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

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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 μ=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 μ_{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.

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

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 μ_{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_μ 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.

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

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 μ_{max} and filters the measured substrate. Since the model is continuous and the update strategy is discrete this EKF implementation is called

continuous-discrete EKF.

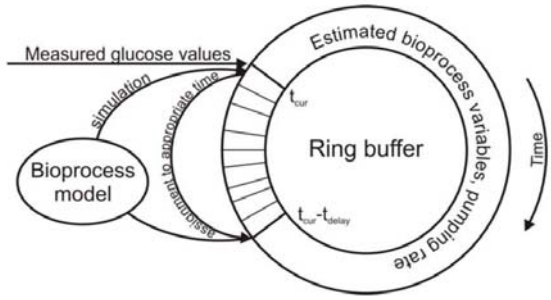

Fig. 1. Data structure of the EKF considering a time delay of measurements of 360 seconds.

Whenever a new value from the on-line glucose measurement system is sent to the EKF, the time delay is considered and the appropriate time point is assigned to the corresponding data in the ring buffer, as shown in Figure 1. The filter algorithm calculates the filtered values using the historic estimated variables, which correspond to the time when the sample for glucose measurement was drawn. Subsequently, the EKF recalculates the current bioprocess variables as well as their estimation error covariances taking the freshly obtained measurement values as well as the historic values of the pumping rate during the time delay into account by numerical integration of the differential equations. Thereby, it corrects deficient estimations from the past, re-evaluates values for the current biomass, glucose concentration, μ_{max} as well as the volume and the estimation error covariances and stores them in the ring buffer. Based on these results, the new feed rate $\dot{V}_f(t)$ is computed. The first term of Equation 3 is deduced from the second component of Equation 1, by setting dS(t)/dt to zero.

$$\dot{V}_f(t_i) = \hat{V}(t)\frac{\hat{\mu}_{\max}(t_i)\hat{S}(t_i)\hat{X}(t_i)}{Y_{X/S}[K_m + \hat{S}(t_i)][S_0 - \hat{S}(t_i)]} + \dot{V}_{PI}(t_i) \quad (3)$$

The first term of Equation 3 represents the feedforward part of the controller where as the second term is the flow rate calculated by the PI controller (feedback). The latter utilizes the estimated glucose concentration as input variable. If negative pump rates are obtained, $\dot{V}_f(t)$ is set to zero. Altogether, the controller calculates the amount of glucose, which is required to maintain the substrate concentration at the set point, and operates the pumps for the feed solution accordingly. Figure 2 outlines the structure of the whole control loop.

The control system was tested during fed batch cultivations of *Saccharomyces cerevisiae* in a 2.5 L stirred tank reactor using a set point of 0.05 g/L. At this glucose concentration, the cells are in a purely oxidative state and no ethanol is produced. Therefore, a high biomass yield is obtained.

Similar control setup was also studied in (Pla et al. 2006), where the authors have worked with a growth rate much lower than the used in this work.

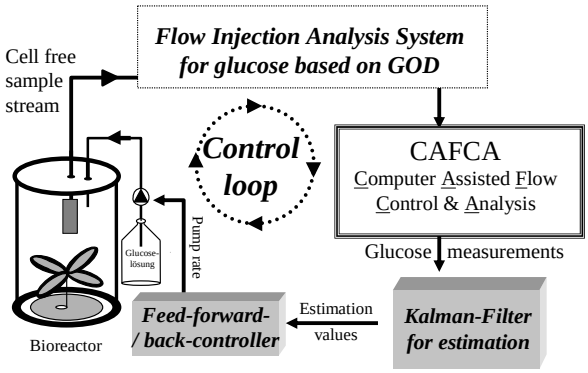

Fig. 2. The control loop consisting of the bioreactor, the FIA measurement system, the Kalman filter, the feed forward-feedback controller, and the feeding system.

3 MATERIALS AND METHODS

3.1 Cultivations

The cultivations were carried out in a 2.5 L stirred tank reactor made of stainless steel (workshop of the Institute of Technical Chemistry, Gottfried Wilhelm Leibniz University Hannover, Germany). A measurement and control unit (Biostat B, BBI, Melsungen, Germany) was engaged for the measurement of the DO and the recording and regulation of temperature, pH and stirring speed. A pH set point of 5.5, a temperature of 30°C and a stirring speed of 1200 rpm were utilised. A wild-type strain of *Saccharomyces cerevisiae* (H620) was employed for the experiments. The process data like temperature, pH, addition of sodium hydroxide solution and hydrochloric acid for pH control, stirring speed and the values from the on-line measurement of oxygen and carbon dioxide (Modular System S710; Sick Maihak, Hamburg, Germany) were collected by the RISP platform (Real Time Integrating Platform, Institute for Technical Chemistry, Gottfried Wilhelm Leibniz University Hannover). The broth was aerated with an air flow of 5 L/min. Table 1 summarizes the medium compounds used during the experiments.

For the pre-culture, a 300 mL flask with 50 mL of medium (modified Schatzmann medium (Schatzmann, 1975), 1.5 g glucose and 0.29 g sodium citrate was prepared, inoculated with yeast cells and incubated for 24 h (30°C, 120 rpm). Afterwards, the medium and cells from the pre-culture (50 mL) were transferred to the reactor. For the batch phase, the reactor was furnished with a certain amount of medium and glucose (20 g/L). After the end of the batch phase, when all substrates were exhausted, the cells of cultivation were starved for four hours before the beginning of the control phase.

Table 1 The medium compounds (modified Schatzmann medium, chemicals from Sigma-Aldrich, Seelze, Germany)

Compound	Pre-culture and batch	Feed Solution
$MgSO_4*7H_2O$ [g/L]	0.34	0.34
$CaCl_2*2H_2O$ [g/L]	0.42	0.42
$(NH_4)_2SO_4$ [g/L]	2.25	11
$(NH_4)_2HPO_4$ [g/L]	14.7	14.7
KCl [g/L]	0.9	0.9
$FeCl_3*6H_2O$ [g/L]	$1.5*10^{-2}$	$1.5*10^{-2}$
$ZnSO_4*7H_2O$ [g/L]	$9.0*10^{-3}$	$9.0*10^{-3}$
$MnSO_4*2*H_2O$ [g/L]	$1.05*10^{-2}$	$1.05*10^{-2}$
$CuSO_4*5*H_2O$ [g/L]	$2.4*10^{-3}$	$2.4*10^{-3}$
m-Inosit [g/L]	$6.0*10^{-2}$	$6.0*10^{-2}$
Ca-Pantothenat [g/L]	$3.0*10^{-2}$	$3.0*10^{-2}$
Pyridoxol*HCl [g/L]	$1.5*10^{-3}$	$1.5*10^{-3}$
Biotin [g/L]	$3.0*10^{-5}$	$3.0*10^{-5}$

Table 2 Initial values and parameter for the Kalman filter as well as the PI controller parameter

Variables	Values
X(t=0) [g/L]	6.4
S(t=0) [g/L]	0.0
μ_{max}(t=0) [h^{-1}]	0.08
V(t=0) [L]	1.6
k_M [g/L]	0.05
Yield factor [$g_{cell}/g_{glucose}$]	0.53
P_{ij}(t=0)	0
Q_{11} [g L^{-2} h^{-1}]	0.001
Q_{22} [g L^{-2} h^{-1}]	0.001
Q_{33} [h^{-3}]	0.05
Q_{44} [L^2 h^{-1}]	0
R [g^2 L^{-2}]	0.002
PI controller p_1	0.9
PI controller p_2	-0.72

During the fed batch phase of the cultivation, the glucose concentration was kept at a set point of 0.05 g/L. With the help of a sampling module (Flownamics E19, IUL Instruments GmbH, Königswinter), a cell free sample stream ($\dot{V}_{sam}$ =0.042 L/h) was continuously drawn from the reactor. The glucose concentration was then analysed by a FIA system (ANASYSCON, Hannover), which was controlled by the software CAFCA (ANASYSCON, Hannover). The resulting data were collected and processed by CAFCA, which sent the glucose concentration values to the Kalman filter via a serial connection. The FIA system essentially consisted of a cartridge filled with an immobilized enzyme (glucose oxidase) and an electrode. The enzyme transforms the glucose and thereby diminishes the oxygen concentration contained in the sample. The oxygen concentration is then analysed by the electrode. Prior to the cultivation the FIA was calibrated with a set of five standard solutions.

During the fed batch phase off-line samples were drawn almost every hour. The biomass (determined gravimetrically as bio dry mass) and ethanol concentration (gas chromatograph GC-14B, Shimadzu) was determined from these samples.

3.2 Controller System

The initial process variables as well as the parameters of the Kalman filter and the digital PI controller are presented in Table 2. The optimal parameters for the Kalman filter and the PI controller were determined in preceding simulations.

4 RESULTS

The controller was evaluated in several cultivations with different set points. In Figure 3 results are presented from the cultivation with a set point of 0.05 g/L.

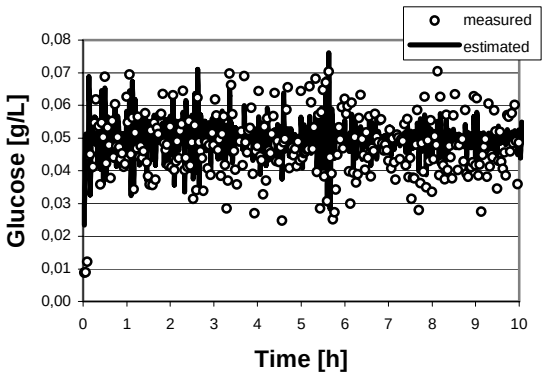

Fig. 3. Measured and estimated glucose concentration.

As one can see, the controller has kept the set point without large deviations in comparision to the high measurement errors. When the control phase started the glucose concentration was zero. It took 0.25 h until the estimated glucose concentration reached the set point and 0.26 h until the measured glucose concentration passed the set point. The mean value of the measurements between 0.26 h and 10 h is 0.0506 g/L with a standard deviation of 0.009 g/L and a maximal difference of 0.025 g/L. The maximal difference occured at 5.6 h cultivation time, where some measurements caused the controller to switch off the pumping rate. This can be seen in Figure 4, where the pumping rate during the whole control phase can be seen.

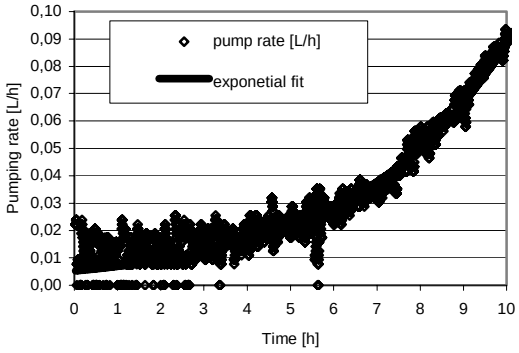

Fig. 4. The pumping rate calculated by the control system. The exponential fit is behind the pump rate data.

At the beginning of the control phase, the pumping rate was switched off several times. The reason for this is, that a very low pumping rate has to be used, which cannot be properly provided by the available pump. After 3.4 h an almost exponential evolution of the pump rate can be seen with an exponential factor of 0.28 h^{-1}. It indicates the success of the controler system.

Although the control results are satisfying, the estimation of the biomass is not. In Figure 5 the estimated and measured biomass can be seen. Due to the fact, that the initial condition of the biomass was not properly chosen, the mean distance of the predicted and measured values is 1.8 g/L. Would the initial value be lowered by a factor of 0.85, then the mean distance between the measured and the predicted values is just 0.56 g/L. Therefore, the dynamic of the process was considered appropriately by the model.

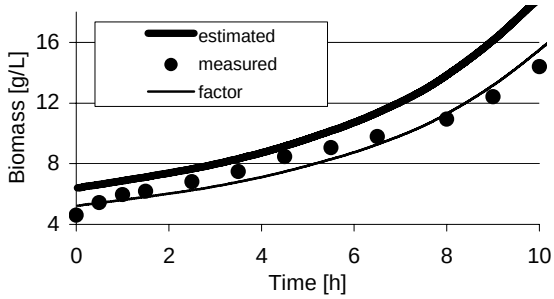

Fig. 5. Measured and estimated biomass concentration as well as the estimated values multiplied by a Factor of 0.85.

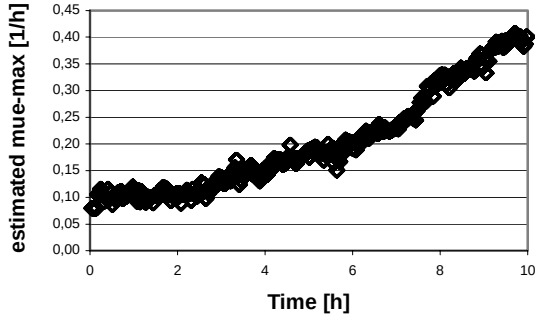

Fig. 6. Estimated specific growth rate.

As one can see in Figure 6, the estimated maximal specific growth rate is increasing during the whole cultivation time. This indicates that the Monod model used in the Kalman filter is not appropriate for this process. Although, the used K_m value might be inappropriate the constant substrate concentration should cause an almost constant specific growth rate. Therefore, another process variable than the substrate concentration is influencing it significantly.

The specific oxygen uptake rate as well as the specific carbon dioxide production rate, which can be seen in Figure 7, indicates the same. If the condition of the cells is not changing too much, then these values should be almost constant too. However, as can be seen in Figure 7 both of these values are increasing almost continuously.

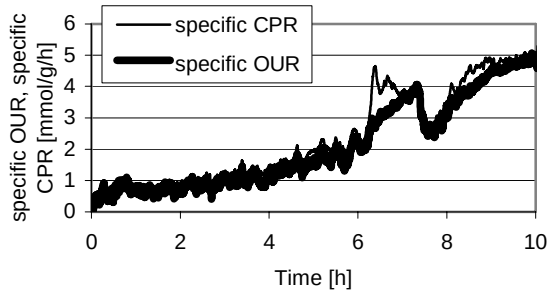

Fig. 7. The specific oxygen uptake rate and the specific dioxide production rate.

In Figure 7 one can see also, that most of the time the values of the specific CPR and the specific OUR are almost the same. However, after 6 h cultivation time, the CPR values increase significantly. Here the metabolism of the cells is different compared to the rest of the cultivation. However not much ethanol is produced; the highest value measured was 0.11 g/L at 8 h cultivation time. In overall these values demonstrate, that the controller performed quit well.

5 CONCLUSIONS

In this contribution a control system based on a FIA measurement system as well as a Kalman filter and a feedforward-feedback controller is presented for the control of yeast cultivation at a set point of 0.05 g/L. The Kalman filter was modified to compensate a time delay of 360 sec. Therefore, in the Kalman filter historic data has to be stored, so that the filtering can be performed with values, which correspond to the measurement time. The overall effort to develop the Kalman filter is therefore larger than usual.

The initial biomass concentration, with which the Kalman filter was supplied, was higher than the real concentration in the reactor. Nevertheless, the process control succeeded and the evolution (but not the values itself) of the biomass was estimated properly. This shows that the control system is robust and can cope with deficient initial parameters.

The control of the cultivation at the set points of 0.05 g/L poses different challenges to the control system.

For small glucose concentrations, the dependency of the growth rate on the substrate concentration is more distinct. Therefore, even a small change in the glucose concentration can result in a significant change in the process dynamic. A rough estimation based on the Monod model shows that a change in the glucose concentration of 10 % (20 %) causes a change of 5 % (10 %) in the growth rate at a glucose set point of 0.05 g/L (with a K_m=0.05 g/L). Therefore, the dynamic of the process is changing significantly if the actual glucose concentration is changing. However, as has been demonstrated the presented control system can handle such situations. Therefore, the Monod model seems to be adequate for the control of this cultivation.

The increasing of the maximal specific growth rate as well as the increasing specific oxygen uptake rate and carbon dioxide production rate indicate a metabolic change in the cells. It shows that the Monod model is in fact not appropriate

REFERENCES

Åkesson, M.; Hagander, P.; Axelsson, J.P. A probing feeding strategy for *Escherichia coli* cultures, *Biotechnology Techniques* **1999**, 13, 523-528

Arndt, M.; Hitzmann, B. Kalman Filter Based Glucose Control at Small Set Points during Fed-Batch Cultivations of *Saccharomyces cerevisiae*, *Biotechnol. Prog.* **2004**, 20, 377-383

Arndt, M.; Kleist, S.; Miksche, G.; Friehs, K.; Flaschel, E.; Trierweiler, J.; Hitzmann, B. A feedforward-feedback substrate controller based on a Kalman filter for a fed-batch cultivation of *Escherichia coli* producing phytase, *Computers and Chemical Engineering* **2005**, 29, 1113-1120

Bastin, G.; Dochhain, D. On-line Estimation and Adaptive Control of Bioreactors, Process Measurement and Control, *Elsevier Science Publishers B. V.*, **1990**

Hantelmann, K.; Kollecker, M.; Hüll, D.; Hitzmann, B.; Scheper, T. Two-dimensional fluorescence spectroscopy: A novel approach for controlling fed-batch cultivations, *Journal of Biotechnology* **2006**, 121, 410-417

Johnston, W.; Cord-Ruwisch, R.; Cooney, M. J. Industrial control of recombinant *E. coli* fed-batch culture: new perspectives on traditional controlled variables, *Bioprocess Biosyst Eng* **2002**, 25, 111-120

Levisauskas, D. Inferential control of the specific growth rate in fed-batch cultivation processes, *Biotechnology Letters* **2001**, 23: 1189-1195

Lidgren, L.; Lilja, O.; Krook, M.; Kriz, D. Automatic fermentation control based on a real-time in situ SIRE biosensor regulated glucose feed, *Biosensors and Bioelectronics* **2006**, 21: 2010-2013

Miskiewicz, T.; Kasperski, A. A fuzzy logic controller to control nutrient dosage in a fed-batch baker's yeast process, *Biotechnology Letters* **2000**, 22:1685-1691

Pla, I. A., Damasceno, L. M., Vannelli, T., Ritter, G., Batt, C. A., and Shuler, M. L. (2006). Evaluation of *Mut*$^+$ and *Mut*^{S} *Pichia pastoris* Phenotypes for High Level Extracellular scFv Expression under Feedback Control of the Methanol Concentration.. *Biotechnol. Prog* **2006**, 22(3), 881-888.

Rani, K.Y.; Rao, V.S.R. Control of fermenters – a review, *Bioprocess Engineering* **1999**, *21*, 77-88

Renard, F.; Wouwer, A.V.; Valentinotti, S.; Dumur, D. A practical robust control scheme for yeast fed-batch cultures – An experimental validation, Journal of Process Control **2006**, 16: 855-864

Schatzmann, H. Anaerobes Wachstum von *Saccharomyces cerevisiae*, Dissertation **1975**, ETH Zürich No. 5504

Sonnleitner, B.; Käppeli, O. Growth of *Saccharomyces cerevisiae* is Controlled by Its Limited Respiratory Capacity: Formulation and Verification of a Hypothesis, *Biotechnology and Bioengineering* **1986**, 28: 927-937