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# DOE Bioenergy Technologies Office (BETO)
# 2015 Project Peer Review

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

2/26/2015
Biochemical Platform Review

Jim Collett
Pacific Northwest National Laboratory

# Goal Statement



- **The Challenge:** Hydrocarbon biofuel production will require advanced supervisory control and data acquisition (SCADA) systems for bioconversion of **highly variable biomass feedstocks with high levels of suspended solids** such as corn stover or switchgrass.

- **The Solution**: We will accelerate **technology to market** transfer of **Process Analytical Technologies (PAT)** that enable biorefineries to optimize fed-batch bioconversion of variable, high-solids biomass feedstocks

- **The Importance**: A robust fleet of biorefineries will support **a new, nationwide bioeconomy** for the production and distribution of renewable fuels, chemicals, and materials from biomass that will **reduce greenhouse gas emissions and our dependence on foreign oil**.

# Quad Chart Overview

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## Timeline

- Project start date: 10/1/2014
- Project end date: 9/30/2017
- Percent complete: 6.25%

## Budget

| | Total Costs FY 10– FY 12 | FY 13 Costs | FY 14 Costs (seed funding) | Total Planned Funding (FY 15-Project End Date |
|---|---|---|---|---|
| DOE Funded | -- | -- | $195 K | $1006 K |
| Project Cost Share (Comp.)* | | | $50 K | $25 K |

## MYPP Barriers Addressed

- Bt-A. Biomass and Feedstock Variability
- Bt-J. Biochemical Conversion Process Integration
- Bt-K. Product Acceptability and Performance
- Im-E Cost of Production

## Partners

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(biopharmaceutical experts in dielectric spectroscopy )

sartorius

(major supplier of industrial bioreactors and PAT tools for near-infrared spectroscopy)

# 1 - Project Overview



- **Project Objectives:**

  - Enable real-time tracking of **critical process parameters for fed-batch control** of bioconversions of variable, high-solids feedstocks via the integration of Process Analytical Technologies (PAT) into industrial bioreactor operations.

  - **Reduce biorefinery scale-up risks** by using PAT to optimize bioreactor process control systems in the laboratory in a 30-liter bioreactor that simulates industrial operating conditions.

  - Assemble PAT control systems from **commercial-off-the-shelf (COTS) equipment** that will directly integrate into the Supervisory Control and Data Acquisition (SCADA) networks of commercial biorefineries.

# 1 - Project Overview



- FY 13
  - PNNL and Bend Research leverage the DOE's Technical Assistance Program to **evaluate dielectric spectroscopy (DS) tools** for tracking cell mass growth and lipid synthesis during bioconversion.
- FY 14
  - Seed funding from BETO to develop methods for using DS to track *L. starkeyi* growth on **high-solids biomass feedstocks**.
  - Bend Research evaluates frequency scanning DS methods with COTS equipment for **tracking intracellular lipid synthesis**.
  - PNNL evaluates near **infrared spectroscopy (NIRS)** options for monitoring bioconversions of variable, high-solids feedstocks.
  - AOP receives useful feedback from **external Merit Review** panel.
- FY 15 (Q1)
  - PNNL and Sartorius Stedim develop **preliminary NIRS calibration models**.
  - **Bend Research completes subcontract work** on intracellular lipid tracking.
  - **Autosampling system** for PNNL 30 L bioreactors is specified and purchased.

# 2 – Approach (Technical)

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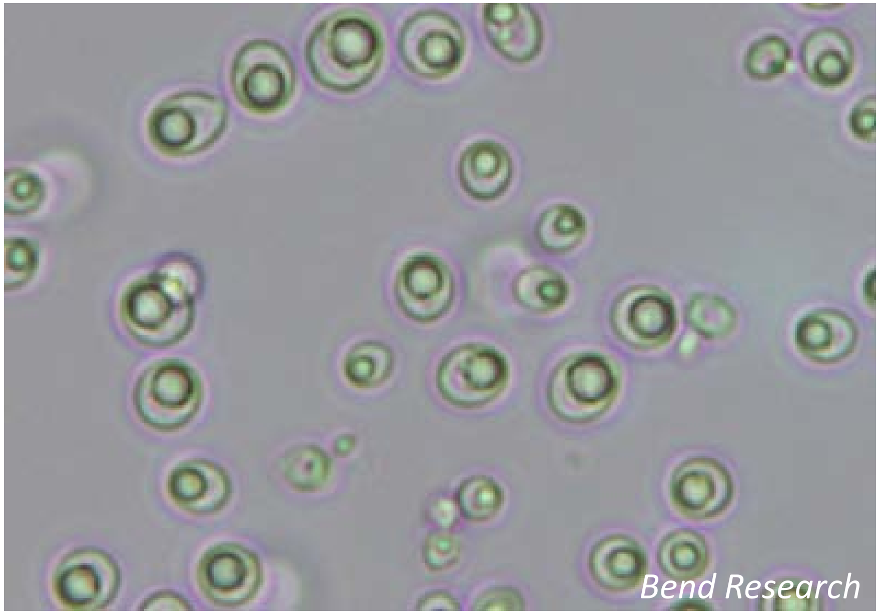
**Challenge**: Lignin separation prior to bioconversion is expensive. The **cost and performance tradeoffs between sugar stream purity and microbial hydrocarbon production** must be quantified and controlled.

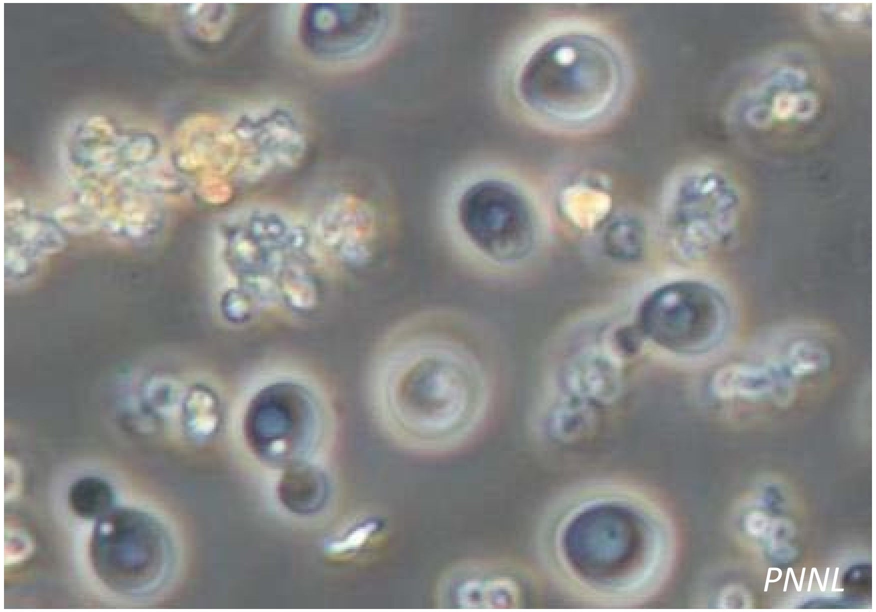
| Table 1. Corn Stover Compositional Variation* | | |
|---|---|---|
| *Parameter* | *Minimum* | *Maximum* |
| Ash | 0.8% | 6.6% |
| Xylan | 14.8% | 22.7% |
| Lignin | 11.2% | 17.8% |

*500 samples were taken from 47 locations over three years

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




*Lipomyces starkeyi* is being developed for hydrocarbon production at PNNL and NREL.




Solids in pretreated corn stover (PCS) impede optical tracking of cell mass growth.

# 2 – Approach (Technical)

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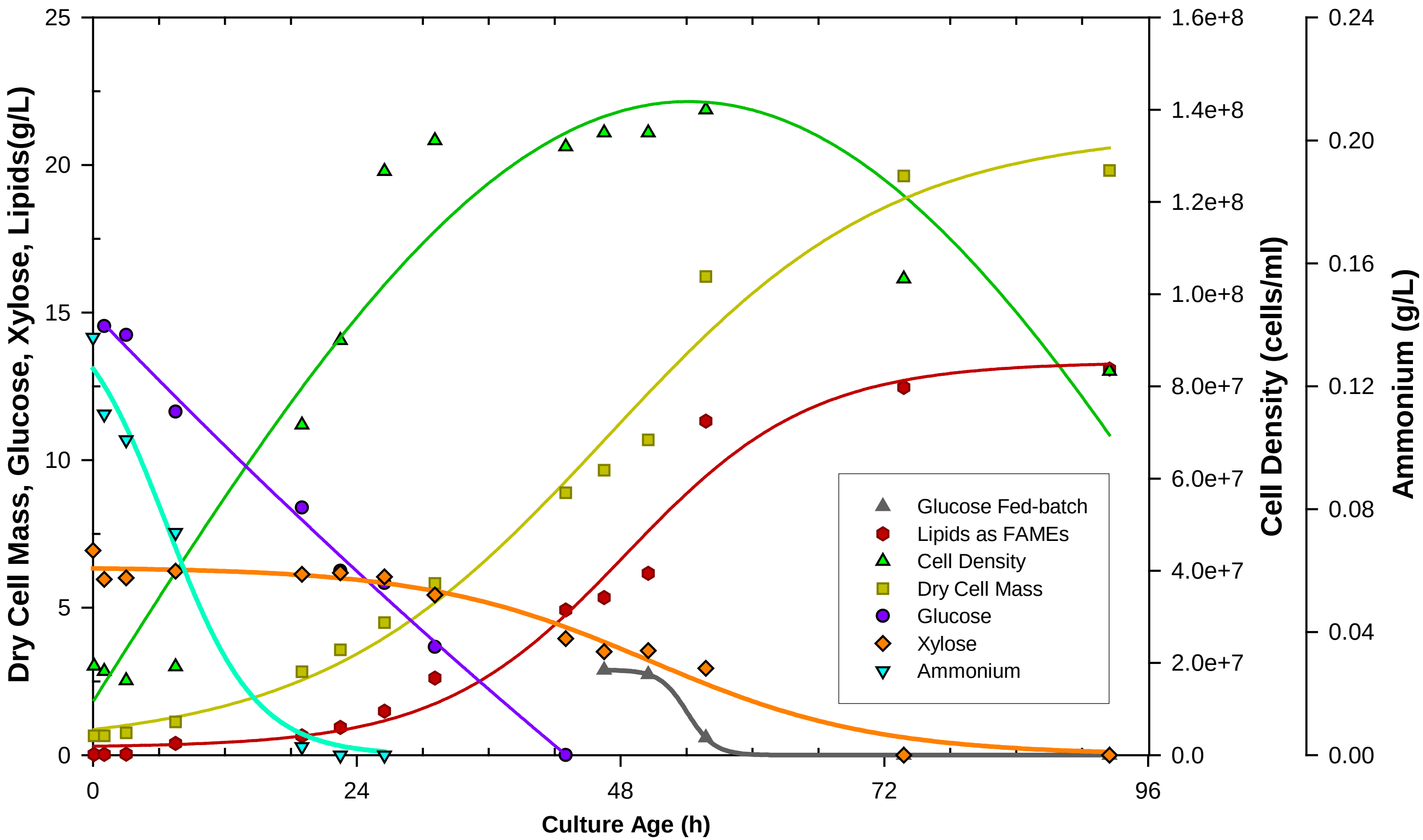
**Challenge**: Fed-batch bioconversion of hydrocarbons from variable, high-solids feedstocks requires **complex multivariate analysis for fed-batch control.**

- Differential sugar consumption rates
- Dependency of lipid synthesis on nitrogen levels

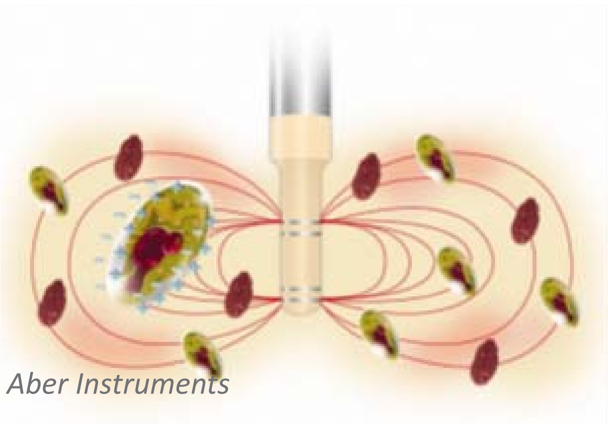



(Above) **Bioreactor cultivation of *Lipomyces starkeyi*** on 2% glucose/xylose minimal medium. Lines show regression models of sample data.

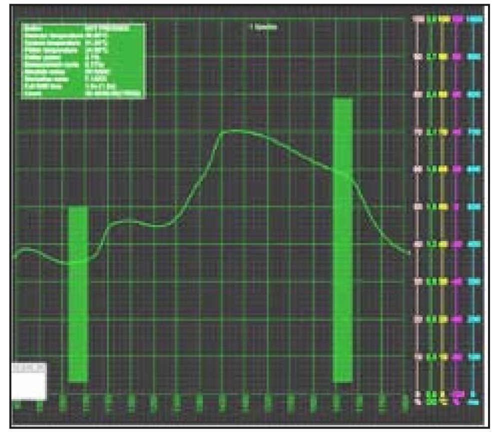
# 2 – Approach (Technical)



**Solution**: **Tech transfer of Process Analytical Technology** (PAT) from the biopharmaceutical sector to industrial biotechnology in collaboration with Bend Research and Sartorius Stedim.




**Dielectric spectroscopy (DS)** for estimating viable cell volume in high-solids biomass feedstocks.



**Near infrared spectroscopy (NIRS)** for online tracking of sugars and nutrients during bioconversion.

**30-liter Bioreactor** to integrate DS and NIRS with SCADA.

## Critical Success Factors and Technical Challenges

- Use industrial **COTS equipment for immediate deployment** within biorefinery SCADA systems.
- Must increase resolution of **Critical Process Parameters** while reducing offline analysis expense
- Use of NIRS in lignocellulosic hydrolysates with **high spectral backgrounds will be challenging**.

# 2 – Approach (Management)

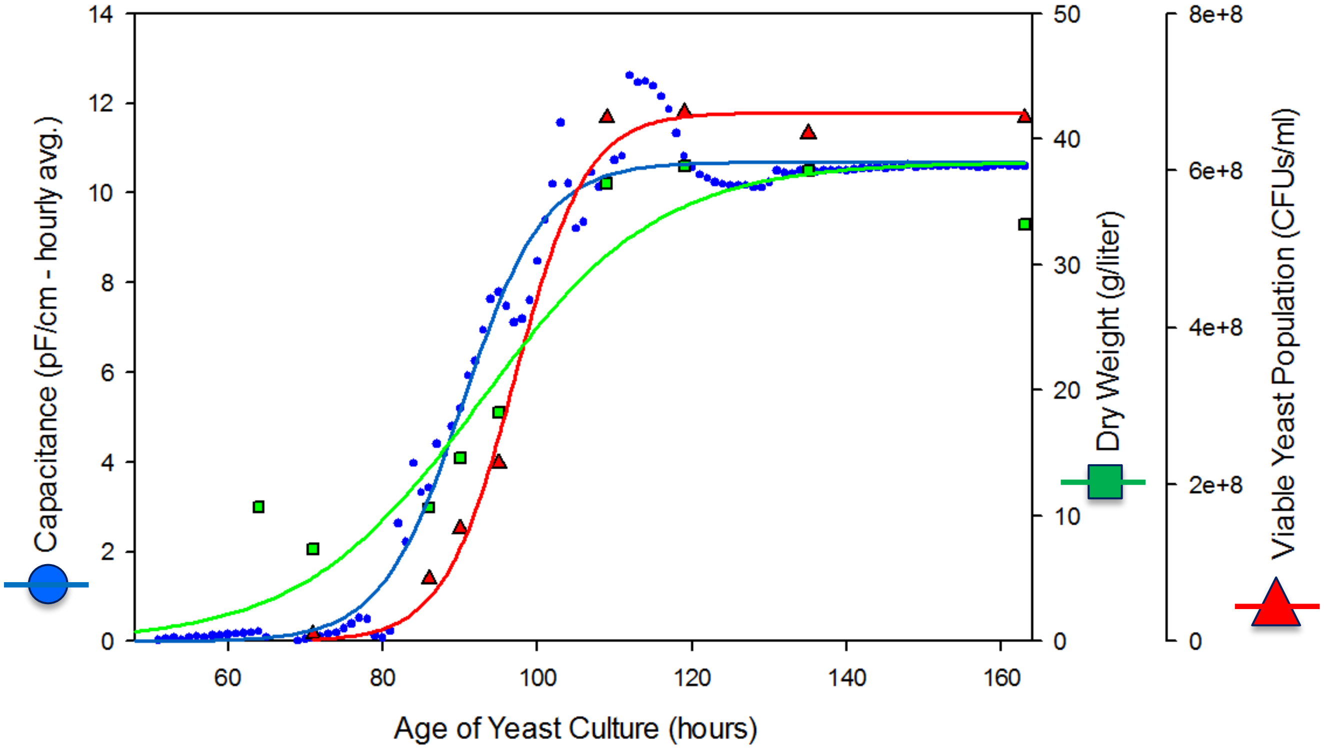
**Standard DOE project management procedures:**

- Develop project management plans
  - Statement of work and how it relates to DOE goals
  - Quarterly milestones to minimize project slippage
  - Go/No Go decision point

- Frequent project communications
  - Teleconferences with BETO platform leads
  - Quarterly formal reporting to BETO
  - Regular meetings with industrial collaborators

# 3 – Technical Accomplishments/ Progress/Results – FY 2014






All regular milestones were met on time and within budget.

| | |
|---|---|
| Q1 | Bench-top estimation of *L. starkeyi* culture growth with 70% confidence |
| Q2 | Bioreactor estimation of *L. starkeyi* culture growth with 90% confidence |
| Q3 | Bench-top estimation of *L. starkeyi* culture growth with 90% confidence in PCS |
| Q4 | Bioreactor estimation of *L. starkeyi* culture growth with 90% confidence in PCS |

**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 solids (*right*).

# 3 – Technical Accomplishments/ Progress/Results – FY 2014

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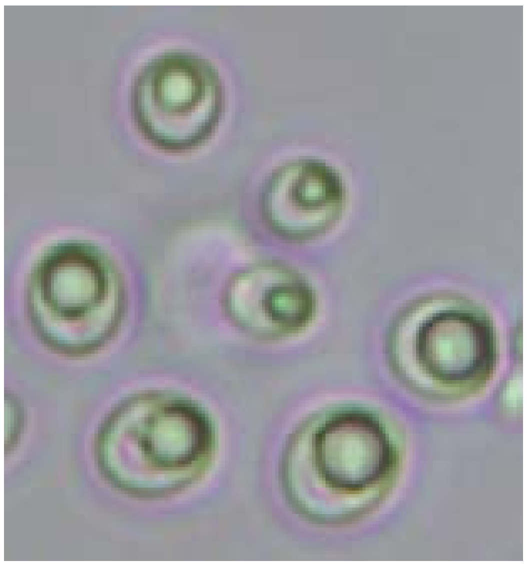
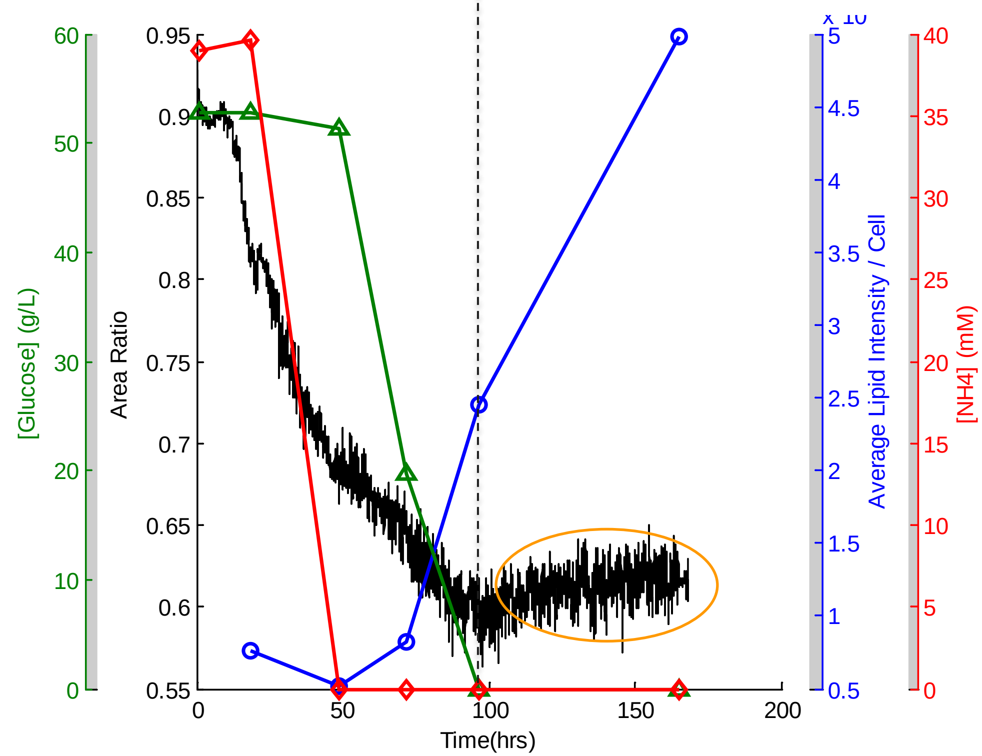
**Stretch Milestone:** DS frequency scanning for tracking **intracellular lipid content**



*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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# 3 – Technical Accomplishments/ Progress/Results – FY 2015

## FY 2015 Milestones

- Q1 - Integrate software and equipment for DS frequency scanning, bioreactor process control, NIRS chemometrics, and automated sampling and analysis. *(partially completed)*
- Q2 - Correlate DS cell density in PCS with NIRS calibration model with < 10% error. *(ongoing)*
- Q3 - Correlate offline glucose and xylose in PCS with NIRS calibration models with < 10% error.
- Q4 - Correlate offline ammonium in PCS with NIRS calibration model with < 10% error.

Flownamics 8000i Autosampling System for collecting data to build **NIRS calibration models.**

# 3 – Technical Accomplishments/ Progress/Results – FY 2015

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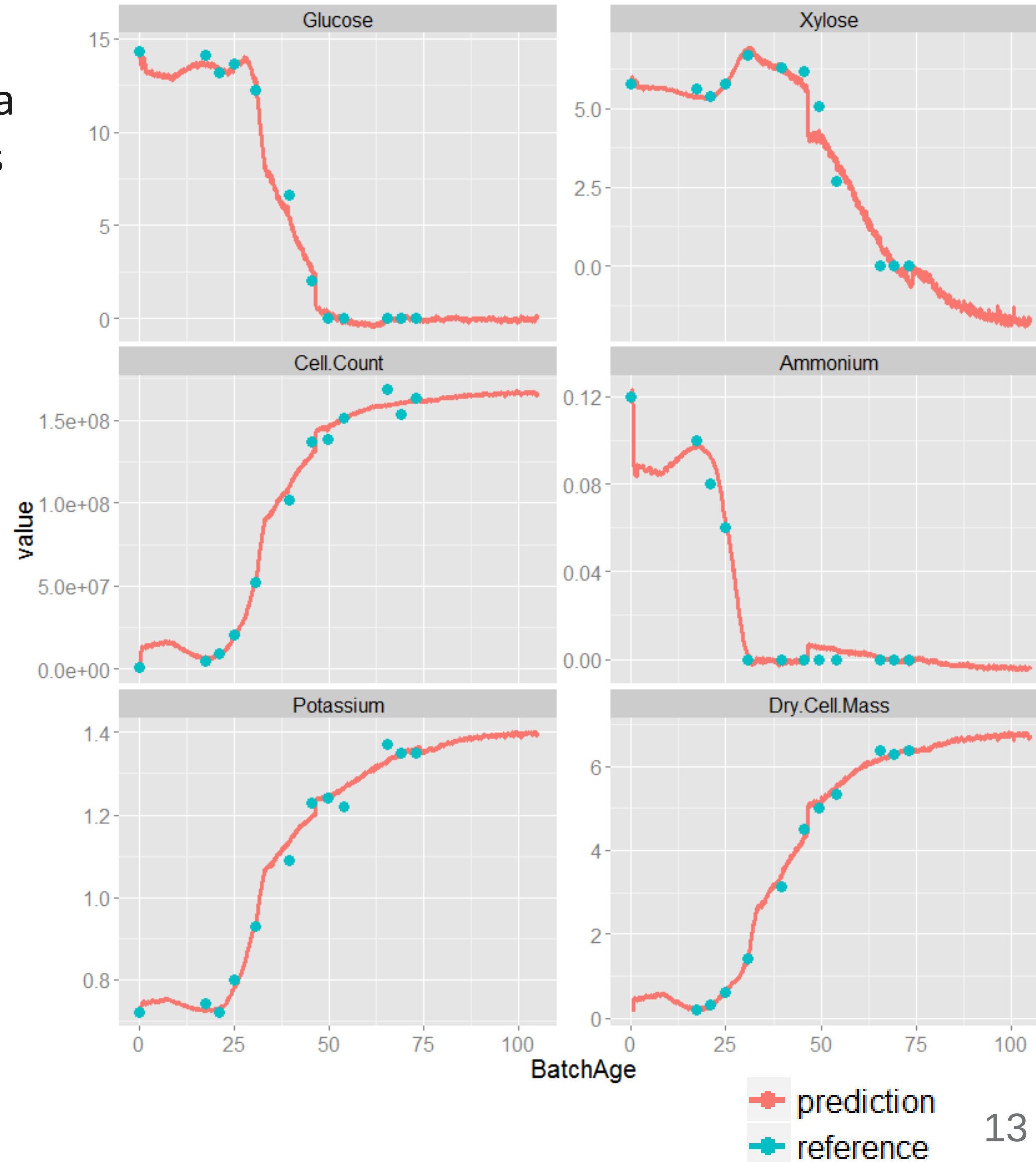
Initial **NIRS calibration models for *L. starkeyi* cultivation** on defined media have been constructed in the SX-Plus chemometrics software package.


sartorius
mechatronics



# 4 – Relevance

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This project follows 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 the expected particle sizes.
- Optimization focused on **industrially relevant organisms**.


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

This project also addresses needs defined by the **NREL 2013 bioconversion design case** (Davis, *et al., 2013):*

- **Understanding the tolerance of hydrocarbon-producing microbes to lignin** and other impurities, including organic acids, salts, and other potential inhibitors.
- **Cost and performance tradeoffs** between sugar stream purity and microbial hydrocarbon production.

# 5 – Future Work



- **Batch and chemostat kinetic models** of lipid accumulation in oleaginous yeast in bioconversions of lignocellulosic hydrolysates
- NIRS calibration models for **bioconversion inhibitors** in lignocellulosic hydrolysates
- NIRS calibration models for components of **corn steep liquor** (CSL) added to bioconversions of lignocellulosic hydrolysates
- Development of a **fed-batch process control system** for optimal production of hydrocarbons from hydrolyzed PCS.
- Go/No Go: *Are the accuracies of the online DS/NIRS PAT tools sufficient to justify a 50% reduction in the frequency of bioreactor culture sampling and offline analysis?*

# Summary

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1. 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.
2. Approach: **Dielectric and Near Infrared Spectroscopy** are being integrated into a set PAT tools and methods using COTS equipment and software that will directly integrate into biorefinery SCADA systems.
3. Technical Accomplishments:
   - DS for tracking cell mass growth in **high-solids, pretreated corn stover.**
   - Progress in applying DS to measure **intracellular lipid content**.
   - **NIRS calibration models of Critical Process Parameters** for hydrocarbon production from corn stover.
4. Relevance: This work develops **Conversion Enabling Technologies** that will improve the profitability of biorefineries and supports the growth of a nationwide bioeconomy.
5. Future work: Development of **kinetic models and fed-batch control algorithms** for hydrocarbon production, and NIRS calibration models for **growth inhibitors** in pretreated corn stover and for nutrients in **corn steep liquor**.

# Acknowledgements

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Pacific Northwest NATIONAL LABORATORY

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*Related Projects*

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

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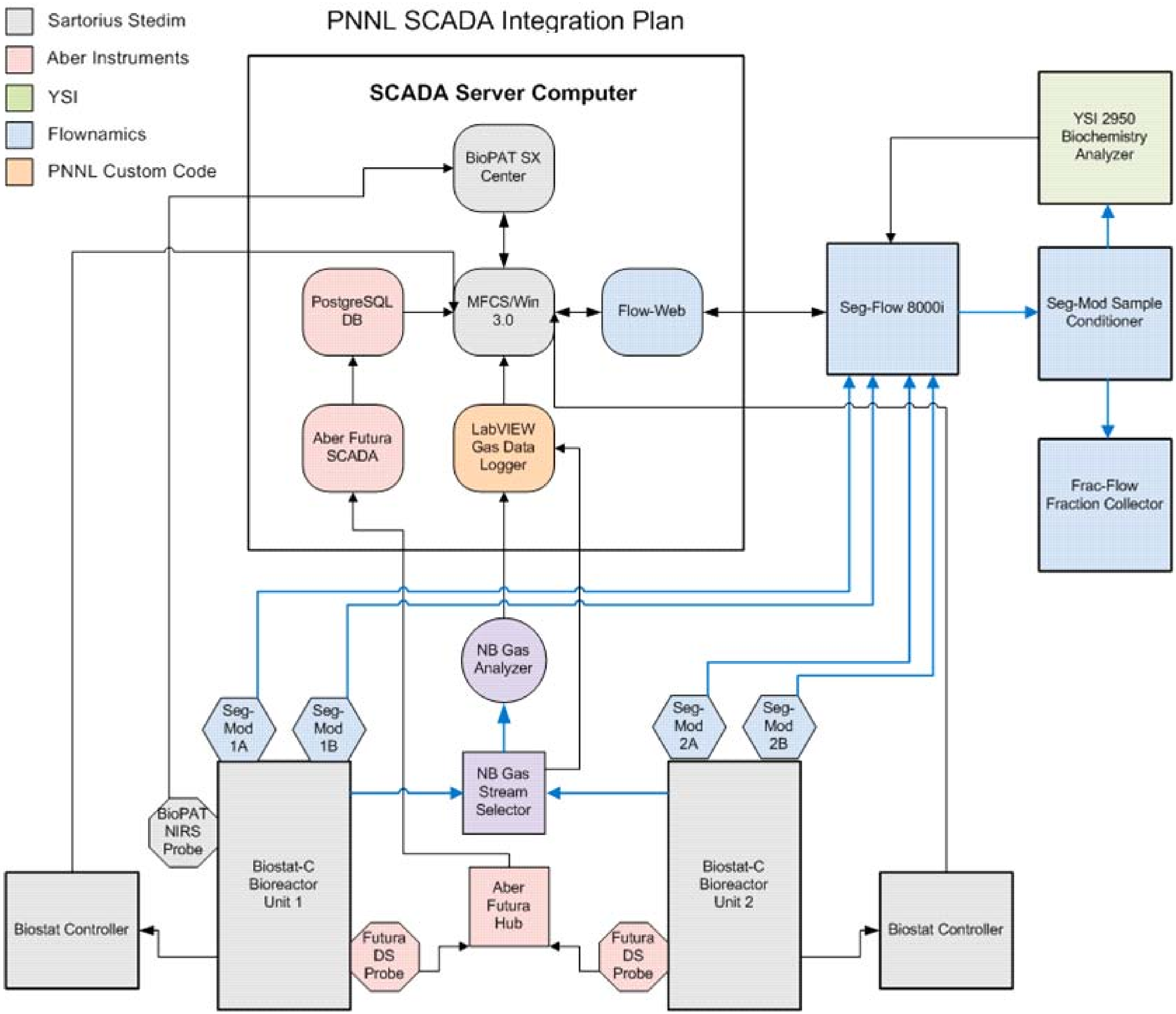
# Additional Slides

# SCADA Integration Plan



# *Lipomyces starkeyi* bioreactor kinetics

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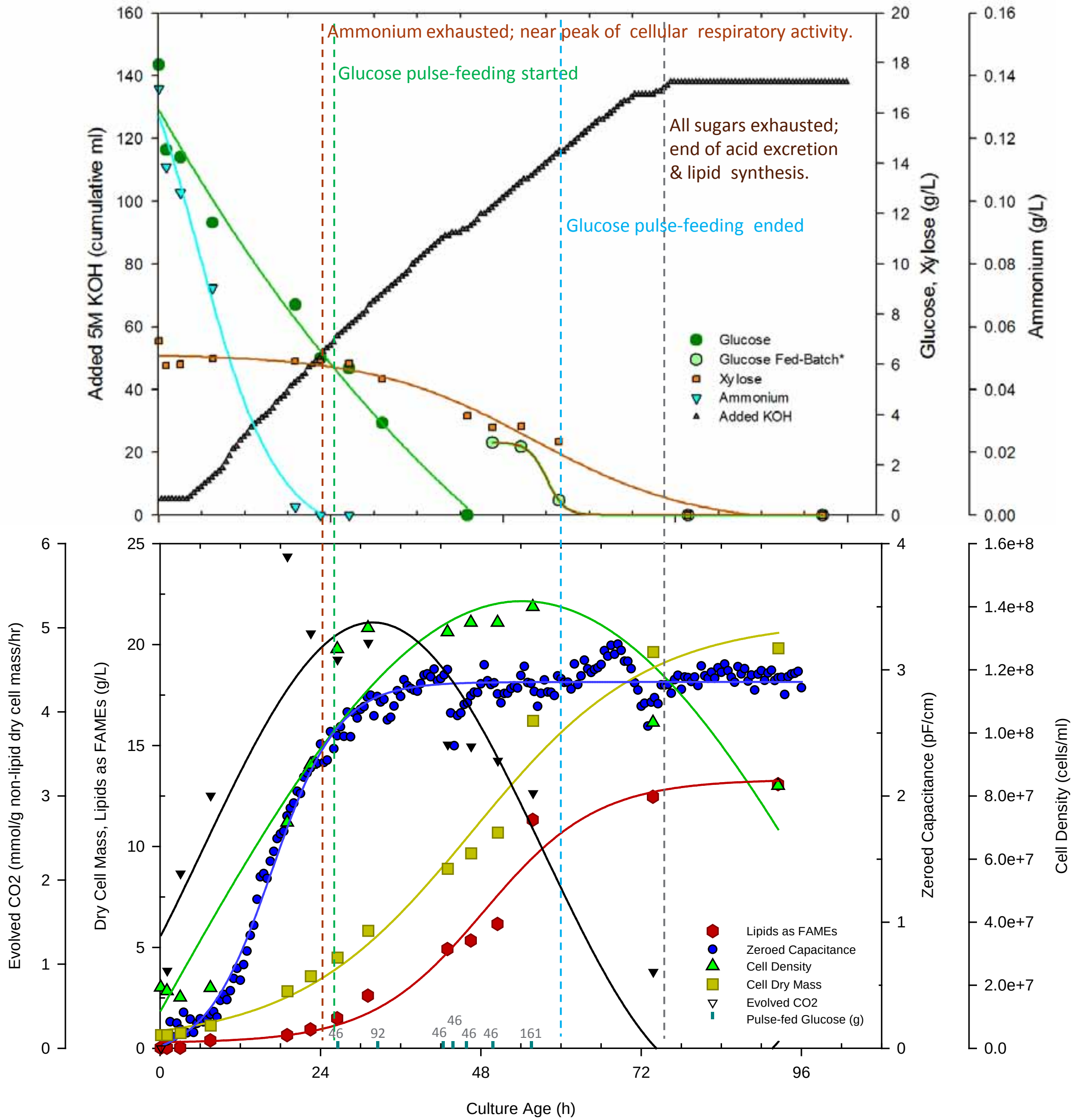
Pulsed fed-batch cultivation of *L. starkeyi* on 2% sugar (2:1 glucose/xylose) minimal medium in a 20-liter stirred tank bioreactor. The agitation rate was set at 600 RPM, the pH controlled at 5.5 via addition of 5 M KOH, and the DO maintained at 50% via variable aeration at 0.2-2.5 vvm.

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

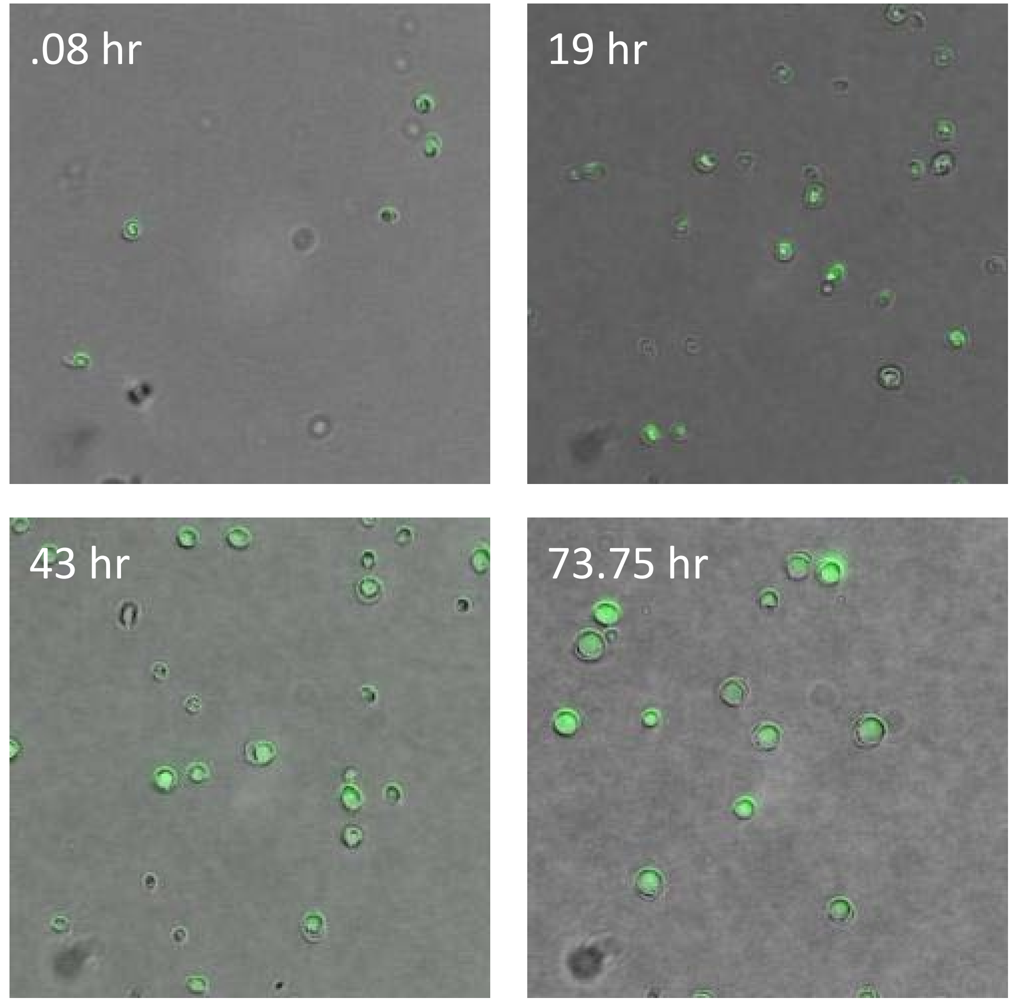
| Hours after inoculation | Glucose added (g) |
|---|---|
| 26.50 | 46 |
| 32.50 | 92 |
| 42.25 | 46 |
| 43.75 | 46 |
| 45.75 | 46 |
| 49.75 | 46 |
| 55.50 | 161 |
| Total | 483 |



# *Lipomyces starkeyi* micrographs



400x micrographs of samples from a pulsed fed-batch cultivation of *L. starkeyi* grown on 2% sugar (2:1 glucose/xylose) minimal medium in a 20-liter stirred tank bioreactor. The lipid droplets within the cells are stained green with LipidTox neutral lipid stain.



# DS Frequency Scan Analysis



Total cell mass and its electrical properties are primary factors

Dielectric increment (Δε) relates to "amount" of charging

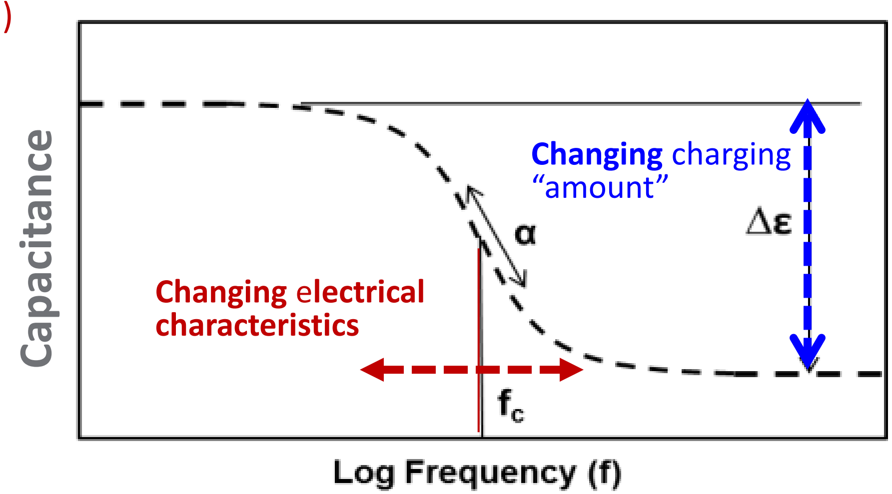
$$\Delta\varepsilon = \frac{9PrC_m}{4\varepsilon_0}$$

**Where:**
Δε = Dielectric increment
**P = Biomass volume fraction**
r = Representative cell radius
**$C_m$=Specific membrane capacitance**
$\varepsilon_0$=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
**$\sigma_i$ = Intracellular conductivity**
$\sigma_m$ = Medium conductivity
r = Representative cell radius
**$C_m$=Specific membrane capacitance**



# DS Frequency Scan Analysis

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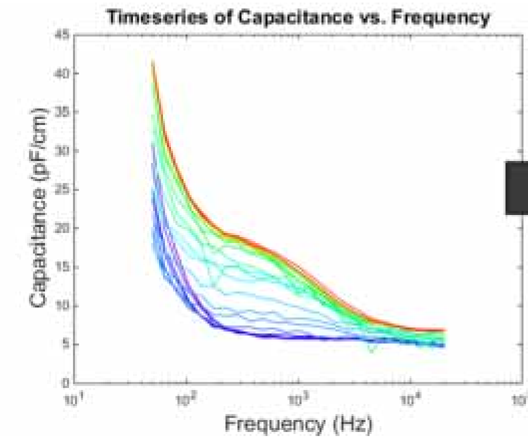
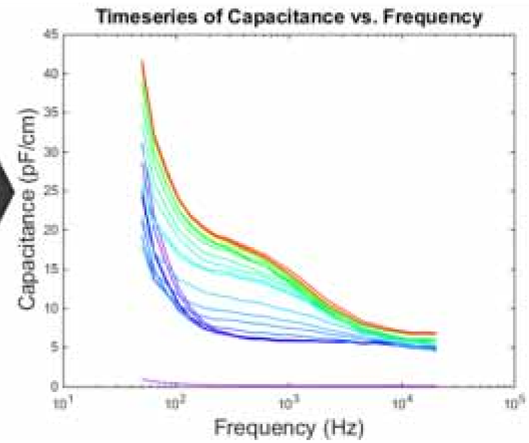
## Raw Frequency Scans

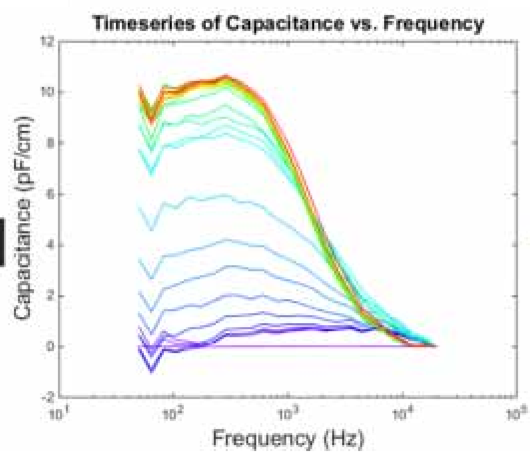



## Moving Average Filter (window = 15min)

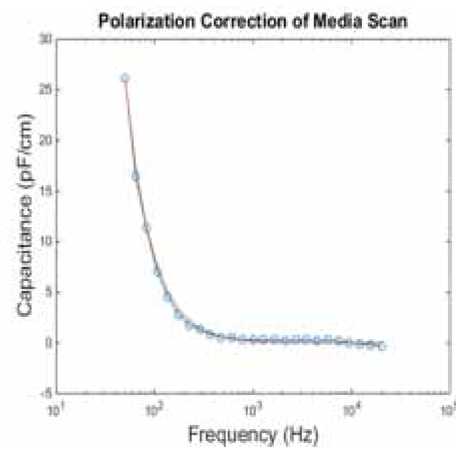



## Polarization Correction

- Area Ratio



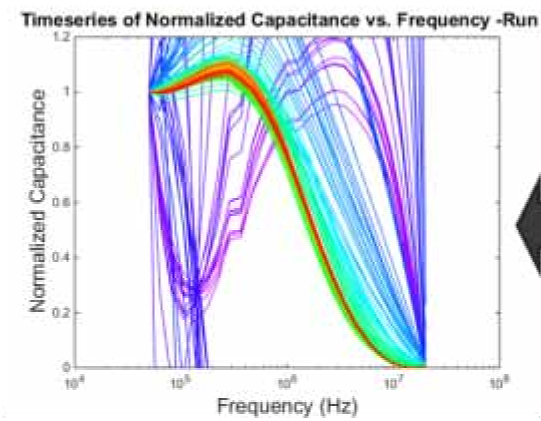

minus




## Sazitsky-Golay+ Normalization

- Order=3
- Window=13

- Cole-Cole Model fits

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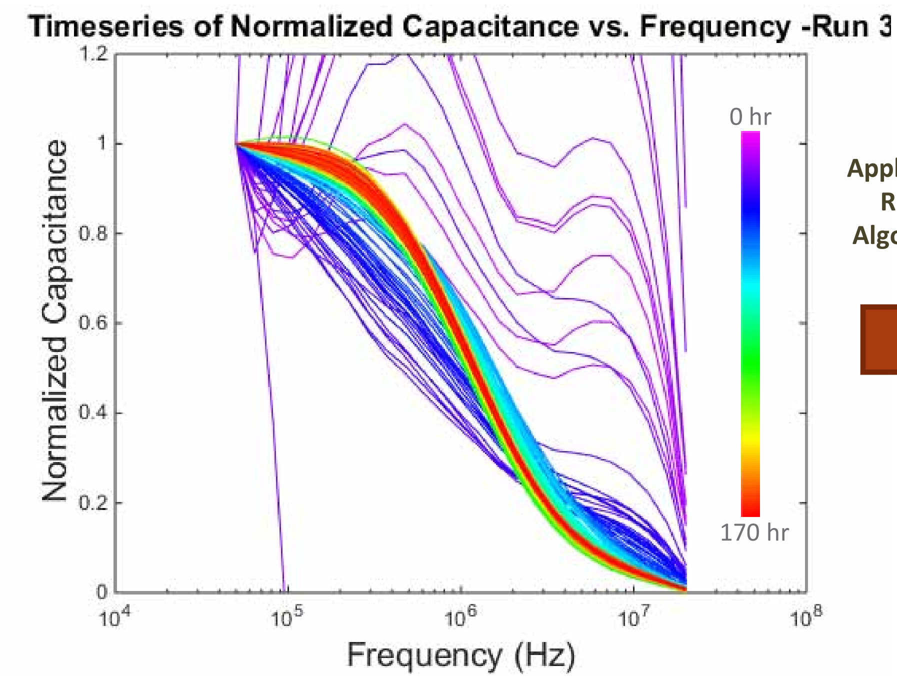
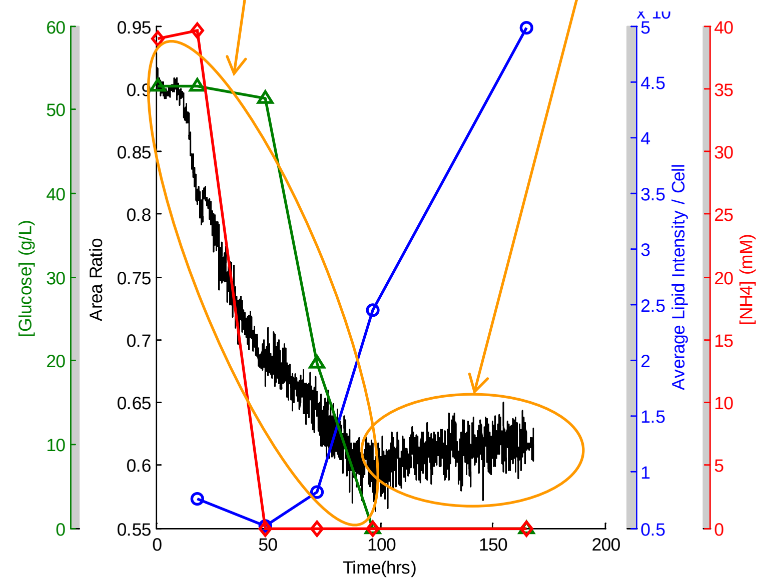
# DS Frequency Scan Analysis




**Apply Area Ratio Algorithm**

Electrode polarization effects

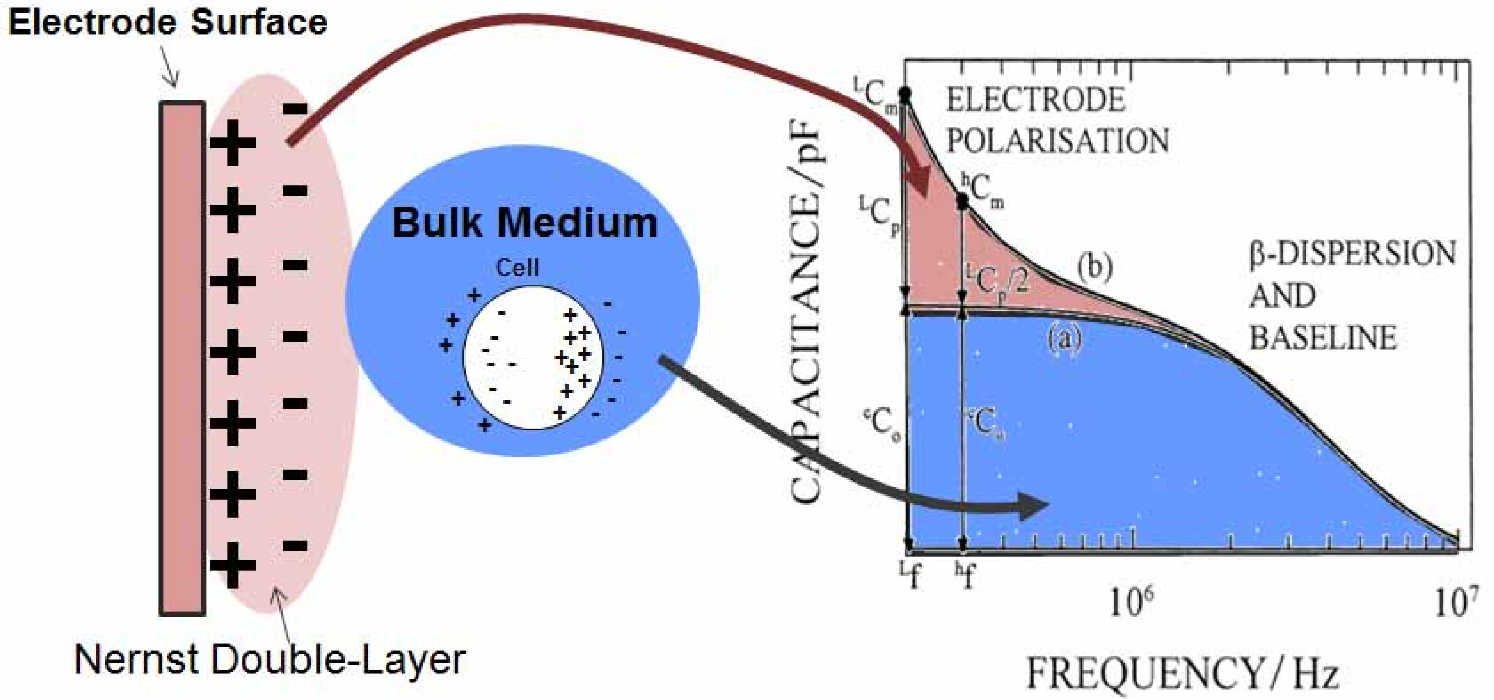
Subtle changes in DS frequency scans during lipid synthesis



# 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.

# NIRS Applications

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## PMD500 Series Process Analyzer with NIR Technology

sartorius mechatronics

- Online moisture analysis
- Process monitoring and control using industrial OPC communications protocol
- Multiparametric concentration measurement
- Homogeneity measurement
- Sample identification
- Purity testing


http://www.sartorius.com/fileadmin/fm-dam/DDM/Bioprocess-Solutions/Process_Analytical_Technology/Data_Sheets/Data_BioPat_Spectro_PMD500_W--2038-e.pdf

# NIRS Calibration Models

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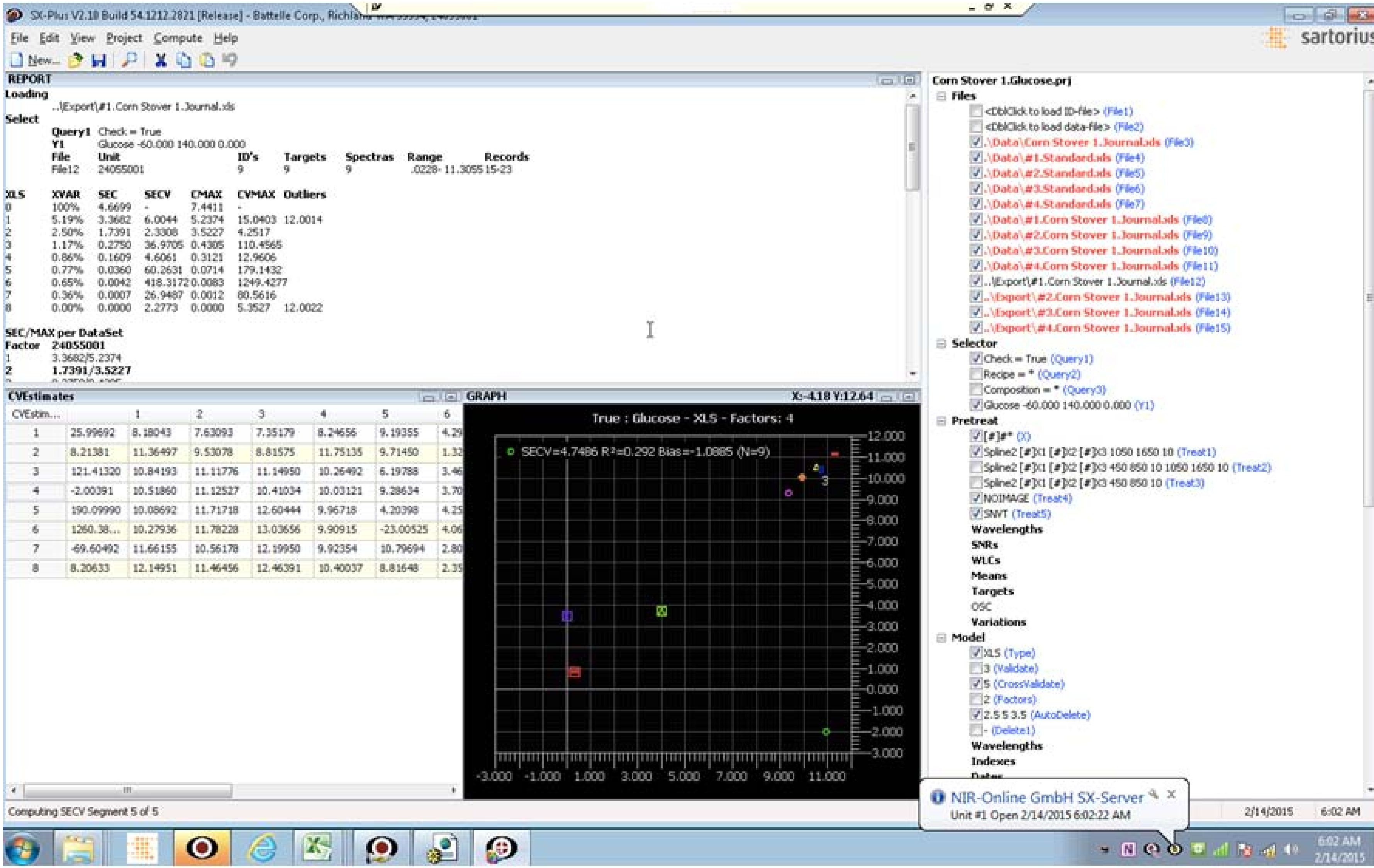
## Sartorius SX-Suite Chemometrics Software



## (Not a template slide – for information purposes only)

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- *You may refer to them during the Q&A period if they are helpful to you in explaining certain points.*

# 2014 External Merit Review Comments and Responses

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| Criterion 1: Technical Merit, Innovation, and Impact |
| --- |
| **Strengths** |
| • The team proposes to develop PAT for industrial fermentations using new online sensing technology (NIRS and DI). If successful, this enabling technology would be applicable to many fermentation technologies currently being developed in the space. The proposed project is a collaborative effort between industry and the labs.<br>• Measurement of process relevant process parameters in lignocellulosic fermentations is difficult due to the large amount of solids present. This project seeks to overcome these difficulties by integrating dielectric spectroscopy (DS) and near infrared spectroscopy (NIRS). The bioprocessing hurdle identified in this proposal seems worthy of investigation to further BETO's mission. The overall approach keeps industrial considerations at the forefront throughout the process by using off the shelf components and involving commercial partners, increasing the likelihood of adoption. Beyond the benefit of being able to more accurately monitor growth, DS has the potential to provide information on the lipid content of cells during fermentation which may find broad application.<br>• This research is focused on the development of a monitoring and control system for high concentrations of suspended solids. This project is highly related to the BETO program goals and will significantly impact the state of bioenergy. This proposed project is highly aware of the current state of the art and provides a superior overview and demonstration of how this project will move the state forward. The proposed project is superior is outlining collaborations with both industry and academics (including all the major players in the field). The technical detail is superior and well founded in literature to support this project. The milestones are superior for this project and nicely link the milestones, and deliverables with the state of the art. |

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# 2014 External Merit Review Comments and Responses



| Criterion 2: Research Approach |
|---|
| **Strengths** |
| • The FY 15 experiments seem reasonable.<br>• The research approach for FY15 and FY16 (subtasks 1.1 and 1.2) were clearly described, the technical risks were identified, and appropriate mitigation strategies were proposed.<br>• The proposed project is very clearly described and the ability to monitor the fermentation process and provide feedback will have a very strong application to the future of biofuel fermentation research. The ability to monitor the fermentation process is a great need and this project will address this issue. The technical risks are well understood and well explained in the proposal, with very strong mitigation solutions. The deliverables are well defined and the equipment that will be developed will be of great need for this industry and all technical partners in the project. |

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# 2014 External Merit Review Comments and Responses

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| Criterion 2: Research Approach |
| --- |
| Weaknesses |

- It is unclear why L.starkeyi was chosen as the oleaginous yeast for study. Comments on why/how information from this organism would be transferable would be useful. For FY16 Subtasks 1.1 it would be nice to have some likely outcomes or potential problems that could be overcome with the proposed experiments to drive home the importance of another round of experiments. Why the kinetic model for Umbelopsis isabellina was chosen would be useful know among other modeled yeasts. FY 16 Subtasks 1.2 and 1.3 are not well defined, though they seem interesting. The subtasks for FY 17 were just not described which makes it hard to justify funding.
    - *L. starkeyi was chosen for this study to provide synergy with another BETO AOP project at PNNL to engineer this microbe for increased lipid productivity. Many strains of L. starkeyi natively produce high levels of lipids, and grow well on xylose. Moreover, this unicellular oleaginous yeast provides a facile physical system for developing our DS and NIRS approaches. We expect our results with L. starkeyi to be highly transferable to Yarrowia lipolytica, which has a more tractable genetic transformation system and is a model organism for lipidogenesis. We may also perform trials of our DS and NIRS methods (in collaborations outside of the present project) using other candidate microbes for advanced biofuel production.*
    - *Biochemical and physiological evidence thus far suggest that many oleaginous yeast and fungi share a similar metabolic basis for their high production of lipids as reserve energy stores under nitrogen starvation conditions. The submerged batch and chemostat kinetic models for Umbelopsis isabellina cited in the proposal are general yet highly specified models of fungal lipid synthesis under nitrogen limitation that appear to be directly applicable to our bioreactor work in L. starkeyi.*

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# Publications, Patents, Presentations, Awards, and Commercialization

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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 37th Symposium on Biotechnology for Fuels and Chemicals, San Diego, CA on April 27, 2015. PNNL-SA-107273.

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