BlueSens

understanding bioprocesses

Application Report

Report from

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Application Note

Real-time estimation of fungal biomass based on off gas analysis

Abstract

Real-time information on biomass concentration is of critical importance for robust control of any bioprocess. In this application report, CO_2 and O_2 off gas concentrations measured by BlueSens gas analyzer technology were used to accurately estimate biomass concentrations during a Penicillium chrysogenum fed-batch process. The estimation method is based on the combination of real-time off gas measurements, dynamic modelling of fungal biomass growth and particle filtering. Using this method, biomass concentrations were continuously monitored with an average prediction error of 3.0 %. Based on this signal process supervision and tight control of nutrient supply is possible, leading to significant savings in raw materials as well as increased process robustness, by maintaining product quality at high levels.

Introduction

Understanding bioprocesses on a mechanistic level and having critical information continuously available during the process, is the basis for rational bioprocess development and control. This information can help to ensure product quality as proposed by the Process Analytical Technology (PAT) guidance of the Food and Drug Administration (U. S. Food and Drug Administration, 2004) and to implement quality by design (QbD) principles (U. S. Food and Drug Administration, 2009). However, measuring key process variables such as biomass concentration is challenging, as off-line laboratory analysis is time consuming and available in-line sensors, e.g. light scattering and transmittance probes, are only valid within a restricted range of concentrations and are highly dependent on other process parameters.

A promising alternative to overcome these limitations, is the use of so-called soft sensors. By combining a mathematical description of the process and available measurements, process variables of interest can be estimated in real-time. In the simplest case, biomass formation can be directly linked to the respiratory activity expressed as Oxygen Uptake Rate (OUR) and Carbon Evolution Rate (CER) measurable with BlueSens gas analyzers. In the case of non-uniform biomass growth, like hyphal growth of fungi, the establishment of a soft sensor requires the implementation of a state observer algorithm (Ali et

al., 2015). These algorithms, including the well-known Kalman filter and the novel particle filter rely on Bayesian principles, which allow to estimate the most probable state based on a mathematical description of the system and partial measurements. Within this application report it is described how BlueSens O2 and CO2 measurements can be used to estimate Penicillium chrysogenum biomass concentrations by employing a particle filter algorithm.

Working Principle and Implementation

A structured model was used to describe growth and production behavior of penicillin producing Penicillium chrysogenum. The model is schematically depictured in Figure 1 and is based on the mathematical descriptions, which can be found in Kager et al. (2018) and Paul et al. (1998). Hyphal growth is hereby characterized by branching (rb) and extension (re) of hyphal tips (A0) and differentiation (rd) into hyphal bodies (A1) under the usage of different substrates (S1 and S2). During growth O_2 (rO2) is consumed and CO_2 (rCO2) is produced which can be quantified by off gas analysis.

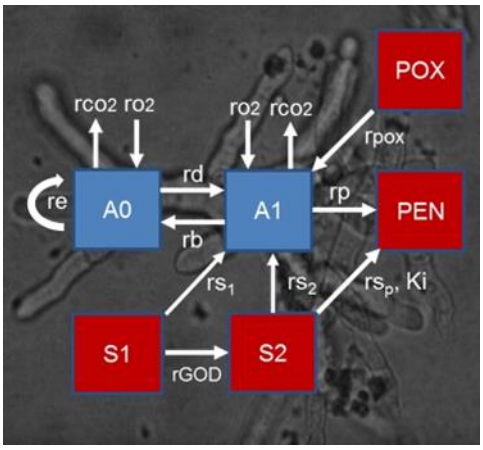

Figure 1: Schematic representation of the mathematical description of hyphal growth and penicillin production.

By solving the model during a running process, a theoretical signal ($\hat{y}$) for OUR (rO2) and CER (rCO2) can be calculated. By comparing them with the actual CER and OUR measurement (y), calculated according to the approach of Aehle et al. (2011), information on the current model validity can be obtained. With the knowledge of measurement and model uncertainty the most probable system state can be calculated by a filtering procedure. The principle of the used particle filter is depicted in Figure 2. Particle filtering is a sequential Monte-Carlo method. The most probable culture state is hereby approximated by a large number of weighted points in the sample space ("particles") which evolve within the known process and measurement noise.

After propagating every single particle in time, particles ($\hat{y}$t+1) can be filtered (selected), which are in good alignment with the measured states (yt+1). As the measured states (y = CER and OUR) are a function of the other, unmeasured states ($\hat{y} = f(A0, A1)$), most probable estimates can be obtained from the filtered particle distribution.

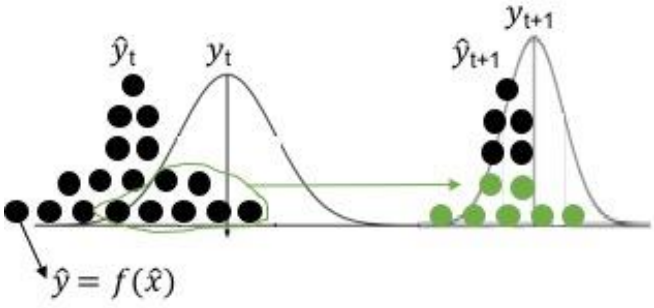

Figure 2: Particle filter principle were the state distribution is approximated by the particles represented as dots and filtered according to their alignment with the measurement distribution (y_t). From the filtered particles a state estimate (x) can be deduced.

For real time application of the described soft sensor, a bidirectional OPC connection was established between BlueVis software, the reactor system (30 L Techfors; Infors AG, Switzerland) and a numerical computing software (MATLAB R2017b; Mathworks, USA). Data was imported into MATLAB every 30 seconds. Besides off gas data, feed rates based on balance signals as well as gas flow rates based on mass flow controller data were needed as input data for the particle filter. Figure 3 gives an overview on the setup. A particle filter method with 500 particles based on the bootstrap algorithm as described by Gordon et al. (1993) was used for estimation of unmeasured biomass concentrations.

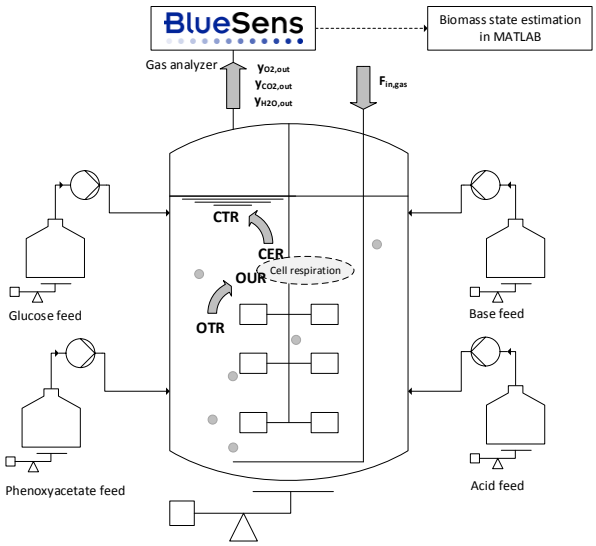

Figure 3: Overview of setup for real-time biomass state estimation using off gas data during a Penicillium chrysogenum fed-batch process.

Fermentations using the spore suspension of an industrial Penicillium chrysogenum strain were performed in a stirred tank bioreactor in batch and fed-batch mode. After the batch phase, cells were harvested and the reactor was filled up with 11 L sterilized defined medium and inoculated with 160 mL L-1 cultivation broth from the batch culture. The culture was controlled at a temperature of 25 °C, pH of 6.5 and a dissolved oxygen concentration of above 40 % by stirrer speed (350-800 rpm). Air inflow was controlled at 1 vvm by a mass flow controller (Voegtlin GmbH, Switzerland). Glucose (500 g L-1) and phenoxyacetate (160 g L-1) were used as feeding solutions. Further details on the process are described in Posch and Herwig, 2014. At-line samples were taken every 12 hours and biomass dry weight (gravimetrically) and penicillin and phenoxyacetate (HPLC, ZORBAX C-18 Agilent column) were determined in triplicates. Off gas was analyzed by a BlueSens gas analyzer (BlueSens GmbH, Germany) using a ZrO_2 sensor for O_2 and an infrared sensor for CO_2 analysis.

Results and Discussion

Real-time biomass estimation was performed for a high cell density Penicillium chrysogenum penicillin production process. As it can be seen in Figure 4 and Figure 5, the inclusion of real-time CO_2 and O_2 data from a BlueSens off gas analyzer adjusts the modelled CER and OUR outputs in a way that discrepancies to measured CER and OUR are lowered. Through this filtering procedure, based on the described particle filter, the model output can be corrected with the information contained in the off gas measurements during the running process.

A comparison between resulting biomass estimates and model simulation without any adaption is shown in Figure 6. Especially during the first 45 hours considerable differences between offline measured biomass and model simulations without off gas data occur. This results in a model error of 1.42 g L-1, corresponding to a normalized root mean squared error (NRMSE) of 5.7 %. If the model outputs are filtered based on the off gas data, biomass state estimation errors can be reduced by approx. 50 %. Thereby, biomass can be estimated with an error of only 0.75 g L-1, corresponding to a NRMSE of 3.0 %.

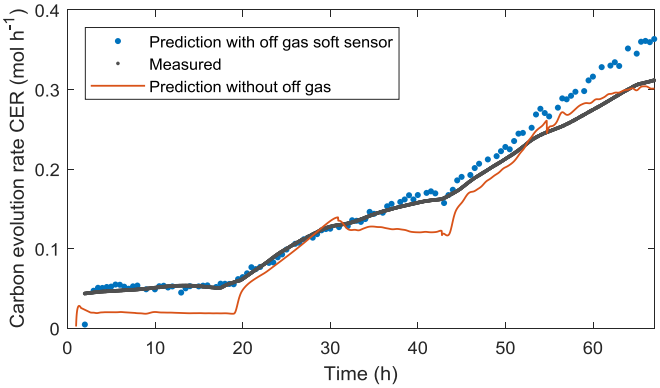

Figure 4: Time course of carbon evolution rate (CER) during a P. chrysogenum fed-batch process, comparing measurements (grey dots) and model-based predictions with real-time off gas data input (blue dots) and without off gas data input (orange line).

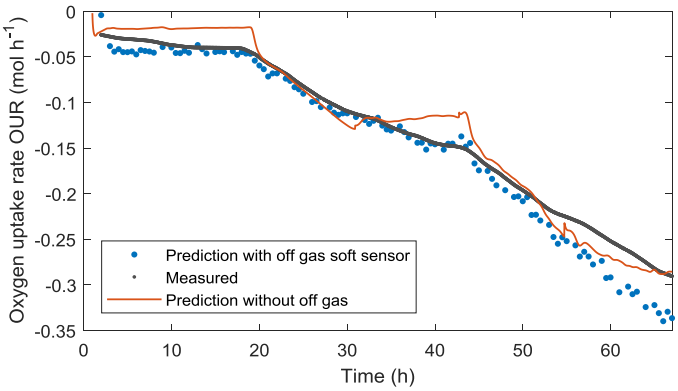

Figure 5: Time course of oxygen uptake rate (OUR) during a P. chrysogenum fed-batch process, comparing measurements (grey dots) and model-based predictions with real-time off gas data input (blue dots) and without off gas data input (orange line).

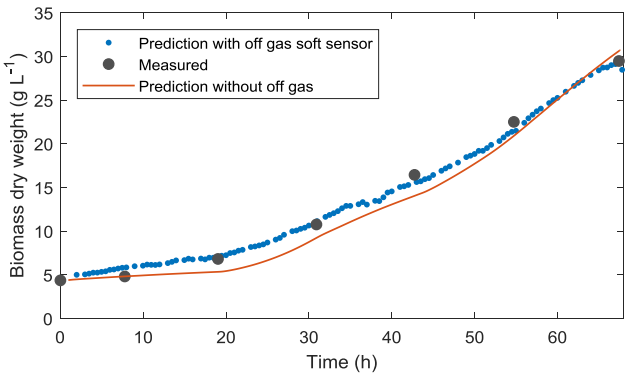

Figure 6: Time course of biomass dry weight during a P. chrysogenum fed-batch process, comparing measurements (grey dots) and model-based predictions with real-time off gas data input (blue dots) and without off gas data input (orange line).

Industrial Relevance

BlueSens based off gas analysis in combination with process models offer the possibility to accurately and continuously estimate culture states, such as biomass formation and nutrient consumption.

This is of considerable relevance for industry as decisions and process control based on these continuous estimates can lead to more efficient and robust processes.

As reported by Biwer et. al, 2005 the raw material costs of raw and starting materials are main costs of the analyzed fermentation process. Therefore, the presented approach was successfully transferred to a large-scale industrial facility with the aim to control the glucose and precursor addition based on the continuous biomass estimate. This successful implementation led to considerable cost savings and increased process robustness. Specifically, batch failures due to wrong nutrient addition could be reduced and the overall raw material needs were lowered, while at the same time maintaining the product quality of the fermentation process.

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