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Model based substrate control of cultivation processes

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Introduction

For the control of glucose concentration during a cultivation process based on its direct measurement just few examples are published. This is due to the fact that glucose measurement with available analysers is afflicted with a significant noise and time delay of more than five minutes. This makes the control task a challenge.

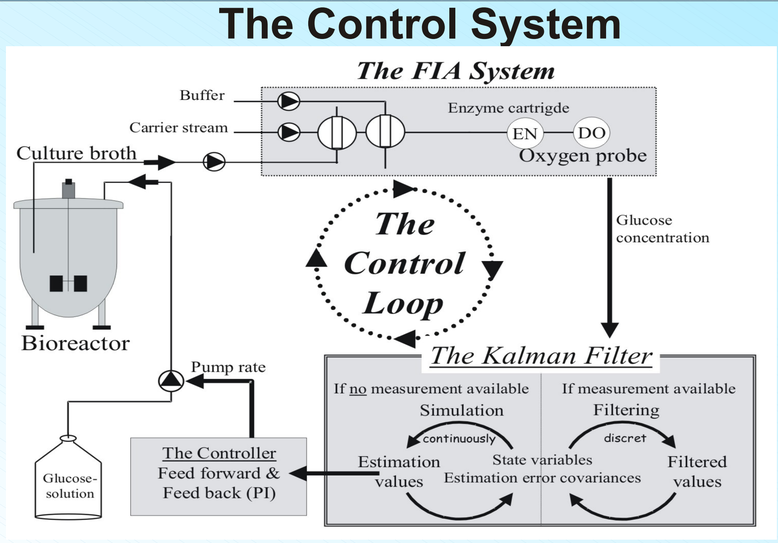
In this contribution we present a control system based on a glucose FIA measurement system. The FIA is supplied with a cell free sample stream *via* a sampling module, which results in a time delay of six minutes. The controller compensates the time delay with the help of an extended Kalman filter with an implemented bioprocess model for the estimation of biomass, substrate and the maximal growth rate.

The system was tested during yeast fed batch cultivations in a 2.5 L stirred tank reactor.

The Control System

The adjoining Figure outlines the structure of the control loop, which consists of the reactor, the FIA system for the glucose measurement, the Kalman filter, the feed forward & feed back controller and of the feeding pump.

The task of the Kalman filter is to provide a current estimation of the bioprocess variables glucose (S(t)), biomass (X(t)), volume (V(t)) and maximal growth rate ($\mu_{max}(t)$). The feed back controller is implemented as a PI controller. The following equations represent the bioprocess:



$$\begin{bmatrix} \frac{d\,X(t)}{d\,t} \\ \frac{d\,S(t)}{d\,t} \\ \frac{d\,\mu_{max}(t)}{d\,t} \\ \frac{d\,V(t)}{d\,t} \end{bmatrix} = \begin{bmatrix} \frac{\mu_{max}\,S(t)}{K_m + S(t)} X(t) - \frac{\dot{V}(t)}{V(t)} X(t) \\ -\frac{\mu_{max}\,S(t)}{K_m + S(t)} \frac{X(t)}{Y} + \frac{\dot{V}(t)}{V(t)} (S_0 - S(t)) \\ 0 \\ \dot{V}_f(t) - \dot{V}_{sam} \end{bmatrix} + \begin{bmatrix} u_X \\ u_S \\ u_\mu \\ u_V \end{bmatrix}$$

As the glucose concentration is analysed every few minutes, the estimation of the substrate concentration is corrected by the Kalman filter considering the measured values.

Methods

The control system was tested during several fed batch cultivations with set points between 0.07 g/L and 0.5 g/L. The cultivation (pH 5.5, aeration 5 L/min, 30°C, stirring speed 1200 rpm) of *Saccharomyces cerevisiae* was carried out in a 2.5 litre stirred tank reactor (Biostat B, Sartorius, Göttingen). A mineral medium with glucose as only carbon source was used. The feed solution was transported from the reservoir to the reactor with a pump, which was operated directly by the controller.

The fed batch phase was preceded by a pre-culture in a shaking flask and a batch phase. During the cultivation with the set point 0.07 g/L, the cells were starved for 4 hours after the end of the batch phase. The fed batch phase of the second cultivation, with a set point of 0.5 g/L, was started directly out of the batch phase.

Results

Glucose set point 0.07 g/L

The controller system was tested during cultivations with various set points. The result of two fed batch cultivations with set points of 0.07 g/L and 0.5 g/L are presented.

Figure 1 shows the measured values of the glucose concentration throughout the controlled phase of the cultivation. The values fluctuate near the set point. The mean value of the measured glucose concentration is 0.070 g/L with a standard deviation of 0.002 g/L or 3 %, not counting the first hour, which is regarded as rise time. This means that the standard deviation is lower than the measurement error of the FIA, which was considered to be 0.045 g/L. The obtained yield coefficient of 0.68 g/g is very high. However, in other cultivations with set points below 0.1 g/L similar values have been achieved. The high yield coefficient is due to an efficient avoidance of side products.

The evolution of the estimated biomass concentration corresponds with the off-line measurement as well (data not shown).

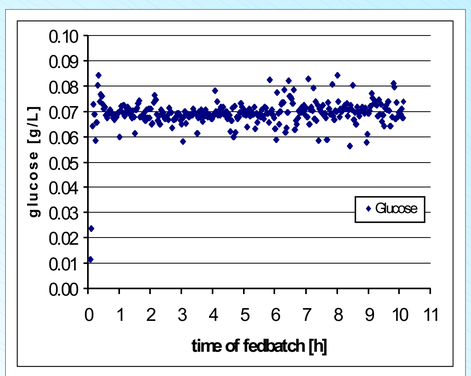


Figure 1: set point 0.07 g/L, on-line FIA measurement

Glucose set point 0.5 g/L

While the yeast metabolism during the cultivation with a set point of 0.07 g/L was purely oxidative, the cells were in an oxidative-reductive state during the cultivation at 0.5 g/L. The process therefore showed a different and higher dynamic. Nevertheless, the Kalman filter proved to be equal to this challenge. The mean value of the measured glucose values was 0.52 g/L with a standard deviation of 0.022 g/L (5 %). As the cells showed an oxidative-reductive metabolism, ethanol and acetate were produced. The maximum ethanol concentration was 11 g/L. Altogether, the estimation for biomass of the Kalman filter and the off-line data match except for the off-line values between 2 h and 3 h, which are a little higher than the estimation (Data not shown). This is probably due to the fact that the real growth rate of the cells rose faster than the estimated growth rate. The yield coefficient was 0.2 g/g for biomass and 0.15 g/g for ethanol.

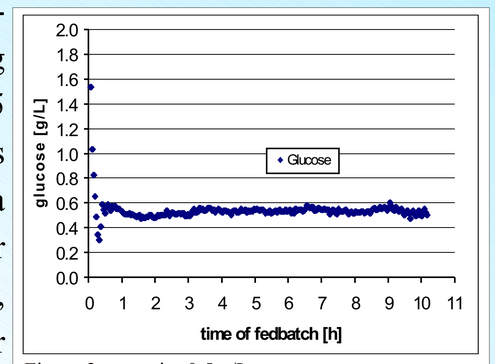


Figure 2: set point 0.5 g/L, on-line FIA measurement

Summary

The controller is capable to compensate the time delay of the measurement unit and to maintain the substrate concentration at the selected set point. During the control phase the standard deviation of the measurements are 0.002 g/L (3%) and 0.022 g/L (5%) for the set points of 0.07 g/L and 0.5 g/L respectively. The dynamic of the process was covered correctly and the estimation of the biomass evolution was accurately, both under oxidative and oxidative-reductive conditions. For the cultivation with a set point of 0.07 g/L, a high yield coefficient of 0.69 g/g was obtained.