

Increasing the predictivity of kinetic models for high-cell-density cultivations

Harvey Arellano-Garcia,^{a+} Anja Drews,^{b+} Udo Schubert,^{a+} Günter Wozny,^{a+}
Matthias Kraume^{b+}

^a Dept. of Process Dynamics and Operation, KWT 9, arellano-garcia@tu-berlin.de

^b Dept. of Chemical Engineering, MA 5-7, anja.drews@tu-berlin.de

⁺ TU Berlin, Straße des 17. Juni 135, 10623 Berlin, Germany.

Abstract

In this work, an optimization-based approach is presented which recognises the switching to new parameters or even to a different model at a certain growth rate improving the quality of model prediction for different time horizon lengths. For the dynamic automatic adjustment to changing kinetics, a moving horizon estimator (MHE) is used. Experimental data from cultivation of *Ustilago maydis* are used for the model-based parameter identification. The embedded MHE was successfully applied to predict changes in biokinetic constants during membrane bioreactor (MBR) fermentation when very low growth rates and therefore changes in metabolism occur. Setting suited horizon lengths and parameter bounds were found to be crucial for convergence and parameter estimation. The expected drop in maintenance parameters at low growth rates was confirmed when using an optimum number of data points.

Keywords: moving horizon estimation, biokinetics, fermentation, membrane bioreactor

1. Introduction

During high-cell-density cultivations, which are becoming increasingly popular in biotechnology and wastewater treatment in membrane bioreactors (MBR),

very low growth rates and changes in cell metabolism occur [1]. While knowledge on near zero-growth states is scarce it is clear that the emerging phenomena cannot be sufficiently described by kinetic models used during earlier phases in the process when growth rates were higher. Therefore, process monitoring and control requires switching to new parameters or even to a different model at a certain growth rate [2]. Growth rate, however, is a value which cannot be determined directly online. A model-based identification approach utilising online data is thus needed [3]. In this work, novel numerical strategies are presented which recognise the switching time and improve the quality of model prediction for different time horizon lengths. For the first time, such methods are applied to biological processes.

2. Problem Statement

For design, monitoring, and control of a biological process, reliable models are required. Balance equations for the individual components (biomass, nutrients, and metabolites) are coupled via yield coefficients Y . These are defined as the rate of change in one concentration over the rate of change in another. Biomass yields from substrate uptake can be considered constant over wide ranges of growth rates. However, especially at very low growth rates, other phenomena must be taken into account. To describe such phenomena, Pirt [4] introduced the maintenance concept whereby part of the substrate is always used for cell survival and not for reproduction, the corresponding substrate uptake rate (expressed as specific rate $k_{m,s}$) therefore only yielding energy for maintenance processes. $Y_{B/S}^g$ represents the true yield which relates the formed biomass B to the substrate mass S used for growth (superscript g) as opposed to maintenance purposes. According to Pirt [4], the substrate uptake rate can be expressed as:

$$-\dot{r}_S = \frac{\dot{r}_B}{Y_{B/S}^g} + k_{m,s} \cdot C_B \quad (1)$$

Fig. 1 clearly shows that long-term limited cultures cannot be described by parameters (in this case $k_{m,s}$) optimised for short-term limited cultures and early process phases. To overcome this problem, a strategy is required to improve the predictivity of kinetic models.

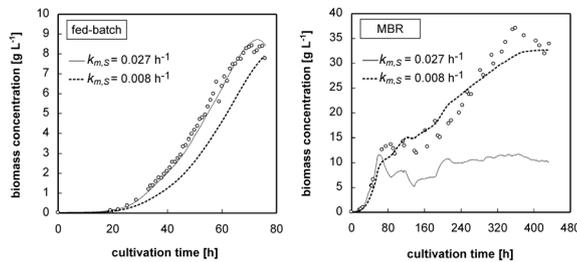


Fig. 1: Model-based prediction of biomass concentration in short-term (fed-batch) and long-term (MBR) limited cultures [2].

3. Solution approach

In this work, experimental data from *Ustilago maydis* cultivations were used for model-based parameter identification and to assess the efficiency of different estimation methods. High cell densities were achieved by using an MBR.

3.1. Methodology

Moving horizon based on-line state estimations have been successfully implemented for several applications [7-10], showing an advantage over extended Kalman-filtering because of robustness despite poor initial values and the comfortable use of constraints on state and parameter variables. Considering only recent measurements for the estimation of kinetic parameters, it is possible to recognize values that change during the progress of the estimation time frame. To overcome the above mentioned problem, we propose an optimization-based approach to improve the predictivity of kinetic models based on available measurements together with a process model. The algorithm presented uses a moving horizon-based approach to estimate kinetic parameters of the nonlinear model. A constrained least squares estimation, acc. to the computational framework we presented in [6], is performed, but without estimation of the input variables and assuming no noise or disturbances in the measurements. The general moving horizon formulation follows [9] in using a fixed number of recent measurements for the estimation, resulting in a moving time frame that keeps progressing as cultivation time proceeds during the tested experiments.

3.2. Experimental arrangement

U. maydis was stored in glycerol stocks (25%) at $-80\text{ }^{\circ}\text{C}$. After a 3-day inoculation on potato-dextrose-agar cells were transferred for approx. 24 h into shaking flasks containing a defined medium with glucose as the main carbon source (100 min^{-1} , $27\text{ }^{\circ}\text{C}$). For cultivation, a 5 L glass fermentor (B.Braun Int., Germany) was used (see Fig. 2). In MBR runs, this was equipped with an external ceramic tubular membrane module for biomass retention (Pall Schumacher, Germany). Temperature was controlled at $27\text{ }^{\circ}\text{C}$, pH at 7.2, and pO_2 at 40 %. Biomass concentration was determined by turbidity measurements at 600 nm (UV-120-01, Shimadzu) calibrated against dry weight measurements. Substrates and nutrients concentrations were determined using test kits (liquicolor, Human GmbH, Germany; LCK 303/304, Dr. Lange GmbH, Germany).

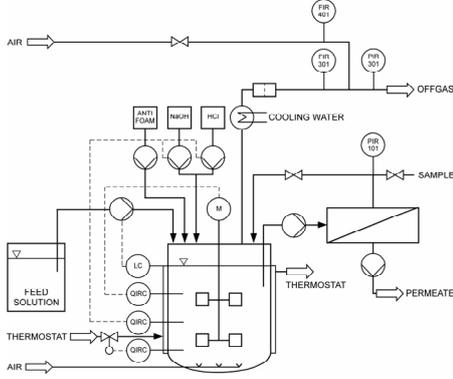


Fig. 2: Experimental set-up (MBR flow sheet).

A model was developed to describe the considered MBR process at the given conditions [5] including mass balances and kinetics (eqs. 2-11), with the kinetic parameters μ_{max} , K_i , Y_i and k_i being subject to possible changes during the fermentation.

$$\frac{dV_R}{dt} = \dot{V}_{in} - \dot{V}_{Permeate} - \dot{V}_B \quad (2)$$

$$V_R \cdot \frac{dc_B}{dt} = -\dot{V}_B \cdot c_B + \dot{r}_B \cdot V_R \quad (3) \quad \dot{r}_B = \mu \cdot c_B \quad (7) \text{ growth rate}$$

$$V_R \cdot \frac{dc_C}{dt} = \dot{V}_{in} \cdot (c_{C,in} - c_C) + \dot{r}_S \cdot V_R \quad (4) \quad -\dot{r}_S = \frac{\dot{r}_B}{Y_{B/S}^g} + k_{m,S} \cdot c_B \quad (8) \text{ substrate uptake rate}$$

$$V_R \cdot \frac{dc_N}{dt} = \dot{V}_{in} \cdot (c_{N,in} - c_N) + \dot{r}_N \cdot V_R \quad (5) \quad -\dot{r}_N = Y_{N/B} \cdot \dot{r}_B \quad (9) \text{ ammonia uptake rate}$$

$$V_R \cdot \frac{dc_P}{dt} = -\dot{V}_{in} \cdot c_P + \dot{r}_P \cdot V_R \quad (6) \quad \dot{r}_P = Y_{P/B}^g \cdot \dot{r}_B + k_P \cdot c_B \quad (10) \text{ production rate}$$

$$\mu = \mu_{max} \cdot \frac{c_C}{c_C + K_{S,C}} \cdot \frac{c_N}{c_N + K_{S,N} + \frac{c_N^2}{K_{I,N}}} \cdot \frac{c_P}{c_P + K_{S,P} + \frac{c_P^2}{K_{I,P}}} \quad (11) \text{ specific growth rate}$$

3.3. Results and discussion

Figs. 3 - 5 show the computed biomass, glucose and ferrichrome concentrations for two fed-batch (FB1 und FB2) and two MBR cultivations (MBR1 and MBR2) along with measurements. In general, experimental data are well represented. As can be seen, the extent of deviations from measurements changes with the used horizon length. Local optima seem to exist for the horizon length: For the product concentration in FB1, e.g., the estimation using 8 data points does not lie between the curves for 9 and 7 data points (see also glucose in FB1 and MBR2).

The changing kinetic parameters are plotted in Fig. 6 for FB2 and MBR2. It was expected that maintenance parameters drop as specific growth rate decreases. This is clearly confirmed for MBR2 when using an optimum number of data points for the moving time frame (in this case 10). At approx. 75 h, $k_{m,S}$ abruptly drops from around 0.045 to 0.022 h⁻¹ and $Y_{B/S}^g$ from 0.55 to 0.3. Using other time frames can cause large overestimations (in this case approx. 50 %). The sensitivity increases with a decreasing number of data points. However, measurements errors can cause large fluctuations here, whereas they get dampened when using more points.

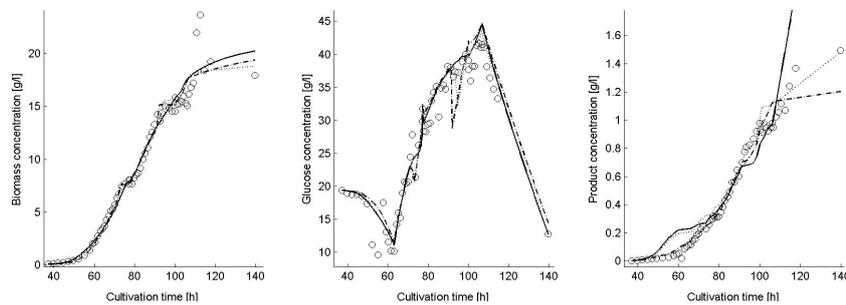


Fig. 3: Ammonium-limited fed-batch experiment FB1 (—: 9, ---: 8,: 7 data points).

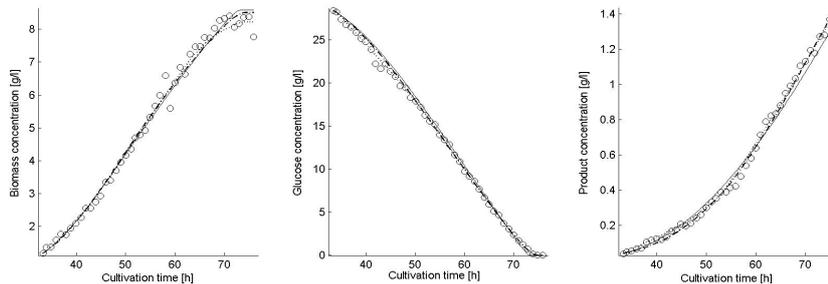


Fig. 4: Glucose-limited fed-batch experiment FB2 (—: 30, ---: 20,: 10 data points)

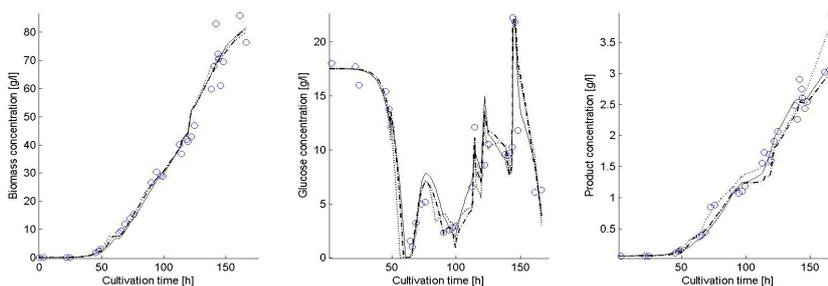


Fig. 5: Continuous cultivation experiment MBR1 (—: 8, ---: 7,: 6 data points).

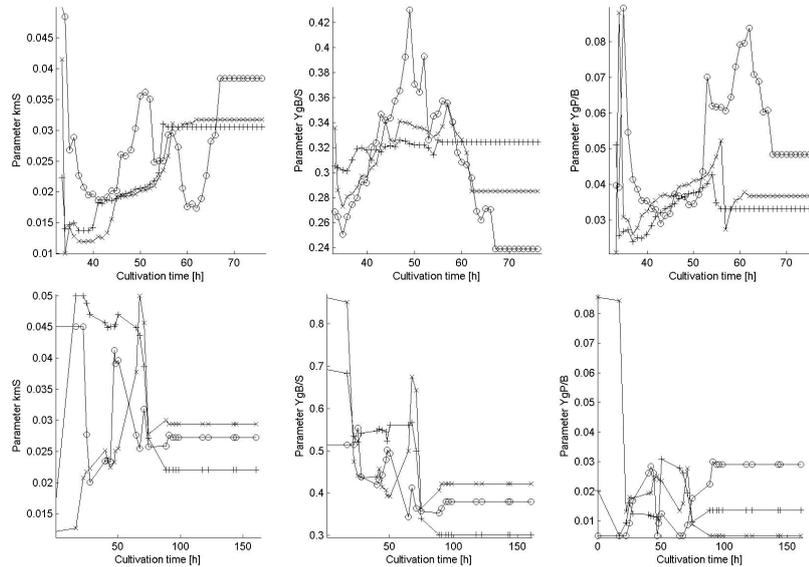


Fig. 6: Parameters estimated for FB2 (o: 10, x: 15, +: 20 Pts) and MBR2 (o: 8, x: 9, +: 10 Pts).

4. Concluding remarks

Moving horizon estimation was successfully applied to predict changes in biokinetic constants during up to 170 hours of fermentation in an MBR. Setting a suited horizon length and parameter bounds was found to be crucial both for convergence of the simulation layer and good estimation results for the parameters. It was expected that maintenance parameters drop as specific growth rate decreases. This was clearly confirmed for MBR2 when using an optimum number of data points. The developed approach is being extended to determine the varying kinetics based on online respiration data to increase the predictivity of long-term limited cultures and to enable a model-based control.

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