IN-SITU BIOREACTOR MONITORING AND CONTROL BASED ON MID-INFRARED SPECTROSCOPIC MEASUREMENTS

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Abstract:

Presented is a flexible bioreactor monitoring and control set-up consisting of a mid-infrared spectroscopic instrument equipped with an *in situ* probe and a customized bioprocess management and control environment. Reaction data addressing calibration of the mid-infrared spectra using partial least-squares regression resulted in the simultaneous estimation of glucose and lactate in animal cell cultures, glucose and biomass in *Phaffia rhodozyma*, and glucose, ethanol, glycerol, acetic acid, and biomass in *Saccharomyces cerevisiae* fermentations. A fed-batch control strategy based on the on-line estimated concentration values from the *S. cerevisiae* fermentations could be implemented to increase biomass productivity.

Introduction:

Bioprocess monitoring and control rely on the use of appropriate sensors. Ideally, these sensors should be *in situ* and measure simultaneously the concentrations of all major bioprocess metabolites with sufficient accuracy and long-term stability under minimum maintenance. Spectroscopic sensors utilizing the mid-infrared (MIR) electromagnetic range offer many advantages, including simultaneous multi-analyte determinations, *in situ* sterilizability, low maintenance during operation, and enhanced information about most biologically important species compared to near-infrared (NIR) spectroscopic sensors. Therefore, they seem to be good candidates for bioprocess monitoring.

The calibration of these sensors in bioprocess monitoring, however, is still challenging due to overlapping absorbance features and metabolic reaction induced correlations among the major metabolites. Partial least-squares regression (PLSR) is often used to calibrate spectra with overlapping absorbance features. So far, however, only a few investigators have pointed out special calibration model design considerations to circumvent the correlation problem for reactive mixtures (Amrhein, 1998; Amrhein et al., 1999) and cell culture reactions (Chung et al., 1996; Riley et al., 1997; Rhiel, 1998).

Materials and Methods:

A ReactIR^{TM} 1000 mid-infrared spectrometer (ASI Applied Systems, Millersville, MD) equipped with a diamond ATR immersion probe (DiComp^{TM} , ASI Applied Systems) and QuantIR^{TM} software (ASI Applied Systems) was used for *in situ* data acquisition and later analysis.

Investigated cultures were (1) CHO/SSF3 cells in ChoMaster HP1 medium (Ferrucio Messi Cell Culture Systems, Zürich, Switzerland) during cultivation in a 2L stirred tank bioreactor (BioLafitte, St-Germain-en-Laye, France), (2) *Phaffia rhodozyma* CBS5905T cells in defined medium (Duboc, 1997) during cultivation in a 2L RC1 calorimeter (Mettler-Toledo, Schwerzenbach, Switzerland), and (3) *Saccharomyces cerevisiae* CBS426 and *S. cerevisiae* W303-1A PVD32 cells in defined (Duboc, 1997) during cultivation in a 16L stirred tank bioreactor (Bioengineering AG, Wald, Switzerland).

A customized bioprocess management and control environment (BioOPT) was developed with LabVIEW^{TM} (National Instruments, Austin, TX).

Results:

Screening of analyte absorbance features in the MIR "fingerprint" region (1800 - 800 cm^{-1}) revealed that important carbohydrates (glucose, fructose, and sucrose), organic acids (lactic acid, acetic acid), amino acids (glutamine, asparagine, alanine), and ions (ammonium, sulfate, phosphate) had distinctive spectra. However, the absorbance features are partially overlapping. Thus, multivariate calibration methods were required.

For the CHO/SSF3 cultures and the *P. rhodozyma* fermentations, PLSR was used for calibration after pretreatment of the data, which included substraction of the initial concentration and spectrum of the respective batch from the other data of that batch

(Amrhein et al., 1999). For each culturing system, reference samples of two batch runs were used for calibration. In the case of the animal cell culture experiments, this resulted in standard errors of calibration (SECs) of 0.59, 0.51, 0.69, and 0.29 mM for glucose, lactate, ammonia, and asparagine, respectively. In the case of the *Phaffia rhodozyma* fermentations, the SECs were 0.86 and 0.27 g/L for glucose and biomass, respectively. Application of the respective calibrations to subsequent bioreactor runs resulted in standard errors of estimation (SEEs) in the same range as the SECs, which are sufficient for bioreactor monitoring.

A novel calibration strategy, combining data sets of pure component spectra with *in situ* collected reaction spectra was used to establish calibration models for the simultaneous concentration estimation of glucose, ethanol, glycerol, acetic acid, and biomass in *S. cerevisiae* fermentations. In subsequent fermentations the estimated metabolite concentrations could be used to implement a fed-batch control strategy to increase biomass productivity.

Simple calibration adjustment are expected to enable immediate monitoring of other fermentation systems, such as two-phase yeast cultures and microbial fermentations, i.e., *Bacillus sphaericus* and *Escherichia coli*.

References:

Amrhein, M. (1998). Reaction and Flow Variants/Invariants for the Analysis of Chemical Reaction Data. Doctoral thesis No. 1861, EPF Lausanne, Switzerland.

Amrhein, M., Srinivasan, B., Bonvin, D., Schumacher, M.M. (1999). Calibration of Spectral Reaction Data. *Chemometrics and Intelligent Laboratory Systems* **46:**249-264.

Chung, H., Arnold, M.A., Rhiel, M., Murhammer, D.W. (1996). Simultaneous Measurements of Glucose, Glutamine, Ammonia, Lactate and Glutamate in Aqueous Solutions by Near-Infrared Spectroscopy. *Applied Spectroscopy* **50:**270-276.

Duboc, P. (1997). Transient Growth of *Saccharomyces cerevisiae*, A Quantitative Approach. Doctoral thesis No. 1648, EPF Lausanne, Switzerland.

Rhiel, M. (1998). Application of Near-Infrared Spectroscopy to Bioreactor Monitoring. Ph.D. thesis, The University of Iowa, Iowa City, Iowa, USA.

Riley, M. R., Rhiel, M., Zhou, X., Arnold, M. A., Murhammer, D. W. (1997). Simultaneous Measurement of Glucose and Glutamine in Insect Cell Culture Media by Near Infrared Spectroscopy. *Biotechnology and Bioengineering* **55:**11-15.