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

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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 quantity 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 3.7L 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 1L fermentor**

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

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**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)

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**Summary/ Conclusion:**

FT-NIR on-line process monitoring capabilities are important for automatic control systems, process optimization, and off-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.

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