Online Monitoring Using FT-NIR to Facilitate Dynamic Fed-Batch Fermentations

Lucas Sandoval, Debjani Mitra, Wei He, Jessica Wong, James Gardner, and Pavan Kambam*

Advanced Biofuels Process Demonstration Unit, Lawrence Berkeley National Laboratory, Emeryville, CA 94608

Introduction

- **Fed-batch fermentations** involve controlled feeding of a growth limiting nutrient to a batch culture enabling higher cell densities. Some classic fed-batch strategies employ pH, OUR (oxygen uptake rate), DO (Dissolved oxygen) etc, as control parameters, based on their indirect correlations with growth. This indirect feedback control could lead to an un-optimized process with compromised productivity.
- **FT-NIR spectroscopy** is an analytical tool that uses electromagnetic spectrum (~800 - 2500 nm) to cause vibrational energy changes in matter resulting from fluctuations in molecular dipole moment and provides a response that can be used to quantify composition of the material.
- The FT-NIR probe can penetrate much farther into a medium allowing instant measurements, and little to no sample preparation. However, its meaningful implementation as a standard practice requires vigorous optimization.
- In this study, we present direct online monitoring of cell density, substrate and product profiles using a single FT-NIR (Fourier Transform Near Infrared) probe during yeast fermentation. This online measurement can then be used to directly control fed batch fermentations, resulting in a precisely optimized process.

FT-NIR Method Development and Validation

- FT-NIR has three basic measurement modes – (1) Off-line, (2) At-line (or rapid off-line), and (3) On-line (includes in-situ and ex-situ monitoring)
- At-line FT-NIR measurements of 3.7 L batch fermentation of *S. cerevisiae* were used for method development. Samples taken every 2 hours were tested for O.D. at 600nm (using spectrophotometer) and scanned using FT-NIR probe. Samples were then centrifuged (4000 rpm, 5 min) and supernatant was scanned again with FT-NIR probe. Supernatant was then filtered (0.2 µ) before testing for glucose and ethanol concentration using YSI
- The spectral data collected from at-line measurements were loaded onto the OPUS Quant 2 method development wizard. Results obtained from spectrophotometric and YSI measurements were also entered and a method for each component was developed separately
- The OPUS software optimized many different methods (~400). Five methods with lowest RMSECV and Rank Values were selected. Each method was then cross validated to produce the calibration curves

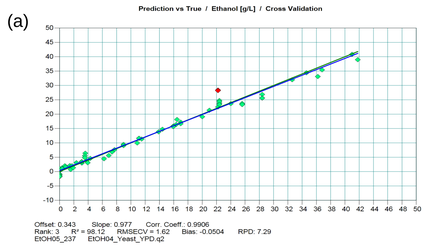
Batch fermentation in 19L fermentor

- Predictions from continuous in-situ FT-NIR monitoring of a yeast batch fermentation were validated against results obtained from spectrophotometer and YSI

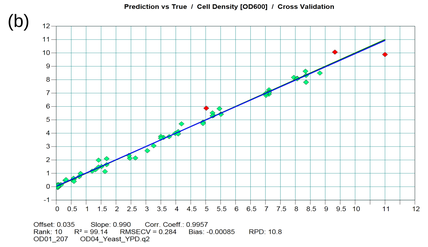
FT-NIR Calibrations for ethanol, glucose, and cell density exhibit close correlation with offline measurements

Preprocessing of spectral data was done using the second derivative method for ethanol (Figs. 1a and 2a), the first derivative method for cell density (OD600) (Figs.1b and 2b) and the straight line subtraction method for glucose (Figs.1c and 2c)

(a)



(b)



(c)

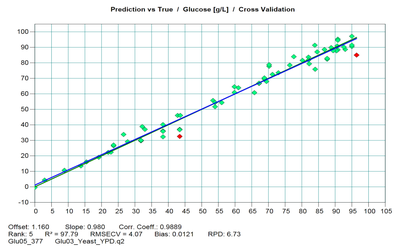
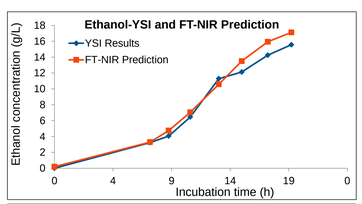
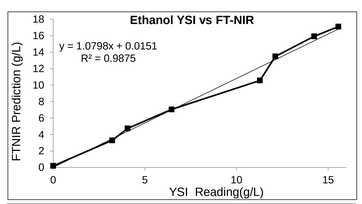


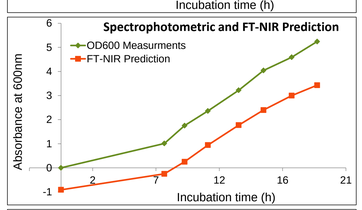
Figure 1: Method development using calibration curves for glucose, ethanol and optical density prepared using off-line and FT-NIR measurements

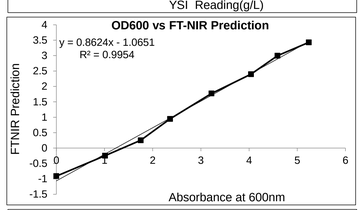
(a)



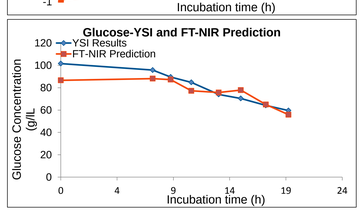


(b)





(c)



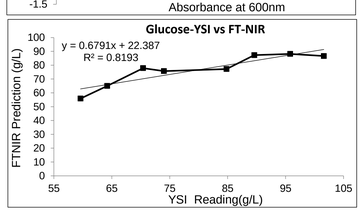


Figure 2: FT-NIR method validation results indicate close correlation between FT-NIR & off-line measurements

Figure 3: Bruker Optics Matrix-F FT-NIR with Flex Transflexion probe

Linear and Exponential Fed-Batch Fermentation

- Batch and fed-batch fermentations of *E. coli* HB101 were conducted in 3.7L fermentors using LB medium supplemented with 1% (w/v) and 10% (w/v) glucose concentrations, respectively
- Fed-batch fermentation was started when cells reached stationary phase and dissolved oxygen dropped to 0%.
- In duplicate reactors, the cell culture was fed with LB supplemented with 100g/L glucose at a constant rate of 2 mL/min for 24 h, calculated based on the equation - $V.x = F.S_F.Y_{(x/s)}.t + V_0.x_0$; where V = final media volume, x = final cell density, F = feeding rate, S_F = substrate concentration in feed, $Y_{(x/s)}$ = g of cell per g substrate consumed, t = total feeding time, V_0 = initial reactor volume at start of feed, x_0 = initial cell density at start of feed
- In parallel duplicate reactors, LB supplemented with 100g/L glucose was added using an exponential feeding profile and the rate was calculated based on the equation – $F.Y_{(x/s)}.S_F = \mu.V_0.x_0.e^{\mu t}$; where µ = specific growth rate

Summary/ Conclusion:

FT-NIR on-line process monitoring capabilities are important for automatic control systems, process optimization, and on-line process quality assessment applications. We conceptualize the extended use of this technique for dynamic fed-batch fermentation optimization using direct control parameters such as substrate concentration and consumption rate, and product concentration and synthesis rate, to achieve improved rates, yields, and titers. This continuous on-line information would also serve to build reliable kinetic models for further optimization and scale-up studies.

Acknowledgement

The authors are grateful to Office of Biomass Processing, Energy Efficiency and Renewable Energy (EERE), at Department of Energy (DOE) under program BM0101020-05794-1004171 for funding this study at ABPDU.

*Corresponding author: PRKambam@lbl.gov, Biological Engineer - Fermentation

JBEI Annual Summer Retreat: 20th – 22nd August 2012, Monterey, CA