Analysis of a batch fermentation process

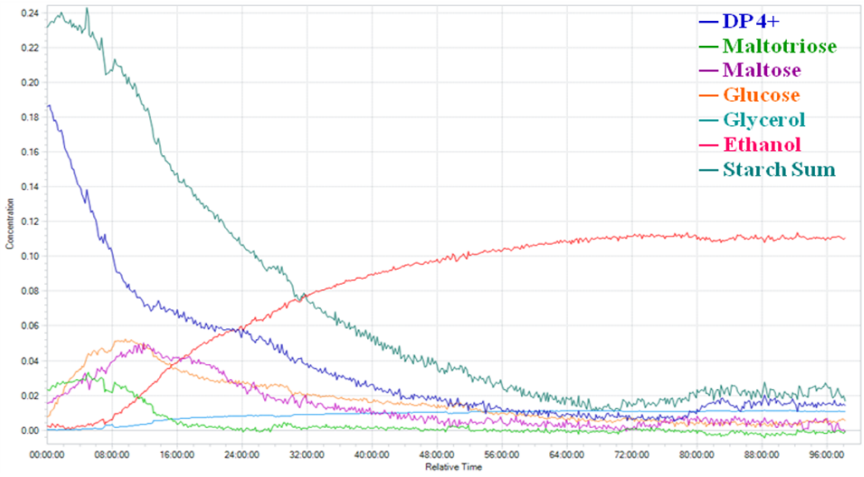

Figure 1: Raman analysis provides quantification on multiple components using a single probe

Benefits at a glance

- Real-time, in-process fermentation understanding
- Quantification of multiple components using a single probe
- Effective reduction of interfering fluorescence signal achieved using 1000 nm excitation
- Raman as a proven Process Analytical Technology (PAT)
- Real-time process and product quality assurance

Introduction

Analyzer technologies are used to gain understanding of industrial processes. This knowledge can be utilized to develop effective control limits, critical quality attributes, and rational specifications. The FDA has extended the original 2004 PAT initiative to allow bioprocess companies to benefit from a Quality by Design (QbD) manufacturing approach.

Development and application of new fermentation analytics in accordance with the aims of QbD are being investigated. Current research is focused on gaining a better understanding of fuel ethanol production via simultaneous saccharification and fermentation of very high gravity corn mash using glucoamylase enzymes and yeast. Understanding, monitoring, and controlling the stages of the fermentation process is vital to ensure healthy progression of the process. Raman spectroscopy can provide compositional information and quantification about a bioreactor's contents.

Raman advantages

Raman spectroscopy is uniquely useful for QbD applications because Raman analyzers enable fast, real-time, *in situ* monitoring and control of process environments. Raman spectroscopy is a type of vibrational spectroscopy, producing similar information to FTIR (Fourier transform infrared spectroscopy). A major advantage of Raman is its ability to measure in aqueous solutions or slurries containing water. In addition, Raman offers ease of use and flexible sampling benefits including remote location of the analyzer and use of non-contact sampling optics.

A previous limitation of using dispersive Raman for monitoring fermentation processes was fluorescence interference. Fluorescence can be reduced or eliminated by using a longer incident wavelength. In this work, a novel dispersive Raman instrument with excitation close to 1000 nm was used to monitor the fermentation of corn mash to ethanol. No fluorescence was observed in the resulting Raman spectra.

ⓘ All Raman analyzers and probes referenced in this application note are Endress+Hauser products powered by Kaiser Raman technology.

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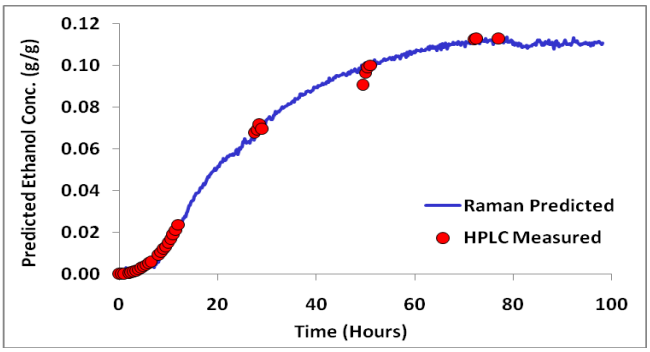

Figure 2: Raman provides quantification of process components similar to HPLC, with the benefits of real-time and in-process measurements.

Experimental

Simultaneous saccharification and fermentation of corn mash was performed using a 2L bioreactor. All conditions and practices used simulated industrial fermentation processes.

The fermentation was continuously monitored over a 72-hour period. A Raman analyzer equipped with an InGaAs array detector and a fiber-coupled *in situ* probe was used. Raman spectra were collected every 10 minutes during the 100 hour fermentation bioprocess. Grab samples were also collected and analyzed using a High Performance Liquid Chromatograph (HPLC).

A multivariate model was built to correlate Raman spectral features to HPLC measurements. HPLC measured concentrations for the components of interest which included DP4+ (higher sugar), maltotriose, maltose, glucose, glycerol, ethanol, and starch sum were correlated to Raman spectra using Partial Least Squares (PLS) multivariate calibration models. All data were analyzed using GRAMS/AI™ PLSplus/IQ™, using a 5 factor model. Solution was added as needed, in response to on-line Raman data.

Results

Raman-predicted process trends for multiple critical process components are shown in Figure 1. In Figure 2 the results of the Raman prediction are plotted against the results from off-line HPLC. These results show that *in situ* Raman analysis yields comparable results to HPLC without the drawbacks of HPLC analysis. Each of the components analyzed using the Raman method resulted in excellent correlation coefficients and standard error of cross validation values. These results demonstrate the Raman spectrum contains the necessary diagnostic information to allow the fermentation process to be monitored.

Conclusions

The results provided demonstrate the utility of Raman for real-time, *in situ* monitoring of fermentation processes. Raman offers simple and accurate analysis of aqueous-based systems, and by using 1000 nm excitation, effective quantification of the components was achieved. This application demonstrates that Raman analysis can be effectively used in biotechnology QbD settings.

The ability of Raman to measure the process *in situ* allows real-time measurements to be made. These results are available to the operator in order to make timely changes to the process control strategy in order to optimize the process in terms of improved process performance, increased product quality, and reduced operating costs. In comparison to the traditional HPLC method, Raman results can be obtained without sample extraction, without the potential for process contamination, and without the need for standard solutions and dilutions. By using Raman technology, the fermentation industry can simply determine the progress and provide data to control the fermentation process.