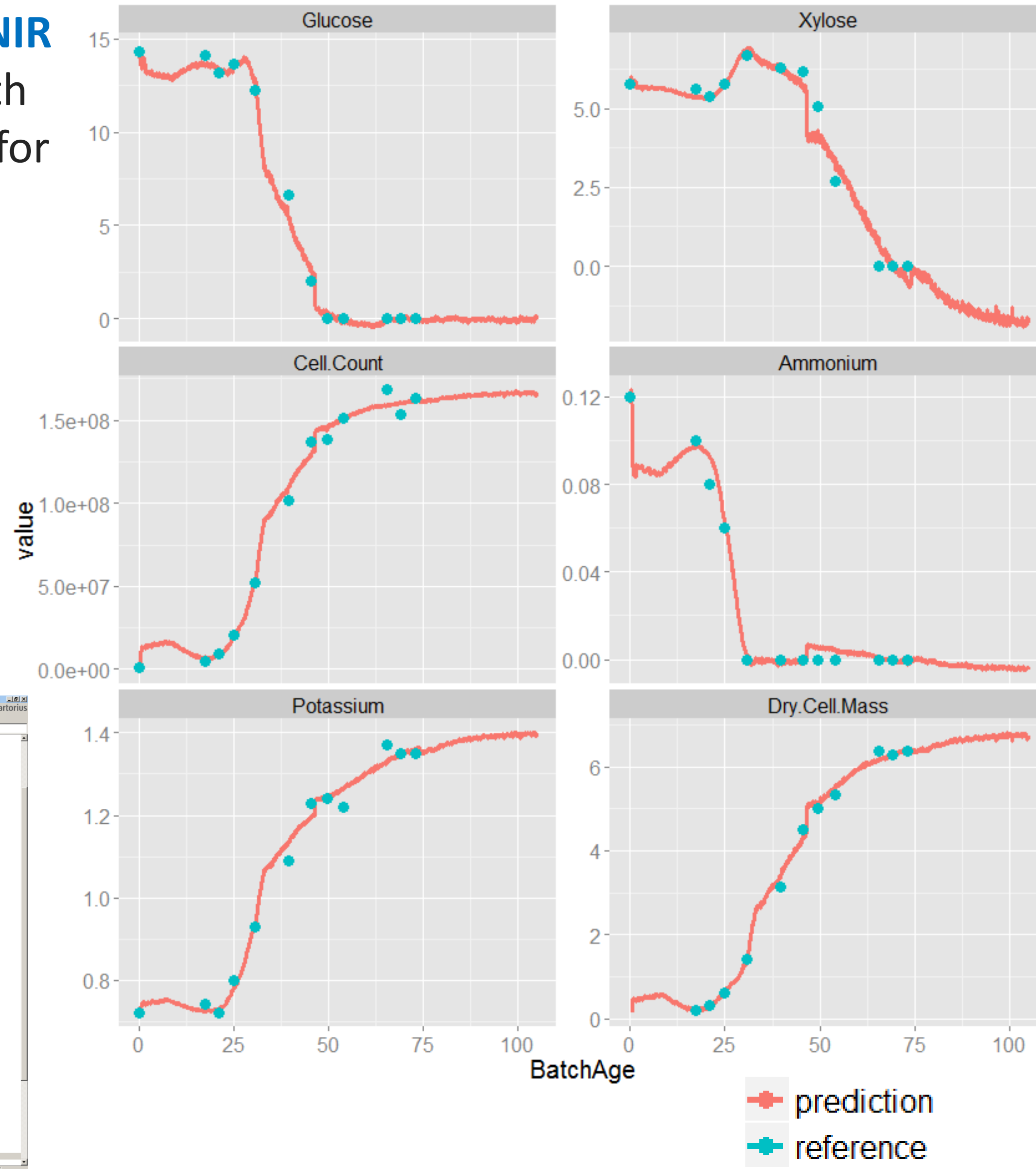

Dielectric Spectroscopy (DS) excels in tracking growth in N-replete PCS, but correlation is reduced during N-limited lipid synthesis.
Actual dry cell mass (regression of offline samples)
Capacitance-based prediction model of dry cell mass
Real-time capacitance reading
Totalized addition of PCS concentrate (23% sugars)
Intracellular lipids as FAMEs (regression of offline samples)
Pretreated corn stover (PCS) stock
Vacuum belt filter cloth for lignin separation
N-limited lipid synthesis alters dielectric signal
10X increase in cell mass in 24 hours
Dry Cell Mass, FAMEs (g/L); Capacitance (pF/cm); Total Added PCS (L)
Culture Age (h)
0
10
20
30
40
0
12
24
36
48
60
72

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

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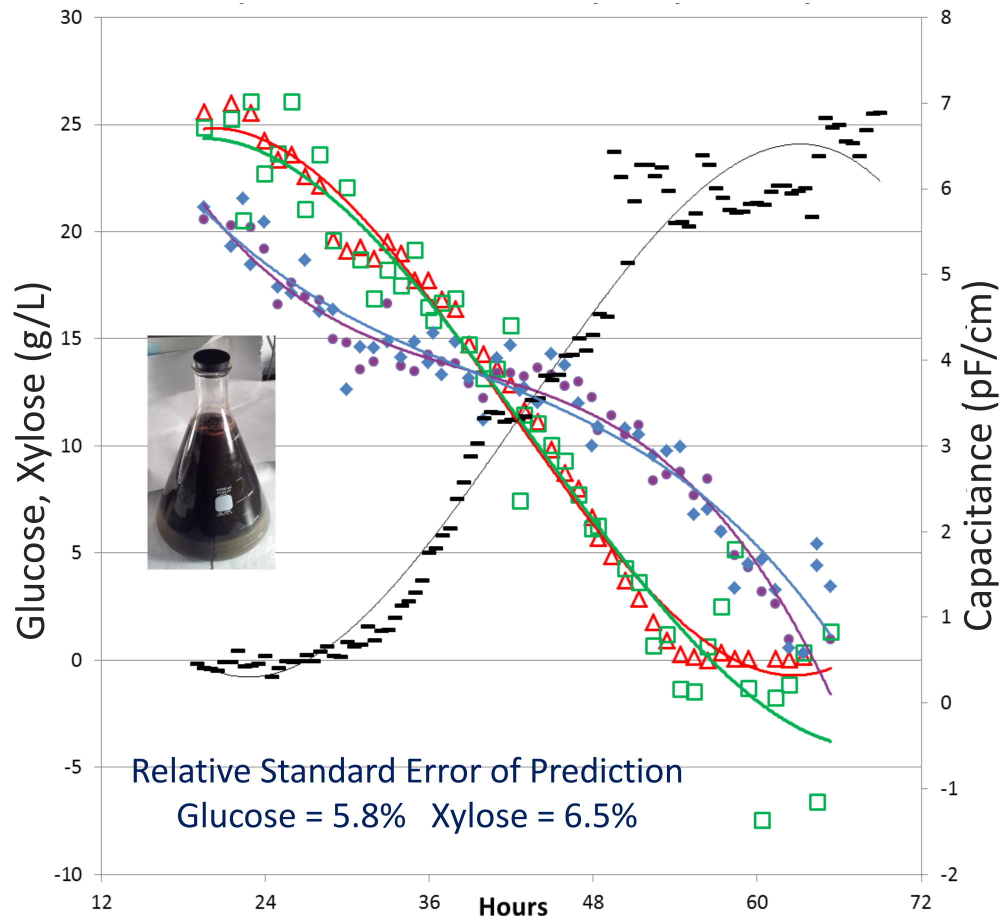
Flownamics 8000i system for high-frequency, 24-hour, cell-free sampling.

Compete.
Council on Competitiveness

Flownamics was featured at the **2015 American Energy Manufacturing Competiveness 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

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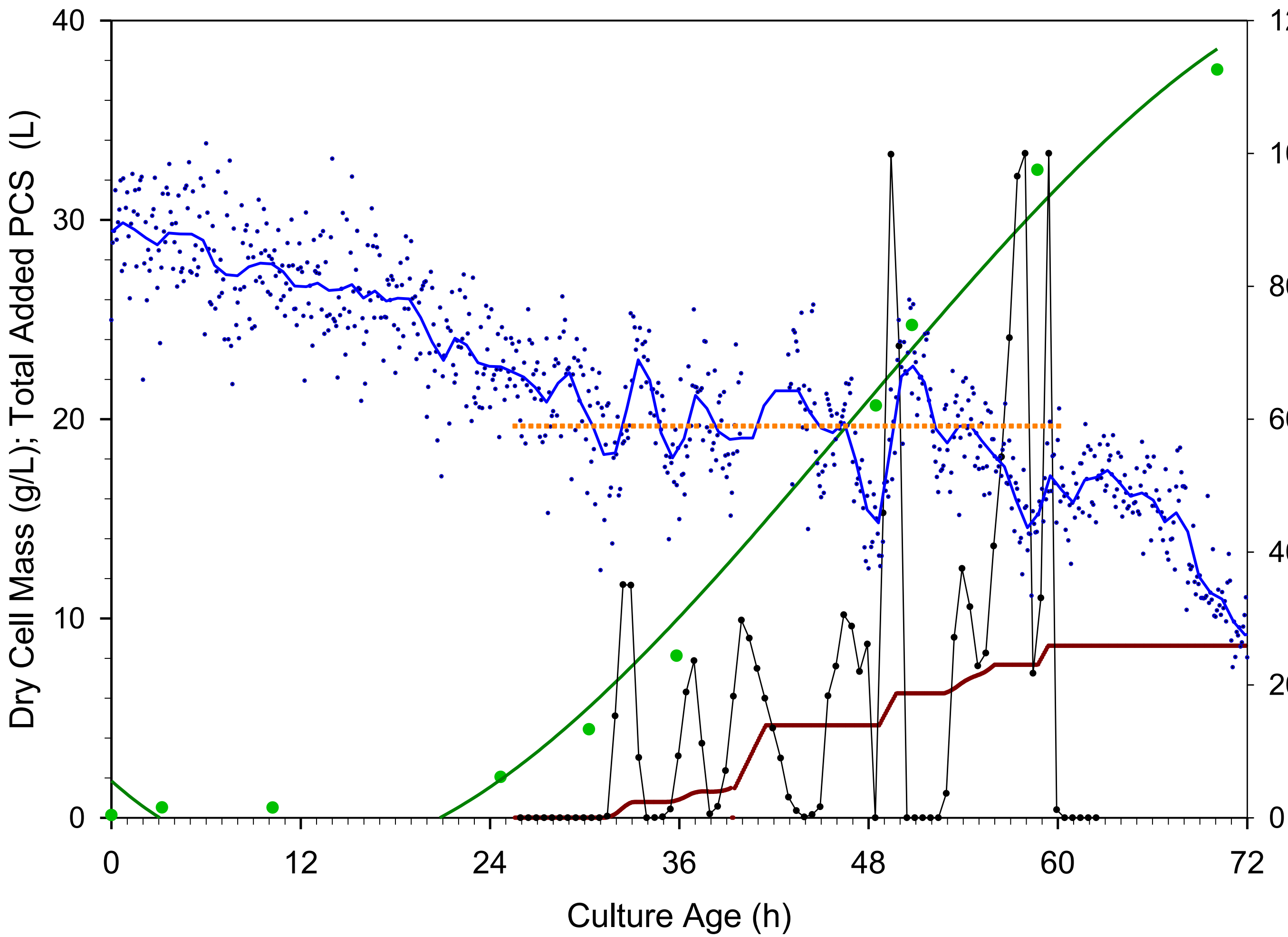
20-liter bioreactor cultivation of *Lipomyces starkeyi* on pretreated corn stover hydrolysate.



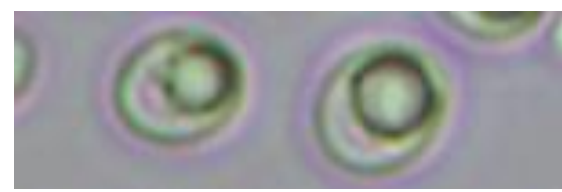
NIRS prediction of glucose concentration (moving average)
Set point of glucose PID control loop
PID Controller Response (% of max output)
Totalized addition of PCS concentrate (22.5% sugar content)
Dry Cell Mass

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.

MARQMETRIX



L. Starkeyi cells with large, intracellular lipid droplets.

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

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

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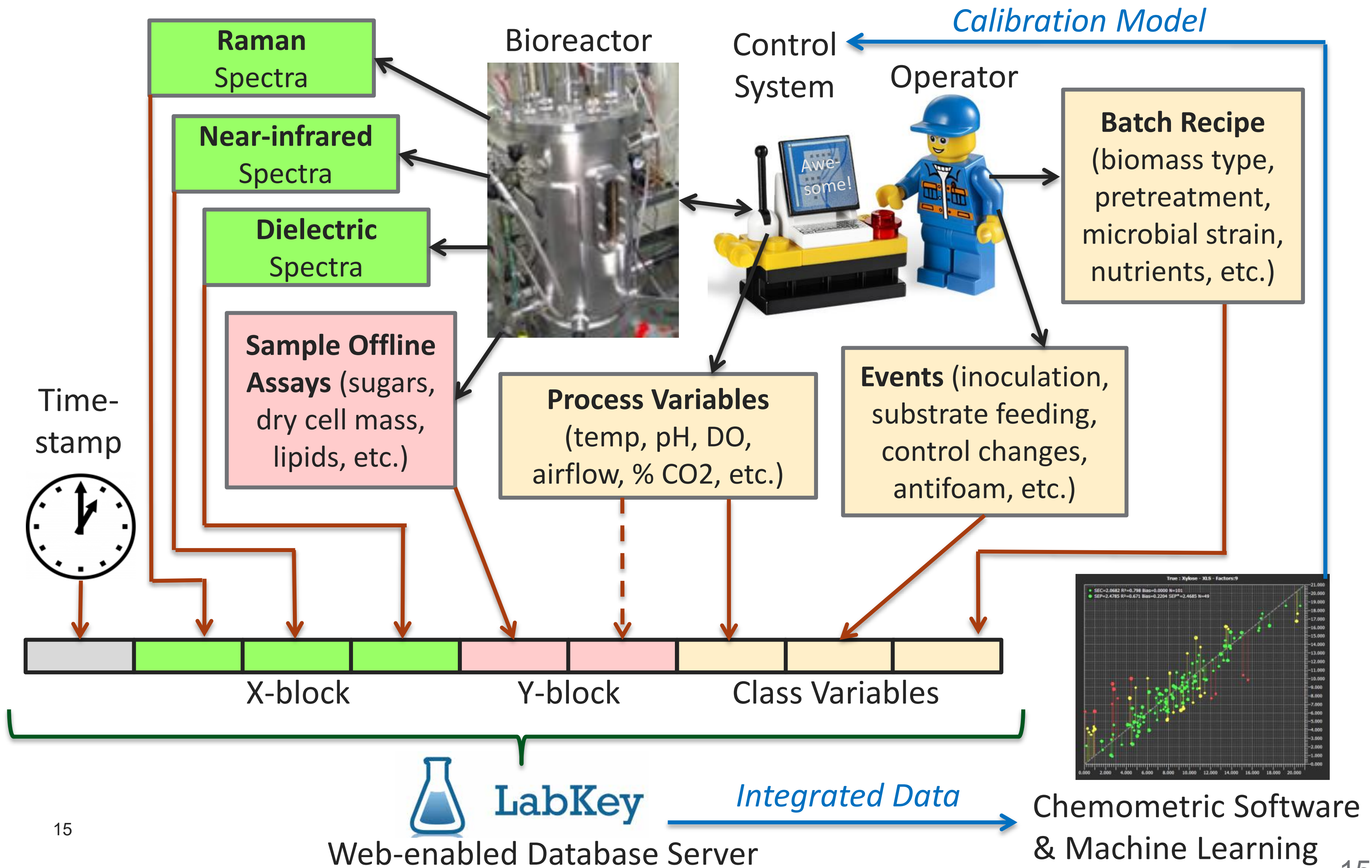

4 – Relevance

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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**.

Summary

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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

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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

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Additional Slides

Laboratory SCADA Integration Plan

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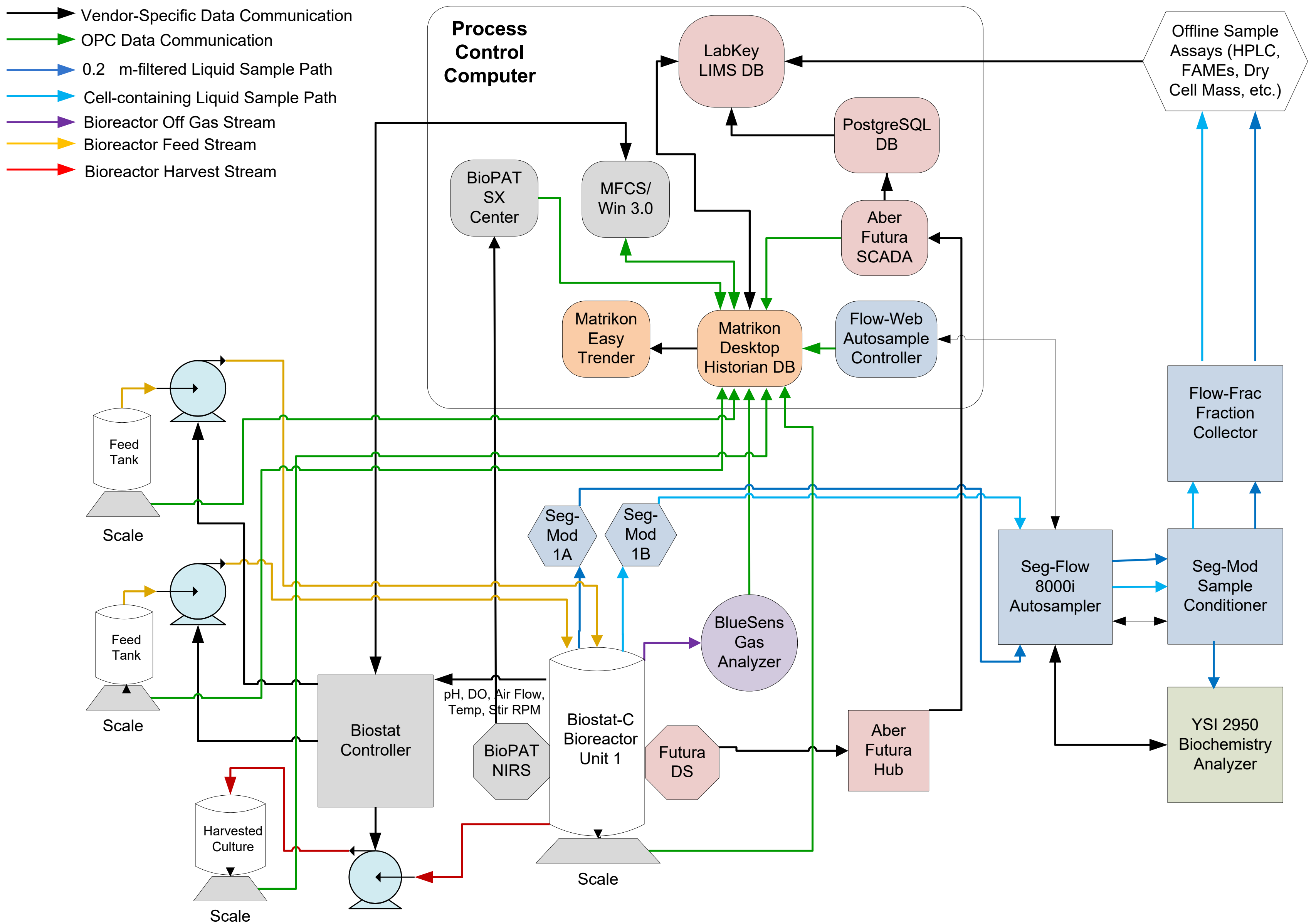
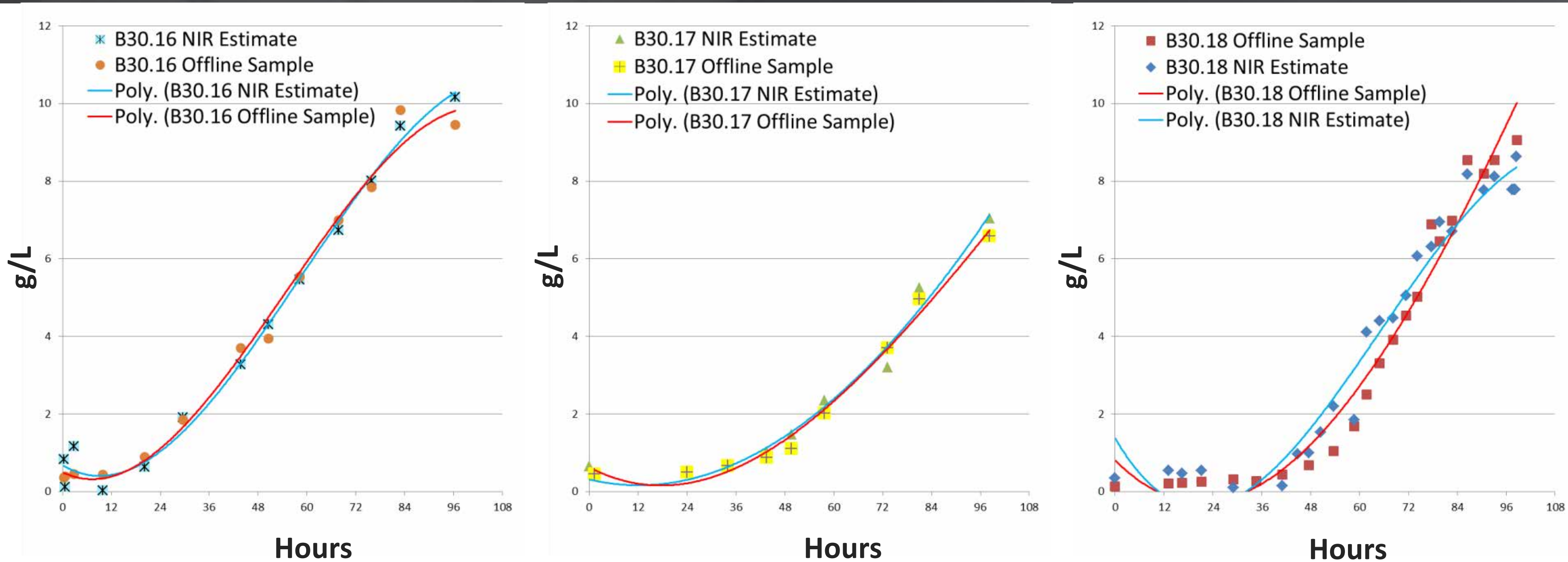


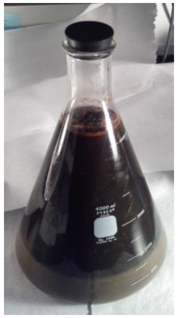
NIRS tracks cell mass growth in PCS



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

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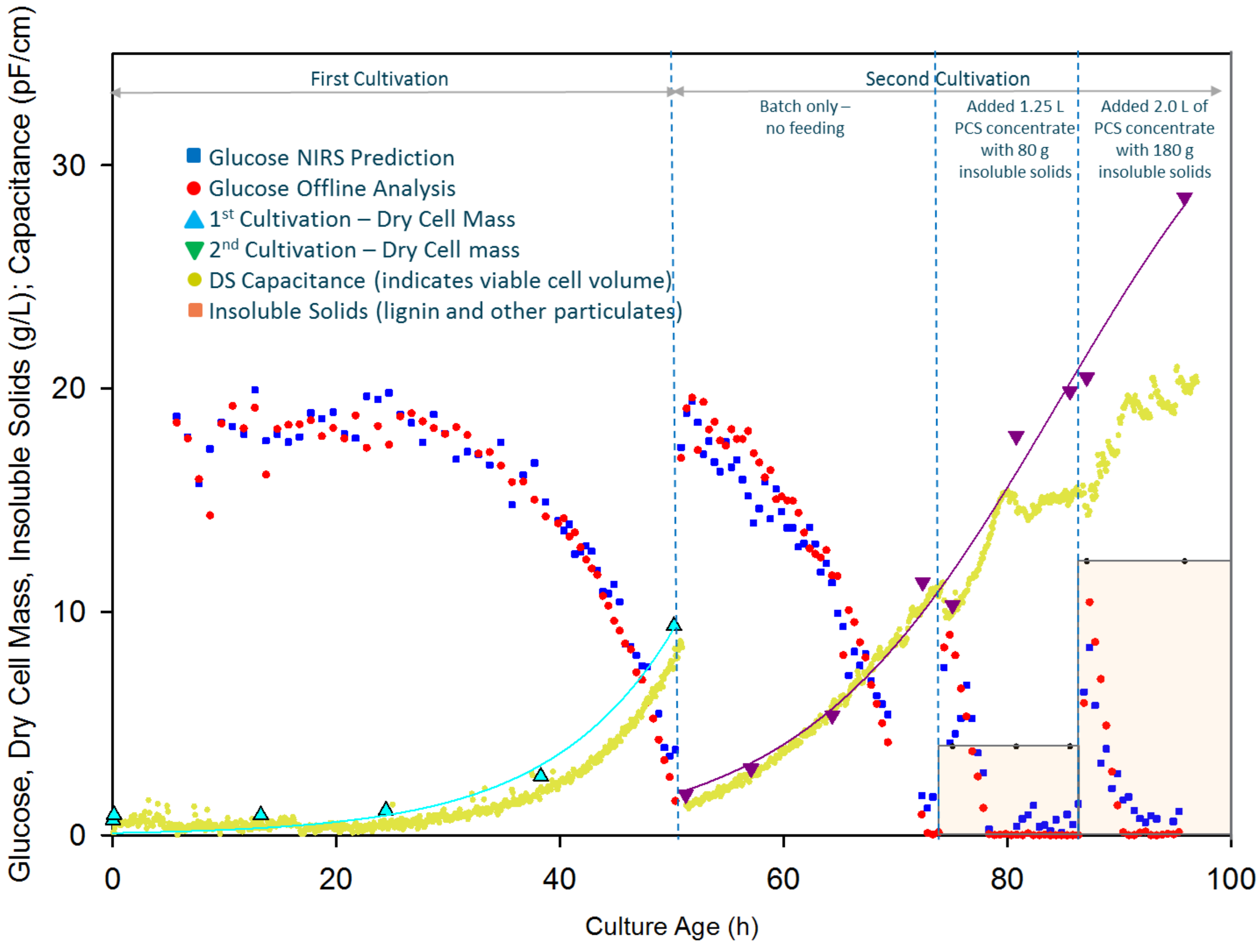

NIRS enables automated fed-batch control of bioconversion of PCS hydrolysate

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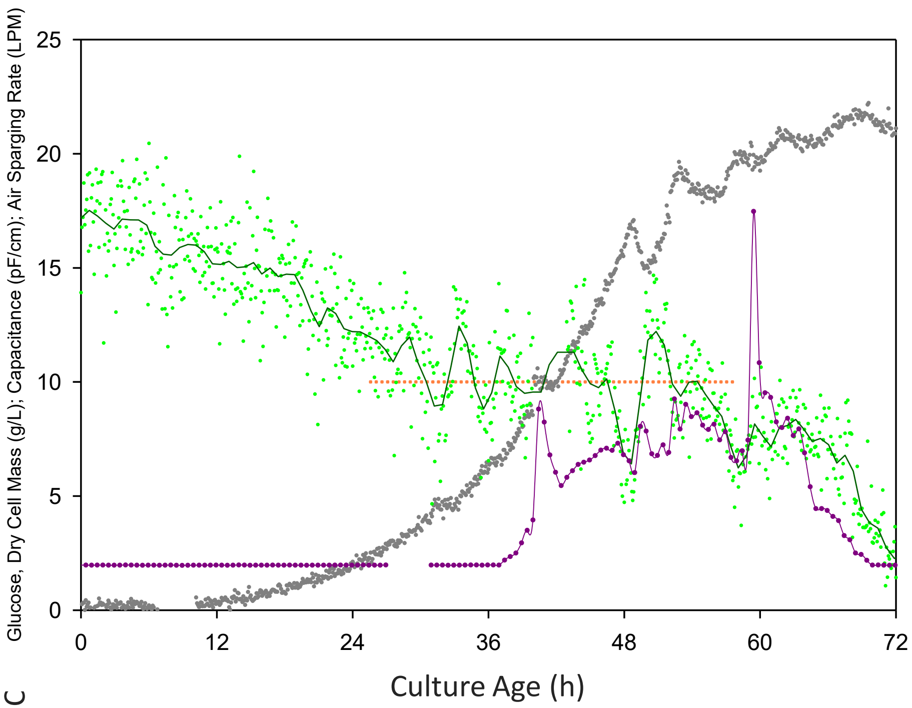
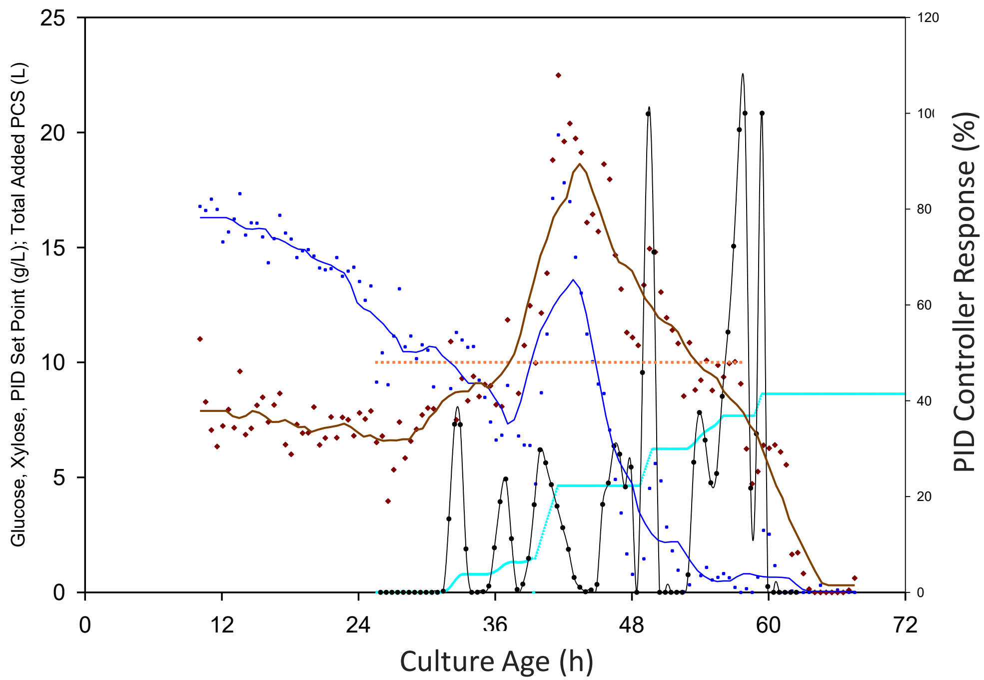
Near-infrared Spectroscopy (NIRS) enabled **PID control of automated, fed-batch cultivation** of *Lipomyces starkeyi* on PCS hydrolysate in a 30-liter bioreactor. Further refinement of chemometric models for glucose and xylose will facilitate tuning of PID control of fed-batch operations.

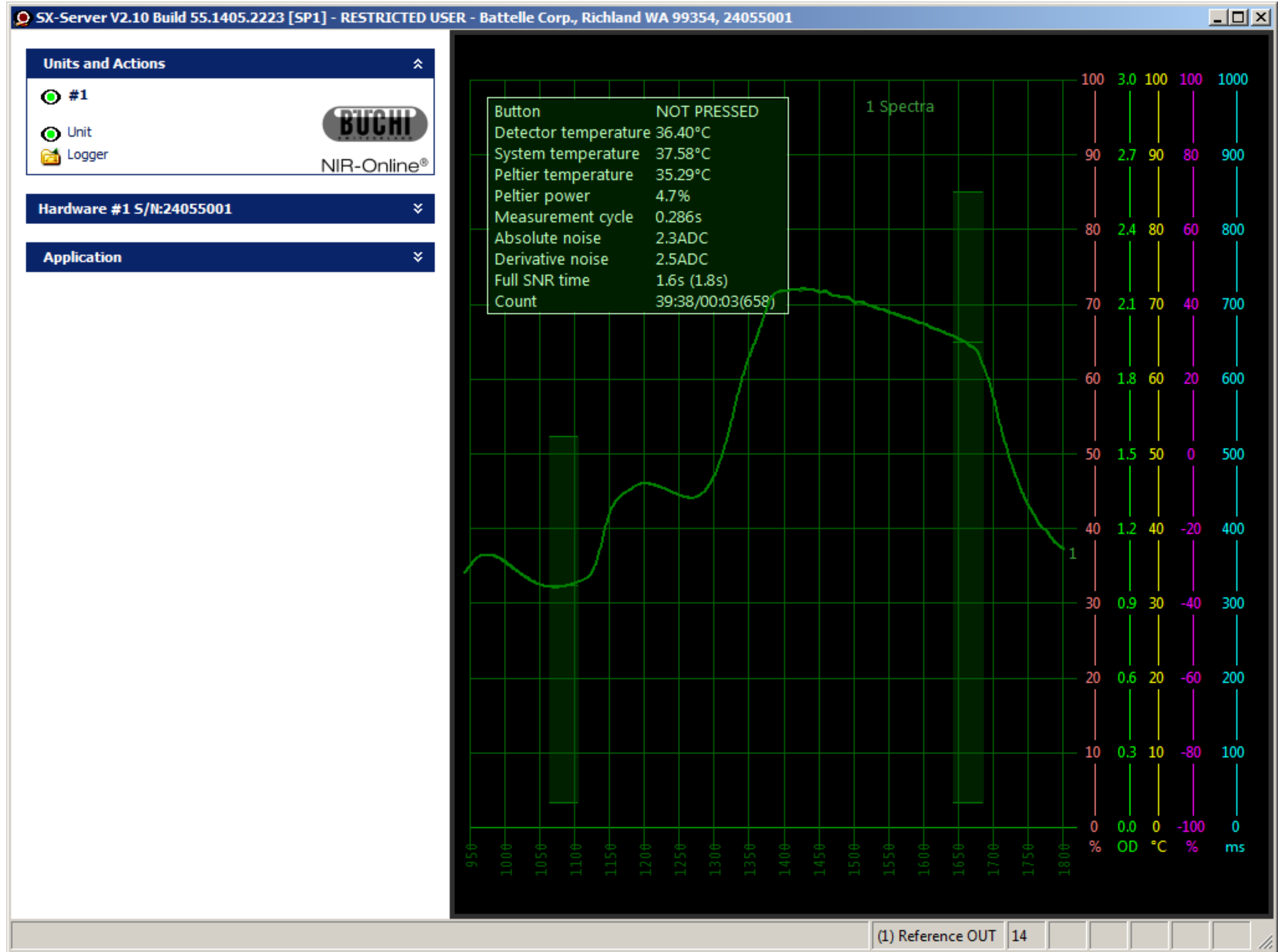


- NIRS prediction of glucose concentration (regression line)
- Set point of glucose PID control loop
- Capacitance
- Air Sparging Rate

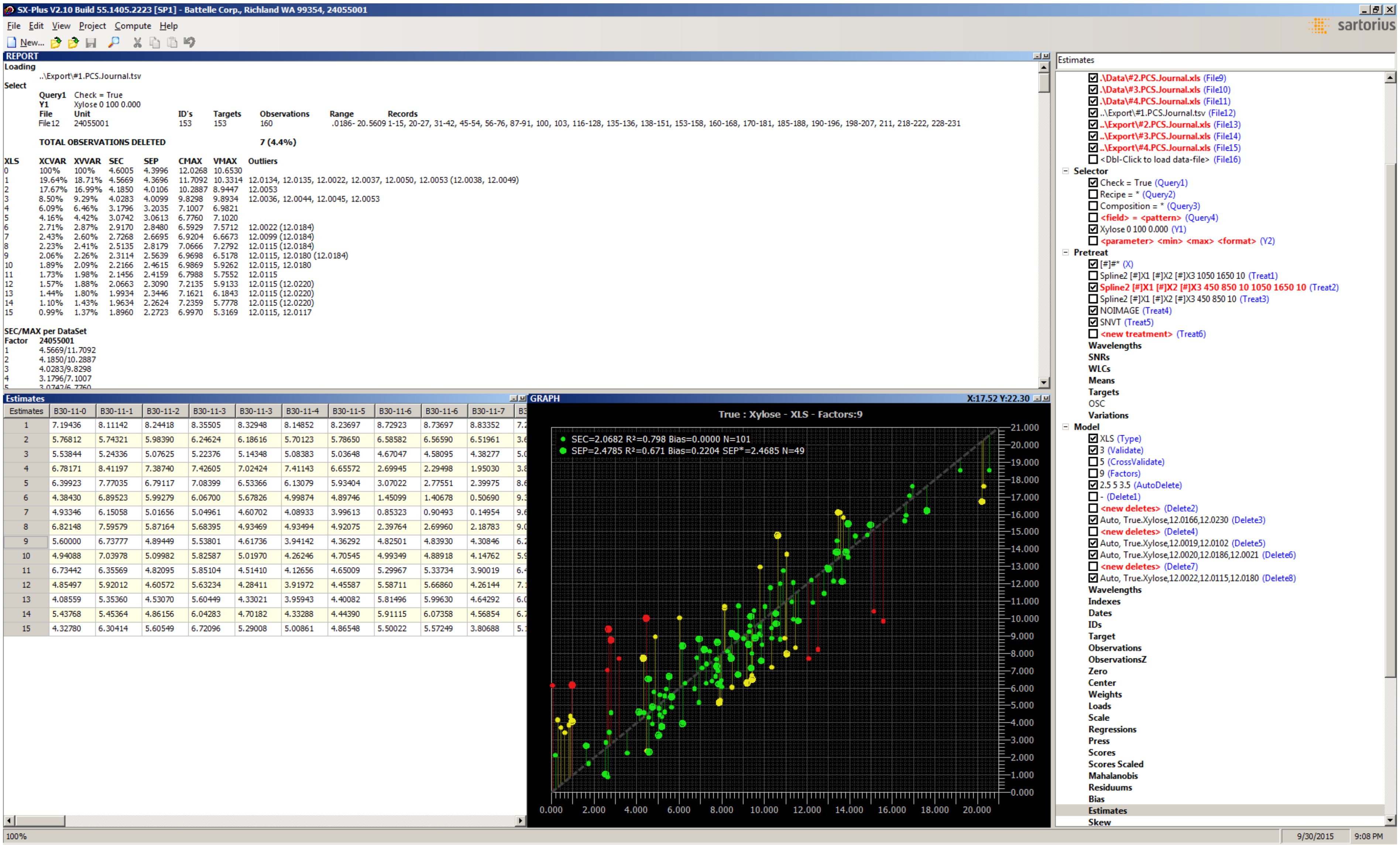


- Offline measures of glucose from autosampler samples
- Offline measures of xylose from autosampler samples
- Set point of glucose PID control loop
- Totalized addition of PCS concentrate
- PID controller response (% of max output)

Sartorius SX-Server



SX-Plus for NIR Calibration Model Development



Lipomyces starkeyi bioreactor kinetics

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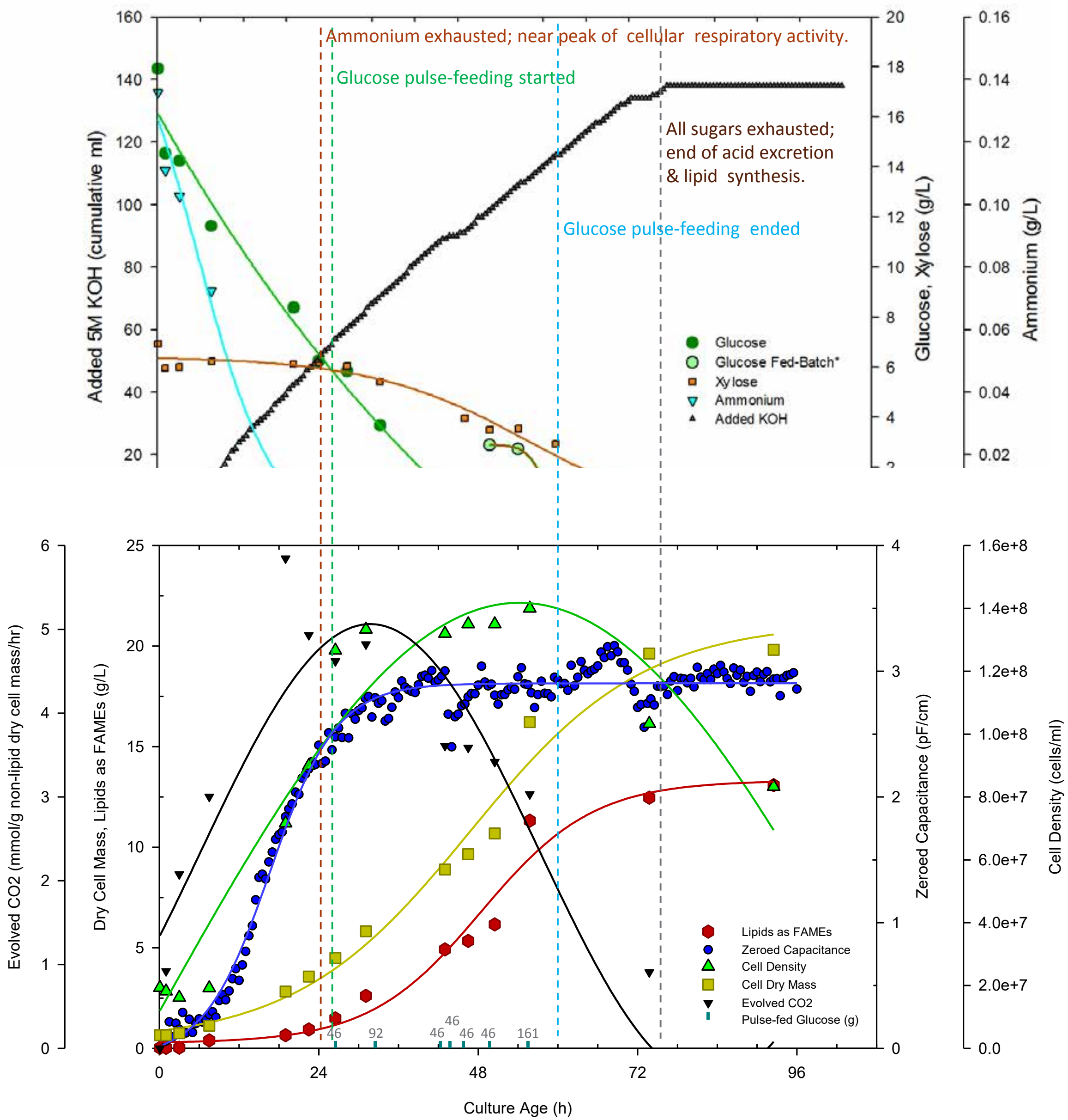
Pulsed fed-batch cultivation of *L. starkeyi* on 2% sugar (2:1 glucose/xylose) minimal medium in a 20-liter stirred tank bioreactor. The agitation rate was set at 600 RPM, the pH controlled at 5.5 via addition of 5 M KOH, and the DO maintained at 50% via variable aeration at 0.2-2.5 vvm.

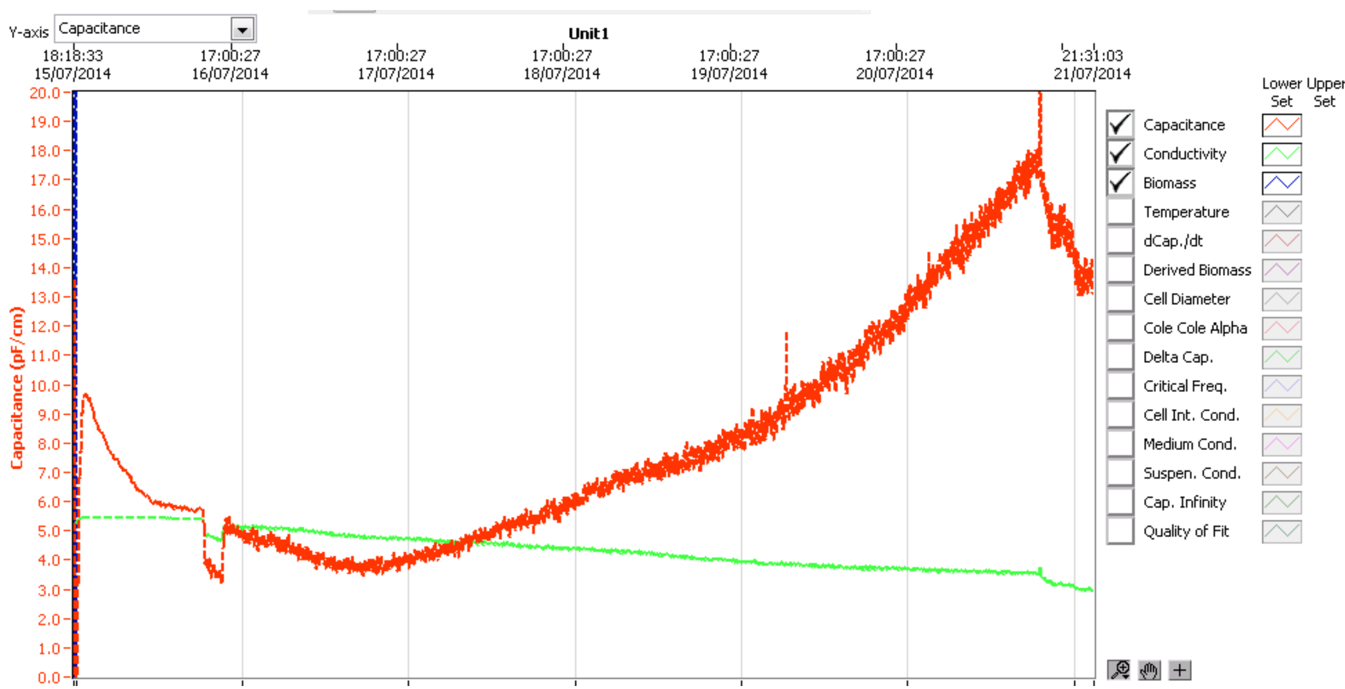
Additional glucose was pulse-fed into the culture after ammonia was exhausted from the medium:

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



FY14-15 Synthetic Biology FOA Project

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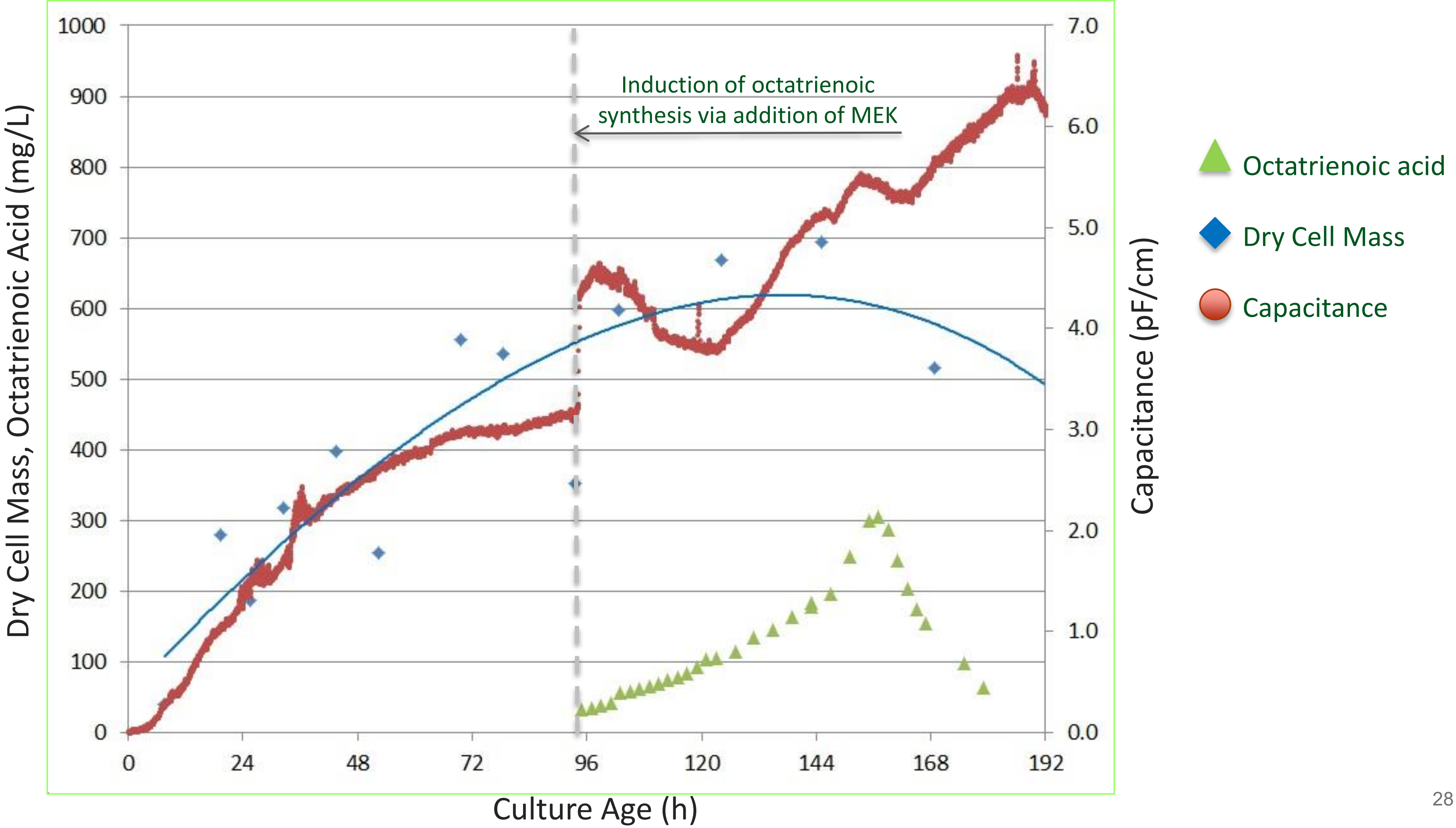
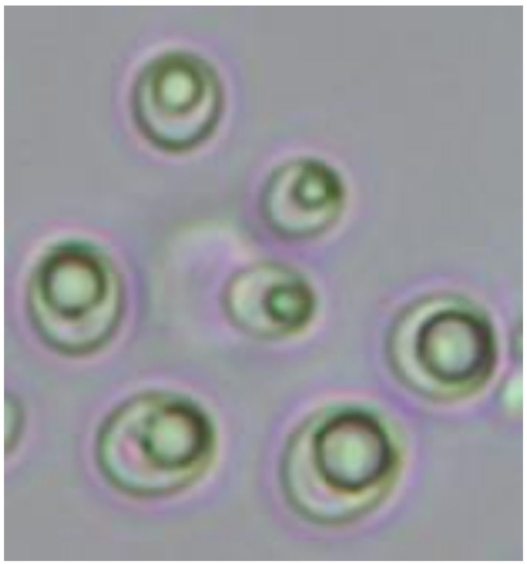
Dielectric spectroscopy tracking of *Aspergillus nidulans* during growth and synthesis of octotrienoic acid on hydrolyzed PCS in a 30-liter bioreactor



3 – Technical Accomplishments/ Progress/Results

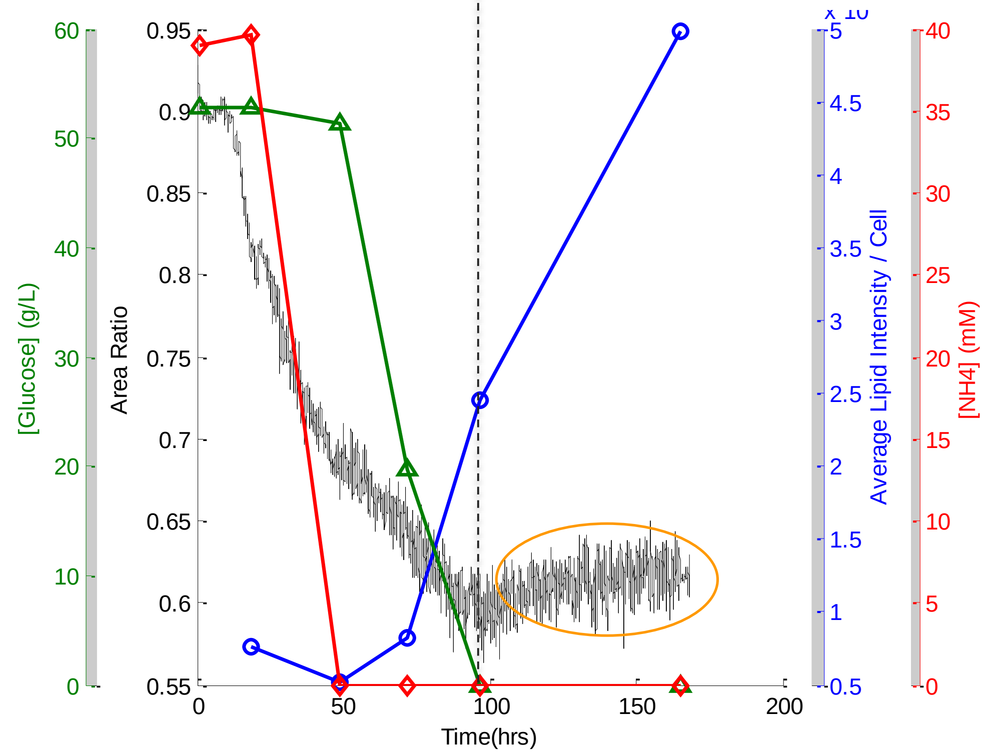
FY15 Stretch Milestone: DS frequency scanning for tracking **intracellular lipid content**



L. starkeyi cells showing internal lipid droplets.

Possible electrode polarization during cell growth phase

Only a subtle increase in signal from DS frequency scans during lipid synthesis



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

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DS Frequency Scan Analysis

Total cell mass and its electrical properties are primary factors

Dielectric increment (Δε) relates to “amount” of charging

$$\Delta\varepsilon = \frac{9PrC_m}{4\varepsilon_0}$$

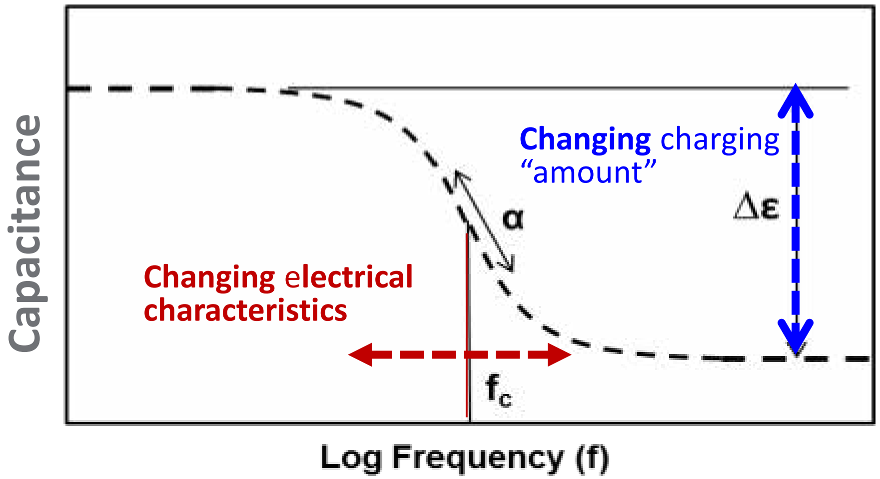
Where:
Δε = Dielectric increment
P = Biomass volume fraction
r = Representative cell radius
C_m=Specific membrane capacitance
ε_0=permittivity of free space (constant)

Critical frequency (fc) relates to frequency dependence of charging

(stemming from electric characteristics of the population)

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

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

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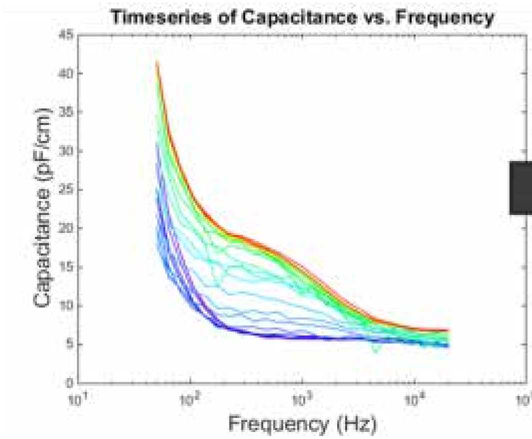
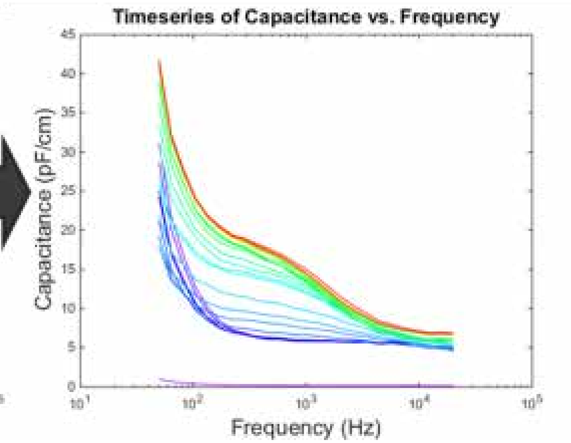
Raw Frequency Scans

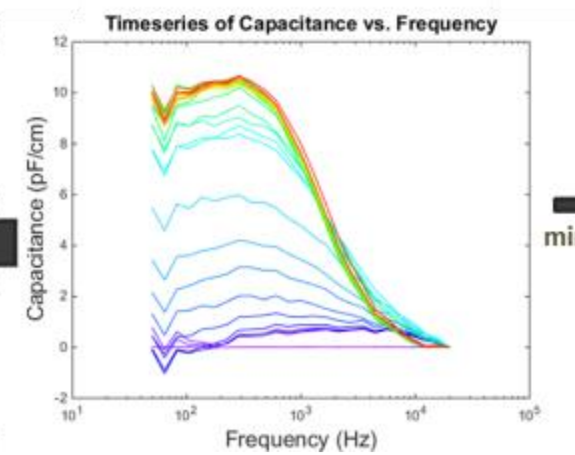


Moving Average Filter (window = 15min)

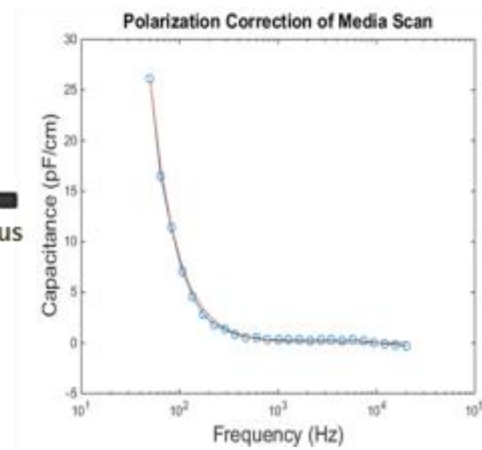


- Area Ratio

Polarization Correction



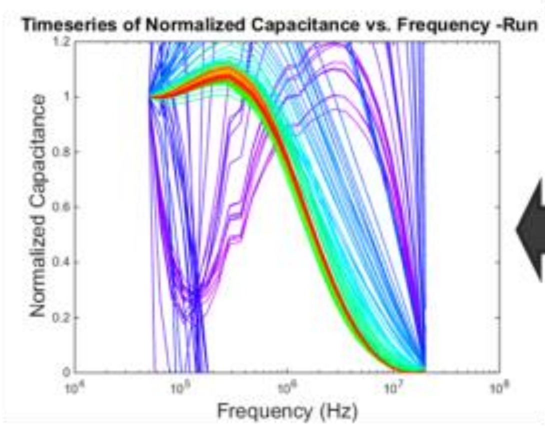
minus



- Order=3
- Window=13

Sazitsky-Golay+ Normalization

- Cole-Cole Model fits



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DS Frequency Scan Analysis

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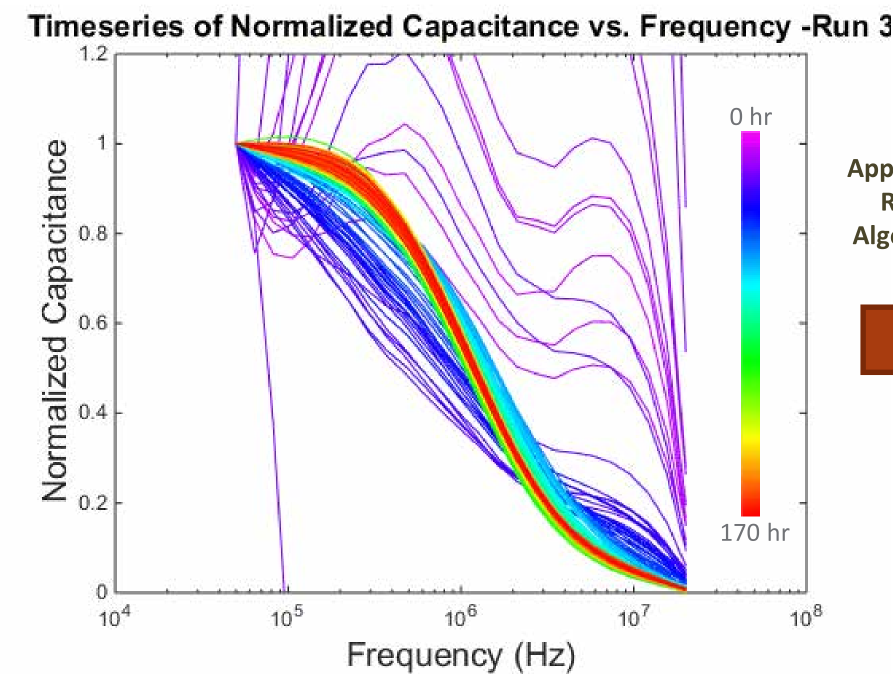
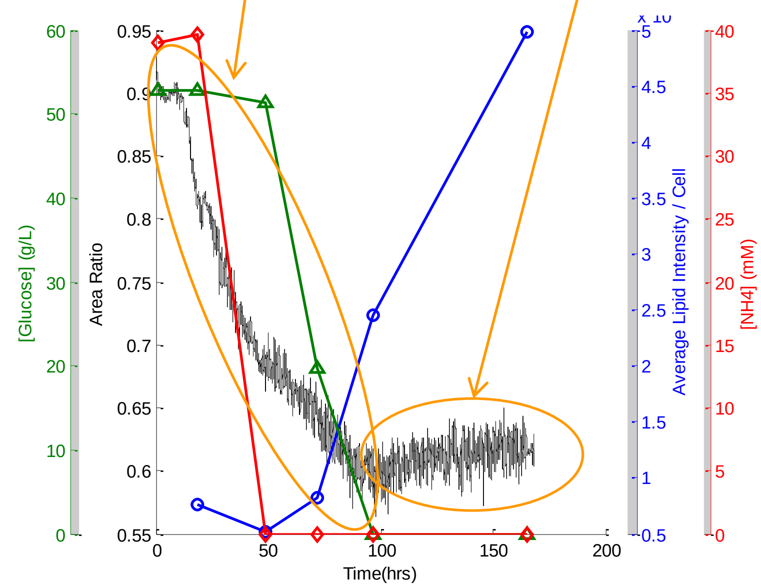


Apply Area Ratio Algorithm

Electrode polarization effects

Subtle changes in DS frequency scans during lipid synthesis



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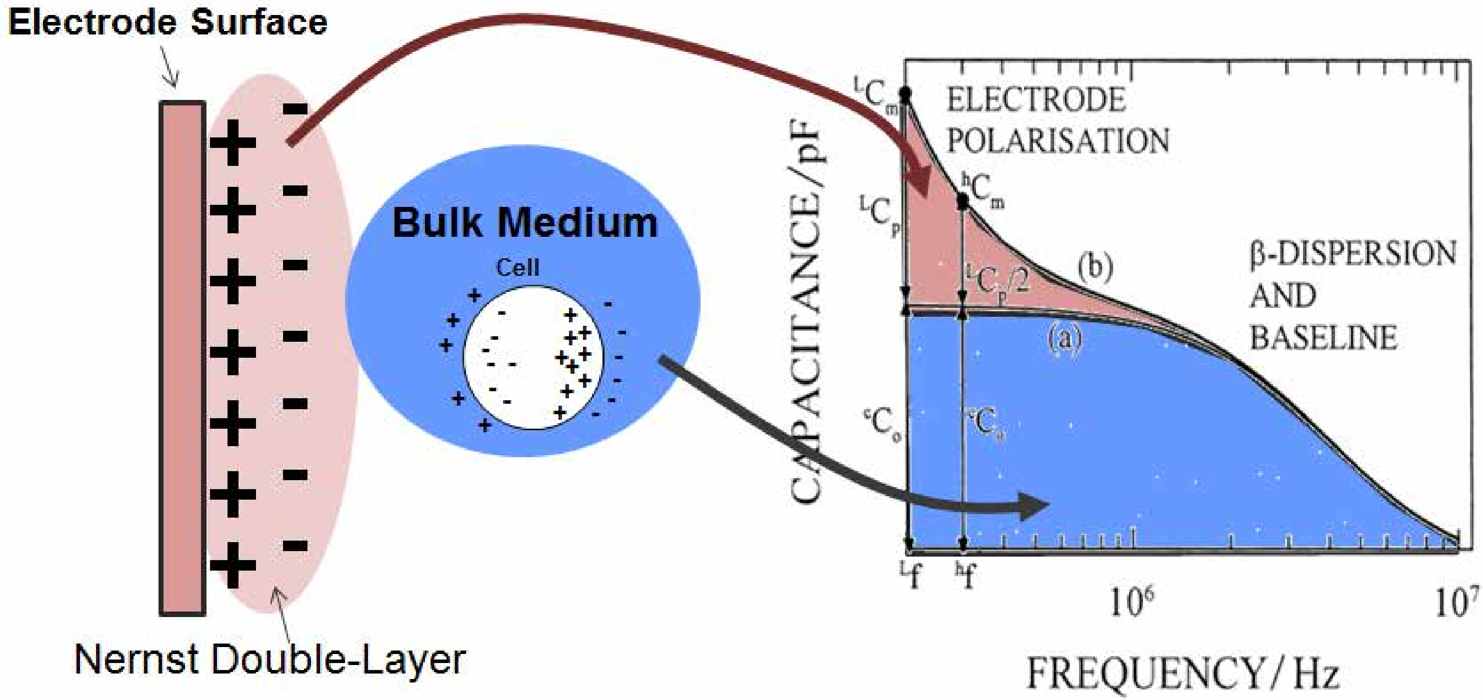
DS Electrode Polarization Effects

- 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

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