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Model-based determination of changing kinetics in high cell density cultures using respiration data

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Abstract

During high cell density cultivations, very low growth rates and changes in cell metabolism occur. These have to be accounted for in the kinetic modelling. In this work, an optimisation-based approach is presented which recognises the switching to new parameters at a certain growth rate and thereby improves the quality of the model prediction for different time horizon lengths. For the dynamic automatic adjustment to changing kinetics, a moving horizon estimator (MHE) is applied. Experimental data from cultivations of *Ustilago maydis* are used for the model-based parameter identification. To validate the method, initially offline data are utilised. In the next step, respiration data, which are available online, are used to enable real-time monitoring. The embedded MHE was successfully applied to predict changes in biokinetic constants during membrane bioreactor (MBR) fermentation. Setting suited horizon lengths and parameter bounds was 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: Biokinetics; Fermentation; Membrane bioreactor; High cell density; Maintenance parameter; Carbon dioxide evolution rate

1. Introduction

During high cell density cultivations, which are becoming increasingly popular in biotechnology and wastewater treatment, e.g., in membrane bioreactors (MBR), very low growth rates and changes in cell metabolism occur (e.g., Ihssen and Egli, 2004). Due to the rising biomass concentration each cell is subjected to an increasing substrate limitation which leads to a decrease in growth rate down to zero growth. In this region, maintenance metabolism where substrates are used for cell survival instead of growth, and which always takes place in parallel to growth metabolism, gains higher importance. Very low growth rates are often encountered in nature, but the consequences for technical applications have not been described as exhaustively as those at higher growth rates or at starvation conditions (Konopka, 2000). By quorum sensing or stringent response, high cell densities or progressing limitations can also lead to a change in metabolism (e.g. van Verseveld et al., 1984). This means that kinetic parameters determined in common experiments like fed-batch and chemostat do not sufficiently describe conditions, e.g., in MBRs or other high cell density processes. If a change in growth behaviour occurs at low growth rates, a change in production behaviour is of course conceivable (Drews and Kraume, 2005), too, and must be borne in mind when designing a production process. 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. Growth rate, however, is a process characteristic which cannot be determined directly online.

A model-based identification approach utilising online data is thus needed (Sun et al., 2006). The realisation of such

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approaches requires information about the current state of the process. It is usually assumed that the state can be gained through measuring essential process variables. In many cases like biotechnological processes, however, it is not possible to measure all required variables, especially in on-line applications where a frequent update of measurements is necessary. A broad variety of methods have been proposed to address this problem, ranging from structural or non-structural observability analysis to various kinds of state observers such as the extended Kalman filter. The drawback of these methods lies in the fact that they either give only information about which variables should be additionally measured so as to be able to compute the unmeasured variables (with the method of structural observability analysis) or are only applicable to linear or linearised process models (with the method of state observers). Biotechnological processes, however, are often of a strong nonlinear nature. In this work, novel model-based numerical strategies are presented which recognise the switching time and improve the quality of model prediction for different time horizon lengths.

2. Biokinetic background

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 are 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 (1965) introduced the maintenance concept whereby part of the substrate is always used for cell survival and not for reproduction. The corresponding specific substrate uptake rate (expressed as specific rate $k_{m,S}$) therefore only yields energy for cell maintenance processes. $Y^g_{B/S}$ is the so-called 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 this, the substrate uptake rate $\dot{r}_S$ can be expressed as:

$$-\dot{r}_S = \frac{\dot{r}_B}{Y^g_{B/S}} + k_{m,S} \cdot c_B. \tag{1}$$

Several other possible mechanisms (endogenous metabolism, cryptic growth) have been reported (Herbert, 1958; Van Loosdrecht and Henze, 1999). Whichever phenomenon prevails, a large number of processes can be satisfactorily described by the Pirt concept. It has often been observed and described that $k_{m,S}$ is not constant (Pirt, 1965; Tijhuis et al., 1993). Deviations from the linear relationship between $\dot{r}_s$ and $\dot{r}_B$ seem to occur when growth is limited by a substrate or nutrient other than the energy substrate (Bulthuis et al., 1989; Pirt, 1982). Ihssen and Egli (2004), however, state that the specific growth rate and neither the nature of the limiting nutrient nor cell density is responsible for the relevant stress response. This is in agreement with Drews and Kraume (2007) who observed no significant differences in maintenance parameters for glucose or ammonia limitation, respectively. Below $\mu_{crit} \approx 10\%\ \mu_{max}$ microorganisms undergo severe changes in metabolism (Van Loosdrecht and Henze, 1999; van Verseveld et al., 1984; Konopka, 2000). Applying the Pirt concept, several authors have reported a significant reduction of maintenance demand at very low growth rates in comparison to chemostat data (Pirt, 1987; Bulthuis et al., 1989; van Verseveld et al., 1984; Low and Chase, 1999; Müller and Babel, 1996; Tros et al., 1996; Drews and Kraume, 2007).

Fig. 1 clearly shows that long-term limited cultures cannot be described by parameters (in this case $k_{m,S}$) determined for short-term limited cultures and early process phases. While the simulation using $k_{m,S} = 0.027\,h^{-1}$ which was found as the optimum parameter for a fed-batch culture (left) is close to the MBR data (right) in the beginning, it strongly underestimates growth after 70 h when limitations get so severe that the specific growth rate μ drops below its critical value. Such a strong limitation only prevailed in the final few hours of the fed-batch run and therefore did not affect the parameter fit. On the other hand, in the MBR run the microorganisms are subjected to strong limitations for most of the cultivation time, and a value of

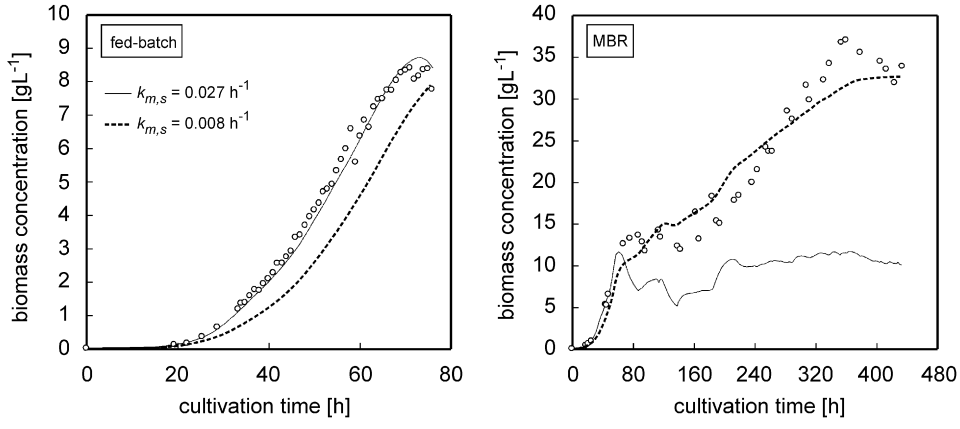

Fig. 1. Model-based prediction of biomass concentration in short-term (fed-batch) and long-term (MBR) limited cultures (Drews and Kraume, 2005).