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

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Biochemical Platform Review

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This presentation does not contain any proprietary, confidential, or otherwise restricted information
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

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.

Budget

<table>
<thead>
<tr>
<th></th>
<th>FY 15 Costs</th>
<th>FY 16 Costs</th>
<th>Total Planned Funding (FY 17-Project End Date)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOE Funded</td>
<td>$300 K</td>
<td>$300 K</td>
<td>$300 K</td>
</tr>
<tr>
<td>Project Cost</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Share (Comp.)*</td>
<td>$30 K</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Partners

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

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

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

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**2017 Target:** Five levels of 10X volume scale-up to 200,000 gal with 24-hour batch time

**2017 Target:** 70 hours for fed-batch conversion of hydrolysate to 9.2% hydrocarbons

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

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

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>0.8%</td>
<td>6.6%</td>
</tr>
<tr>
<td>Xylan</td>
<td>14.8%</td>
<td>22.7%</td>
</tr>
<tr>
<td>Lignin</td>
<td>11.2%</td>
<td>17.8%</td>
</tr>
</tbody>
</table>

*500 samples were taken from 47 locations over three years

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

- Relative Standard Error of Prediction
  - Glucose = 5.8%
  - Xylose = 6.5%

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

- **Raman Spectra**
- **Near-infrared Spectra**
- **Dielectric Spectra**
- **Sample Offline Assays** (sugars, dry cell mass, lipids, etc.)
- **Process Variables** (temp, pH, DO, airflow, % CO2, etc.)
- **Events** (inoculation, substrate feeding, control changes, antifoam, etc.)
- **Batch Recipe** (biomass type, pretreatment, microbial strain, nutrients, etc.)

**Bioreactor**

**Control System**

**Operator**

**Integrated Data**

**LabKey**

**Web-enabled Database Server**

**Chemometric Software & Machine Learning**

**Calibration Model**

**Time-stamp**

**X-block**

**Y-block**

**Class Variables**
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
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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
Additional Slides
Laboratory SCADA Integration Plan

Process Control Computer

- BioPAT SX Center
- MFCS/Win 3.0
- Matrikon Easy Trender
- Matrikon Desktop Historian DB

LabKey LIMS DB

PostgreSQL DB

Aber Futura SCADA

Flow-Web Autosample Controller

Flow-Frac Fraction Collector

Seg-Mod Sample Conditioner

YSI 2950 Biochemistry Analyzer

Vendor-Specific Data Communication

OPC Data Communication

0.2 m-filtered Liquid Sample Path

Cell-containing Liquid Sample Path

Bioreactor Off Gas Stream

Bioreactor Feed Stream

Bioreactor Harvest Stream

Offline Sample Assays (HPLC, FAMEs, Dry Cell Mass, etc.)
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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Calibration Set Count</th>
<th>Prediction Set Count</th>
<th>Relative Standard Error of Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Cell Mass</td>
<td>32</td>
<td>15</td>
<td>3.25%</td>
</tr>
<tr>
<td>Glucose</td>
<td>49</td>
<td>21</td>
<td>8.49%</td>
</tr>
<tr>
<td>Xylose</td>
<td>70</td>
<td>34</td>
<td>10.25%</td>
</tr>
<tr>
<td>Ammonium</td>
<td>73</td>
<td>33</td>
<td>10.04%</td>
</tr>
</tbody>
</table>
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
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:

<table>
<thead>
<tr>
<th>Hours after inoculation</th>
<th>Glucose added (g)</th>
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<tbody>
<tr>
<td>26.50</td>
<td>46</td>
</tr>
<tr>
<td>32.50</td>
<td>92</td>
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<tr>
<td>42.25</td>
<td>46</td>
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<td>43.75</td>
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</tr>
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<td>45.75</td>
<td>46</td>
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<tr>
<td>49.75</td>
<td>46</td>
</tr>
<tr>
<td>55.50</td>
<td>161</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>483</strong></td>
</tr>
</tbody>
</table>
Dielectric spectroscopy tracking of *Aspergillus nidulans* filamentous fungi grown on lactose MM in a 30-liter bioreactor.
Dielectric spectroscopy tracking of *Aspergillus nidulans* during growth and synthesis of octotrienoic acid on hydrolyzed PCS in a 30-liter bioreactor

Induction of octotrienoic synthesis via addition of MEK

![Graph showing dry cell mass, octotrienoic acid, and capacitance over culture age.](image-url)
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 (Δε) relates to “amount” of charging**

\[
\Delta \varepsilon = \frac{9 P r C_m}{4 \varepsilon_0}
\]

Where:
- \(\Delta \varepsilon\) = 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)

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

Where:
- \(f_c\) = Critical frequency
- \(\sigma_i\) = Intracellular conductivity
- \(\sigma_m\) = Medium conductivity
- \(r\) = Representative cell radius
- \(C_m\) = Specific membrane capacitance

Changing electrical characteristics

Changing charging “amount”
DS Frequency Scan Analysis

Raw Frequency Scans

Moving Average Filter (window = 15min)

Timeseries of Capacitance vs. Frequency

- Order=3
- Window=13

Sazitsky-Golay+ Normalization

Cole-Cole Model fits

Polarization Correction

Timeseries of Normalized Capacitance vs. Frequency - Run

Timeseries of Capacitance vs. Frequency

Polarization Correction of Media Scan

Normalized Capacitance

Capacitance (pF/cm)

Frequency (Hz)

Frequency (Hz)

Polarization Correction of Media Scan

Capacitance (pF/cm)

Frequency (Hz)
DS Frequency Scan Analysis

Electrode polarization effects

Subtle changes in DS frequency scans during lipid synthesis

Timeseries of Normalized Capacitance vs. Frequency - Run 3

Apply Area Ratio Algorithm

Normalized Capacitance

Frequency (Hz)

[Glucose] (g/L)

[NH4] (mM)

Average Lipid Intensity / Cell

Time(hrs)
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.
Publications, Patents, Presentations, Awards, and Commercialization

