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# ARE MONOD MODELS ENOUGH FOR BIOREACTOR CONTROL? PART I – EXPERIMENTAL RESULTS

**C. Klockow[1], D. Hüll[1], L. S. Ferreira[2], J. O. Trierweiler[2], B. Hitzmann[1]**

[1]*Institut für Technische Chemie, Leibniz Universität Hannover,*
*Callinstr. 3, 30167 Hannover, Germany*
*Phone: 0049 511 762 2963 - Fax: 0049 511 762 3004*
*E-MAIL: Hitzmann@iftc.uni-hannover.de (corresponding author)*

[2]*Grupo de Integração, Modelagem, Simulação, Controle e Otimização de Processos (GIMSCOP)*
*Departamento de Engenharia Química, Universidade Federal do Rio Grande do Sul (UFRGS)*
*R. Luis Englert, s/n. Campus Central. CEP: 90040-000 - Porto Alegre - RS - BRASIL,*
*E-MAIL: {luciane, jorge}@eng.ufrgs.br*

***Abstract:*** A model based substrate control system for yeast fed batch cultivations is presented. The control system consists of a FIA measurement system, which get a cell free sample stream from the culture broth due to a probing system with a time delay of 360 s. The glucose measurement values were utilized by a discrete-continuous extended Kalman filter, which applied the typical Monod model. The estimated values were applied to run a feedforward as well as feedback PI controller for set point control at 0.05 g/L glucose. The mean glucose measurement was 0.051 g/L with a standard deviation of 0.009 g/L. Therefore, the Monod model seems to be adequate for control, however the specific growth rate itself was not constant neither was the specific uptake rate of oxygen and the specific production rate of carbon dioxide constant, which indicate metabolic changes during the cultivation. 

***Keyword:*** Extended Kalman filter, yeast fermentation, substrate control, time delay estimation, feedforward feedback controller, PI controller

## 1 INTRODUCTION

The set point control of the substrate concentration during bioprocesses is a matter of particular economic and scientific interest. It plays an important role for industrial processes such as yeast fermentation or biotransformations in general (Cooney *et al.*, 2002; Miskiewicz & Kasperski, 2000; Rani & Rao, 1999). Regarding the production of yeast, the biomass yield can be raised from roughly 20 % to approximately 50 % if the glucose concentration is kept below a certain level. This is due to the fact that yeast changes its metabolism from oxidative to oxidative-reductive and produces by-products like ethanol and acetate, if the substrate concentration is above the critical level (Sonnleitner

& Käppeli, 1986). The research takes an interest in exploring metabolic mechanisms or the specific expression of genes and proteins in dependency on the substrate concentration. Yeast also acts as a valuable model organism for 58 eukaryotic microorganisms. A large range of control systems has been tested for substrate control (Bastin & Dochain, 1990; Renard *et al*., 2006; Lidgren *et al*., 2006). Diverse feeding strategies are based upon the detection of changes in the metabolism (Åkesson *et al*., 1999; Hantelmann *et al.*, 2006) or the growth rate (Levisauskas, 2001). Fast FIA-systems, which use a cell containing sampling stream, have also been introduced (Arndt & Hitzmann, 2004). However, this solution contains the risk of blocking as the cells may form clusters in the tubes and thereby cause an illfunction of the measurement. In addition, the cells continue to consume glucose on their way to as well as in the FIA, which results in an inaccurate measurement, which is systematically too low. The control system presented by Åkesson (1999) can just provide set points at a level were the metabolism is changing. This is different to the present investigation, where the set point can be selected arbitrarily.

## 2 KALMAN-FILTER WITH TIME DELAY OF MEASUREMENTS

The control system presented in this work combines a FIA-system furnished with a sampling module for the on-line analysis of glucose with a controller composed of a bioprocess model and an extended Kalman filter for the estimation of biomass, glucose concentration, broth volume and growth rate. Based on the estimated glucose concentration a feed forward controller is implemented. In addition, a PI-controller is employed to fix the substrate concentration at the desired set point. The FIA system is supplied with a cell free sample stream assayed by a sampling device with a ceramic membrane. The data output is collected and processed by the software CAFCA that determines the glucose concentration and forwards the result to the controller system.

In contrast to systems proposed in literature (Arndt & Hitzmann, 2004; Arndt *et al.*, 2005), the Kalman filter implemented here accounts for the time delay of the measurements. In this investigation the time delay is six minutes, but it can be even much more. During six minutes the glucose consumption of a cell concentration of X=10 g/L at a growth rate of $\mu$=0.1 1/h and a yield factor of Y=0.5 $g_{cell}/g_{glucose}$ is $c_{glucose}$=0.2 g/L. This is four times the amount of the set point of 0.05 g/L, which is used in this application. Therefore, it has to be compensated by the controller system. To consider it, the Kalman filter contains a ring buffer in which the historic estimated bioprocess variables are stored. This will be discussed in detail below.

In the Kalman filter the bioprocess variables are obtained via simulations with a bioprocess model which estimates the non-measurable variables maximum growth rate, biomass and volume as well as the current glucose concentration. For the specific growth rate the Monod model was used. As can be seen in Equation 1, the model of the process is complemented with terms for the feed and the sample stream. The filter is supplied with the initial values for the biomass X, the broth volume V and the glucose concentration S and a suitable initial value for the maximal growth rate $\mu_{max}$ is provided.

In the first component of the vector equation the evolution of the biomass is considered. It is composed of a term for the growth based on the Monod model plus a term for the dilution by the feed solution.

$$\begin{bmatrix} \frac{d\,X(t)}{d\,t} \\ \frac{d\,S(t)}{d\,t} \\ \frac{d\,\mu_{max}(t)}{d\,t} \\ \frac{d\,V(t)}{d\,t} \end{bmatrix} = \begin{bmatrix} \frac{\mu_{max}\,S(t)}{K_m + S(t)} X(t) - \frac{\dot{V}_f(t)}{V(t)} X(t) \\ -\frac{\mu_{max}\,S(t)}{K_m + S(t)} \frac{X(t)}{Y} + \frac{\dot{V}_f(t)}{V(t)} (S_0 - S(t)) \\ 0 \\ \dot{V}_f(t) - \dot{V}_{sam} \end{bmatrix} + \begin{bmatrix} u_X \\ u_S \\ u_\mu \\ u_V \end{bmatrix} \tag{1}$$

The experimental setup measures online the substrate concentration, $S(t)$, which is descbribed in the model by the second component of Equation 1. The substrate is degraded due to the growth of the cells and also altered by the feed stream. The maximal specific growth rate $\mu_{max}$ is designed as a flexible variable. The extended Kalman filter (EKF) re-estimates it continuously in order to compensate a certain inadequacy of the theoretical model. The volume of the cultivation broth V increases by the addition of feed solution $\dot{V}_f(t)$ and is reduced by the removal of broth $\dot{V}_{sam}$ for on- and off-line samples. The $u_X$, $u_S$, $u_\mu$ and $u_V$ are the model errors for biomass, substrate, maximal specific growth rate and the culture broth volume respectively.

The EKF estimates the state variables continuously based on the current parameters and the feeding rate, which is calculated by the coltroller. The integration of the differential equations as well as their estimation covariances P, which is presented in equation 2 (F the Jacobian matrix of the state equations; Q is the process noise power matrix), is performed by the Runge-Kutta method.

$$\frac{d\,P(t)}{d\,t} = F(t)P(t) + P(t)F^T(t) + Q \tag{2}$$

All simulated values as well as the pumping rate are stored in the ring buffer as shown in Figure 1. The pumping rate is adjusted every 10 seconds by the feedforward-feedback controller. The measured substrate is also a discrete variable and when a new value is available, the EKF updates the parameter $\mu_{max}$ and filters the measured substrate. Since the model is continuous and the update strategy is discrete this EKF implementation is called