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A feedforward–feedback substrate controller based on a Kalman filter for a fed-batch cultivation of *Escherichia coli* producing phytase

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Abstract

For the feeding-phase of a batch/fed-batch cultivation of a recombinant *Escherichia coli* producing extracellular phytase, a controller has been developed. Based on the estimated process variables by a Kalman filter, a feedforward–feedback controller has been implemented in order to maximize the phytase production and to minimize acetate production. The Kalman filter was used to reduce the noise of the glucose measurements and to estimate the biomass concentration, the substrate (glucose) concentration, the maximal growth rate as well as the reaction broth volume, which are used to calculate the feedforward controller contribution. A PI controller was applied to adjust the glucose concentration to the desired set point of 0.2 g/L. The secretion of phytase into the medium is increased at low glucose concentration whereas the acetate production is reduced, due to a low concentration avoiding significant overflow metabolism. The glucose concentration, as the sole measured variable used by the controller, is determined using flow injection analysis (FIA). The operation of the controller as well as its application to the *E. coli* cultivation is presented. The average on-line measured glucose concentration is 0.208 g/L with a standard deviation of 0.066 g/L. During the cultivation a fault occurred in the measurement system. The response of the controller system with respect to this fault is discussed in detail. Compared to a controller based on oxygen measurements, the yield of phytase is the same with the presented system.

Keywords: Glucose control; Feedforward–feedback control; Kalman filter; *Escherichia coli*; Phytase production

1. Introduction

The automation of cultivation processes still lack robust process analysers and controllers for important variables. Until now commercially available controller exist only for well established measurement systems like pH, temperature, stirrer speed, anti foam as well as dissolved oxygen and oxygen in the exhaust gas. Research for robust controllers based on these variables is going on (e.g. Aziz, Hussain, & Mujtaba, 2000; Babuska, Oosterhoff, Oudshoorn, & Bruijn, 2002). Reasons for this fact are the highly changing dynamics of most bioprocesses, which is caused by the non-linear growth of the cells, the metabolic changes due to a depletion of substrates consumed preferentially as well as changes in the overall metabolism, such as changes in uptake rates, depending on substrate concentration (transition from above to below a critical value). However, the metabolic network of the microorganisms itself is a regulatory unit, which allows the cells to adapt to various environmental situations; with the consequence, that the yield of a desired product is not optimal. This adaptability of the microorganism makes the control task even more difficult. For instance, insufficient oxygen supply or the excess of glucose will not cause *Escherichia coli* to stop growing but the cells will produce acetate, which will inhibit the growth of the cells at higher concentrations (Lee, 1996). Control systems are therefore required, which can prevent acetate formation at a maximum growth rate.

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The most important variables like mRNA concentrations, which determine the transcriptome of the cells and therefore their behaviour, cannot be measured on-line. But even process variables, like substrate, product, by-products and also biomass concentrations are difficult to measure. For most bioprocesses these variables cannot be measured using robust and simple to handle process analysers. A time delay of more than 5 min for the measurement of substrate and product concentration is not unusual. If an HPLC is used, the time delay may be more than 15 min. This time delay makes the control task even more difficult.

Using the above-mentioned measurements, a variety of substrate feeding strategies have been used. For an overview see Rani and Rao (1999). Hisbullah and Ramachandran (2002) demonstrate the unsatisfactory performance of conventional controllers due to significant oscillation and offsets. Fixed-gain PI controllers as well as scheduled-gain PI and adaptive neural-network controllers were not able to solve these problems. However, they showed that a hybrid neural-network PI controller is able to overcome oscillation and offsets. Johnston, Cord-Ruwisch and Cooney (2002) reported about the control strategies of recombinant *E. coli* cultures. They judged two DO-transient control systems to be superior to a pH-stat in detecting and tracking the acetate threshold and concluded that the choice of controller has a great impact on acetate threshold and productivity.

To limit acetate accumulation during the cultivation of *E. coli* Åkesson, Hagander and Axelsson (2001) used a probing technique, which – using a dissolved oxygen sensor – is able to detect and avoid overflow metabolism. They state that their method may be applied generally, as no strain-specific information is needed and the only sensor required is a standard dissolved oxygen probe.

There are only a few examples, where the closed loop control of glucose based on its direct measurements has been applied for bacteria. Kleman, Chalmers, Luli and Strohl (1991) published a predictive and feedback control for *E. coli* cells with a constant glucose concentration of 0.49 ± 0.04 g/L. The predictive control algorithm is based on linear regression to calculate the demand of glucose of the cells.

Due to the growth of the microorganisms time-varying characteristics of the cultivation process occur and have to, be taken into consideration if a controller is implemented. Using a feedforward–feedback controller these time-varying characteristics have to be kept in view in the feedforward contribution. Therefore a model is required, which represents the time-varying characteristics sufficiently and might restrict the application to well known processes. The parameter of the controller have to be determined in simulations, which might be very time consuming.

During the last years phytase production has become increasingly important due to its use as feed additive in the nutrition of monogastric animals. In this contribution a controller is presented to keep glucose concentration at a fixed set point of 0.2 g/L, in order to maximize phytase production and to minimize acetate production during fed-batch cultivation of a phytase expressing recombinant *E. coli*. The secretion of phytase into the medium is increased at low glucose concentrations, whereas the acetate production is reduced by avoiding a significant overflow metabolism, which occurs normally at higher concentrations. The glucose concentration was measured using a special flow injection analysis (FIA) system, which does not require a probing device. An extended Kalman filter was applied to reduce the basic noise and predict other process variables. Based on the estimated values of biomass and glucose concentration, as well as maximal specific growth rate and volume of the cultivation broth, a feedforward–feedback controller has been used to maintain the glucose concentration at the set point.

2. Materials and methods

2.1. The bioprocess

The experiments are performed in a 7 L bioreactor (MBR, Zurich, Switzerland) with a working volume of 5 L in a fed-batch mode. The *E. coli* strain BL21(DE3) pPhyt109 was grown to produce phytase under the control of the *bgl* gene from *B. amyloliquefaciens* (Miksch, Kleist, Friehs, & Flaschel, 2002). In addition is the *kil* gene from ColE1 under the control of the stationary-phase promoter *fic* on the plasmid pPhyt109. The phytase is transported into the periplasm and is secreted into the medium due the function of the *kil* gene dependend production of a bacteriocin release protein (Miksch et al., 2002). Therefore, at low glucose concentration this system should work efficiently.

The medium consisted of mineral salt solution with a glucose concentration of 6 g/L. After the glucose concentration has reached a value of 0.2 g/L (end of batch phase) the glucose feeding was started automatically by the controller system. The feed solution had a glucose concentration of 500 g/L (Horn et al., 1996). The pH was adjusted to 6.9 by the addition of 10% phosphorus acid or 4N NaOH and temperature was maintained at 37 °C. The aeration rate was constant at 600 L/h whereas the stirrer speed starting at 150 rpm was varied so that the dissolved oxygen values do not fall below 30%. Antifoam PE8100 (BASF, Ludwigshafen, Germany) was added when necessary to suppress foaming. The process is described in more detail by Kleist et al. (2003).

2.2. The analytical setup

Samples were obtained for measurements of biomass, glucose, acetate and phytase using the automatic sampler FC205 (Gilson, Middleton, USA) and stored at 4 °C until analysed. One millilitre of culture broth was centrifuged in Eppendorf vials at 14,000 rpm for 10 min; the pellet was washed twice in an aqueous NaCl solution and dried overnight at 60 °C under vacuum before bacterial dry mass was determined. Using test

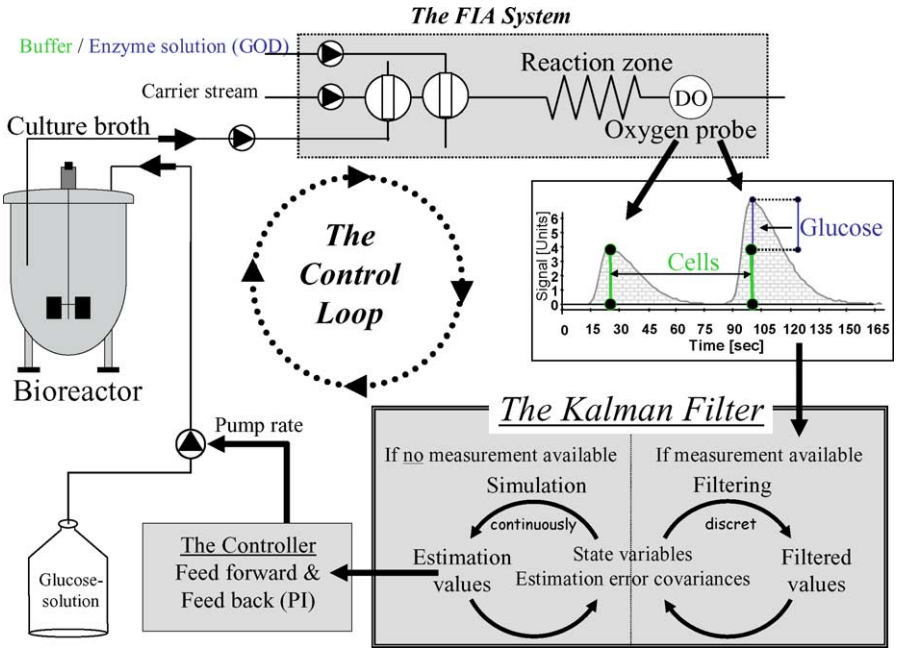

Fig. 1. The operation scheme of the control loop with the bioreactor, the FIA-system, the Kalman filter and the feedforward–feedback controller for glucose feeding.

kits glucose (No. 18–20, Sigma, Deisenhofen, Germany) and acetate (No. 0148261, R-Biopharma, Darmstadt, Germany) were determined. The phytase activity was measured according to the literature (Miksch et al., 2002). Carbon dioxide was measured by URAS 10E gas analyser (Hartmann und Braun, Frankfurt, Germany).

To reduce the time delay of on-line glucose measurements, a modified FIA-system is applied, which does not require a sampling (filtration) system, as previously described for the control of yeast cultivations (Arndt & Hitzmann, 2004; Hitzmann et al., 2000). As can be seen in Fig. 1 the culture broth (sample volume 24 μL) is directly injected (Knauer, Berlin, Germany) into the carrier stream (1.4 mL/min) of the FIA-system and is mixed (reaction coil length 0.5 m, diameter 0.8 mm) by dispersion with an enzyme solution of glucose oxidase (36 μL, 400 kU/L; Fluka, Germany). The oxidase is injected into the carrier stream just in front of the culture broth. Using a dissolved oxygen probe (ANASYSCON, Hannover, Germany), the oxygen consumption caused both by the cells and the enzyme reaction is determined. If the oxygen consumption of the cells alone is measured (no injection of enzyme solution), the difference of the total oxygen consumption by the cells and the enzyme reaction and the pure oxygen uptake by the cells, can be used to determine the glucose concentration. Every 60 s ($\Delta t_{FIA} = 60$) one oxygen measurement was performed, however, every fourth measurement only the oxygen uptake of the cells was determined. The time delay of the glucose measurement is in total 45 s. The operation of the FIA-system is presented in Fig. 1. The automation of the FIA-system and the calculation of the glucose concentration are carried out by the software package CAFCA (ANASYSCON, Hannover, Germany).

2.3. The controller system

In order to prevent high acetate production and to maximize the phytase production the glucose was controlled at low concentration based on glucose measurements. Because two measurements are necessary to determine the glucose concentration (total oxygen consumption and oxygen consumption only by the cells), the measurement noise of the system is double the amount compared to an ordinary glucose measurement FIA-system. Therefore, a Kalman filter is used to convert the measurements in reducing its noise and estimate the process variables for the calculation of the feedforward controller. A Kalman filter uses a dynamic process model to simulate the process variables (state variables), i.e., calculate estimates of the variables. Whenever a new measurement is available, the Kalman filter makes use of a measurement model, with which at least one process variable can be determined. The difference of both values (estimated value by the simulation minus determined value by the measurement according to the measurement model) is used to improve (filter) the estimation of the process variables. This is performed in a way, so that the error covariance of estimating the state variables is minimized. The operation of the Kalman filter is presented in the lower right part of Fig. 1. The Kalman filter is used to estimate the biomass and the glucose concentration, the maximal growth rate, and the culture broth volume. These process variables are necessary for the feedforward part of the controller. Glucose concentration is used as the only variable by the filter. The controller system with the Kalman filter was started, when the glucose concentration was fallen under a limit of $c_{lim} = 0.8$ g/L, which were determined by the optimal measurement range of the FIA-system.

2.4. The mathematical model

For modelling the process an ideal stirred tank reactor in feed-batch mode has been assumed with a cell growth kinetic approximated by the Monod model, where the substrate glucose is the single growth-limiting factor. The dynamic process model consists of four ordinary differential equations:

$$\frac{dX(t)}{dt} = \frac{\mu_{max}(t)S(t)}{K_m + S(t)}X(t) - \frac{\dot{V}_F(t)X(t)}{V_R(t)} \quad (1)$$

$$\frac{dS(t)}{dt} = -\frac{\mu_{max}(t)S(t)X(t)}{[K_m + S(t)]Y_{SX}} + \frac{\dot{V}_F(t)[S_F - S(t)]}{V_R(t)} \quad (2)$$

$$\frac{d\mu_{max}(t)}{dt} = 0 \quad (3)$$

$$\frac{dV_R(t)}{dt} = \dot{V}_F(t) - \dot{V}_S \quad (4)$$

Here X is the biomass, μ_{max} the maximal specific growth rate of the Monod model, S the glucose concentration, K_m the saturation constant, $\dot{V}_F$ the feeding rate, V_R the volume of the culture broth, Y_{SX} the yield coefficient, S_F the glucose concentration of the feed solution, and $\dot{V}_S$ the sample stream for the FIA-system. The parameter values and the list of their sources are presented in Table 1. The sample volumes taken for off-line measurements are neglected. The equation for the maximal specific growth rate has been considered to account for inadequacy caused by the Monod model. Using the maximal specific growth rate as a state variable, the Kalman filter procedure can adjust its values during the filtering procedure, so that the measurements and the model fit together. Although the factor $S/(K_m + S)$ of the Monod model is 0.95 at the set point of 0.2 g/L the Monod model was applied to take into account the principle growth kinetic, but it can be converted easily into a pure exponential growth model.

2.5. The control algorithm

The pumping rate of the feed solution is calculated by using the following equations

$$\dot{V}_F(t_i) = \dot{V}_{ff}(t_i) + \dot{V}_{fb}(t_i) \quad (5)$$

$$\dot{V}_{ff}(t_i) = \frac{\mu_{max}(t_i)S(t_i)X(t_i)V_R(t_i)}{[K_m + S(t_i)]Y_{SX}[S_F - S(t_i)]} \quad (6)$$

$$\dot{V}_{fb}(t_i) = \dot{V}_{fb}(t_{i-1}) + q_0[S_{set} - S(t_i)] + q_1[S_{set} - S(t_{i-1})] \quad (7)$$

Eq. (6) is the feedforward part of the controller, which uses the estimated values of the Kalman filter, derived from Eq. (2), if steady state conditions are assumed ($dS(t)/dt = 0$). Eq. (7) is a digital version of a PI-controller. $\dot{V}_F(t_i)$ is determined with a cycle time of $\Delta t_p = 7.2$ s. If a negative pumping rate was calculated by the controller, then its digital PI-part was initialized (the actual deviation as well as the historic value were all set to zero) and the pumping rate was set to zero too.

Table 1
Parameters and initial conditions used by the Kalman filter and the control system (chosen = value assigned arbitrarily, experiments = value estimated from experiments, simulation = value determined during simulations)

Symbol	Name	Value	Source
K_m	Monod saturation constant	0.01 g/L	Lin, Mathiszik, Xu, Enfors and Neubauer (2001)
S_F	Glucose concentration in feed solution	500 g/L	Chosen
Y_{SX}	Yield coefficient	0.42 g/g	Experiments
$\dot{V}_S$	Sample stream for FIA measurement	1.4 mL/min	Chosen
S_{set}	Set point of glucose control	0.2 g/L	Chosen
q_0	Digital PI-controller parameter 1	1.4 L^2/g h	Simulation
q_1	Digital PI-controller parameter 2	−1.1 L^2/g h	Simulation
Δt_{FIA}	Cycle time of FIA measurements	60 s	Chosen
Δt_p	Cycle time of pumping rate calculation	7.2 s	Chosen
Δt_{RK}	Integration time of Runge-Kutta procedure	10^{-5} h	Chosen
$Q[1, 1]$	Spectral density matrix element of process noise (with respect to X)	0.001 g^2/L^2 h	Simulation
$Q[2, 2]$	Spectral density matrix element of process noise (with respect to S)	0.001 g^2/L^2 h	Simulation
$Q[3, 3]$	Spectral density matrix element of process noise (with respect to S)	0.2 1/h^3	Simulation
$Q[4, 4]$	Spectral density matrix element of process noise (with respect to V_R)	0 L^2/h	Simulation
$Q[i, j]$	Spectral density matrix element of process noise ($i \neq j$)	0	Simulation
R	Measurement error variance	0.0025 g^2/L^2	Experiments
t_0	Start of measurement and Kalman filtering	4.3 h	Chosen
$X(t_0)$	Initial condition of biomass concentration	3.2 g/L	Experiments
$S(t_0)$	Initial condition of glucose concentration	0.78 g/L	Experiments
$\mu_{max}(t_0)$	Initial condition of maximal growth rate	0.35 1/h	Experiments
$V_R(t_0)$	Initial condition of culture broth	2.7 L	Experiments
$P[1, 1]$	Initial condition of estimation error (co)variance (with respect to X)	0.1 g^2/L^2	Simulation
$P[2, 2]$	Initial condition of estimation error (co)variance (with respect to S)	0.02 g^2/L^2	Simulation
$P[3, 3]$	Initial condition of estimation error (co)variance (with respect to μ_{max})	0.2 1/h^2	Simulation
$P[4, 4]$	Initial condition of estimation error (co)variance (with respect to V_R)	0 L^2	Simulation
$P[i, j]$	Initial condition of estimation error covariance ($i \neq j$)	0	Chosen

The parameters of the PI-controller as well as the ones of the Kalman filter (spectral density matrix of process noise Q, the measurement error (co)variance R, the diagonal of the estimation error covariance $P[i, i]$ at $t = 0$) are presented in Table 1. The process noise as well as the measurement noise has been assumed to be Gaussian white noise. For the measurement error variance a measurement error of 0.05 g/L (standard deviation) was assumed, which has been determined during measurements around 0.2 g/L. The other parameter values have been determined during simulation experiments. Here one goal was, that the main PI-controller action should be performed between successive glucose measurements. For the numerical integration of the differential equations the Runge-Kutta procedure is used with $\Delta t = 10^{-5}$ h. The whole control loop can be seen in Fig. 1.

3. Results and discussion

3.1. The glucose concentration

In Fig. 2 the on-line measured and estimated glucose concentrations as well as the off-line measured glucose concentrations can be seen. The end of the batch phase was at 4.5 h cultivation time. Except between 6.4 and 6.9 h cultivation time, the controller was able to keep the glucose concentration at the set point of 0.2 g/L for almost 10 h. In this period the average on-line measured glucose concentration was 0.208 g/L with a standard deviation of 0.066 g/L. Compared to the measurement error variance R of the Kalman filter, which corresponding standard deviation has been set at 0.05 g/L, the calculated standard deviation is too high, but this value resulted not only from measurement errors but also from control errors. Most of the time the off-line glucose measurements gave lower numbers than the corresponding on-line values. One explanation could be that during off-line sample handling and storage glucose is still consumed, although the samples were cooled down fast.

3.2. The malfunction of the analyser

The sample stream clogged up at 6.43 h cultivation time, although no filtration system was used. As a consequence, the FIA-system got no sample solution and lead to wrong measurements of 0 g/L glucose. These values were processed further by the Kalman filter. The time period of this malfunction of the analyser can be seen in more detail in Fig. 3A and B. In sequence eight wrong measurement values were sent to the Kalman filter, each with a concentration of zero. As one can see, these values are not equidistant in time. After three glucose measurements, the oxygen consumption of the cells in the sample is determined by the FIA-system (as described in Section 2); therefore after every three measurements a longer period is carried out without a new glucose measurement.

Due to the wrong measurement values the Kalman filter lowered the estimated glucose concentration by roughly 0.07 g/L, increased the estimated maximal growth rate by 0.04 1/h and increased the estimated biomass by 0.07 g/L. As a consequence the controller immediately increased the pumping rate of the feed solution so that the estimated values quickly returned to the set point (rise time is less than 14 s). Here the dynamics of the feedback PI-part of the controller can be seen. Always an overshoot occurs (average $c_{max} - c_{set\,point} = 0.033$ g/L) and the typical underdamped response can be seen. This emphasizes the importance of the Kalman filter and its estimation of the state variables for the controller as a whole. If the estimation of the glucose concentration is wrong, then the controller cannot work proper. The PI-part of the controller will always quickly adjust the pumping rate, so that the estimated glucose concentration will reach the desired set point almost within one measurement cycle of the FIA-system.

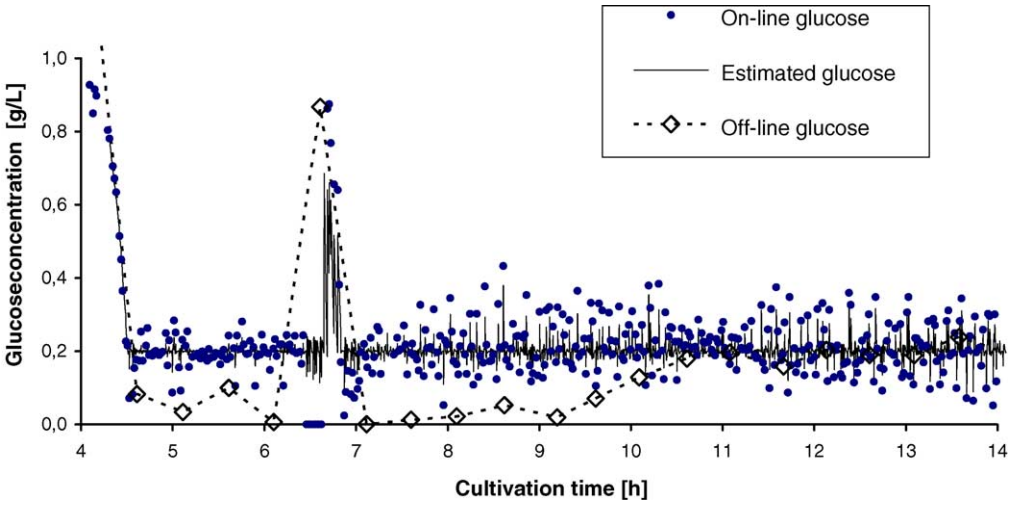

Fig. 2. Glucose concentrations during the feeding phase of the cultivation at the set point 0.2 g/L (feeding starts after the fall of first estimated glucose value below the set point, which is 4.5 h cultivation time).

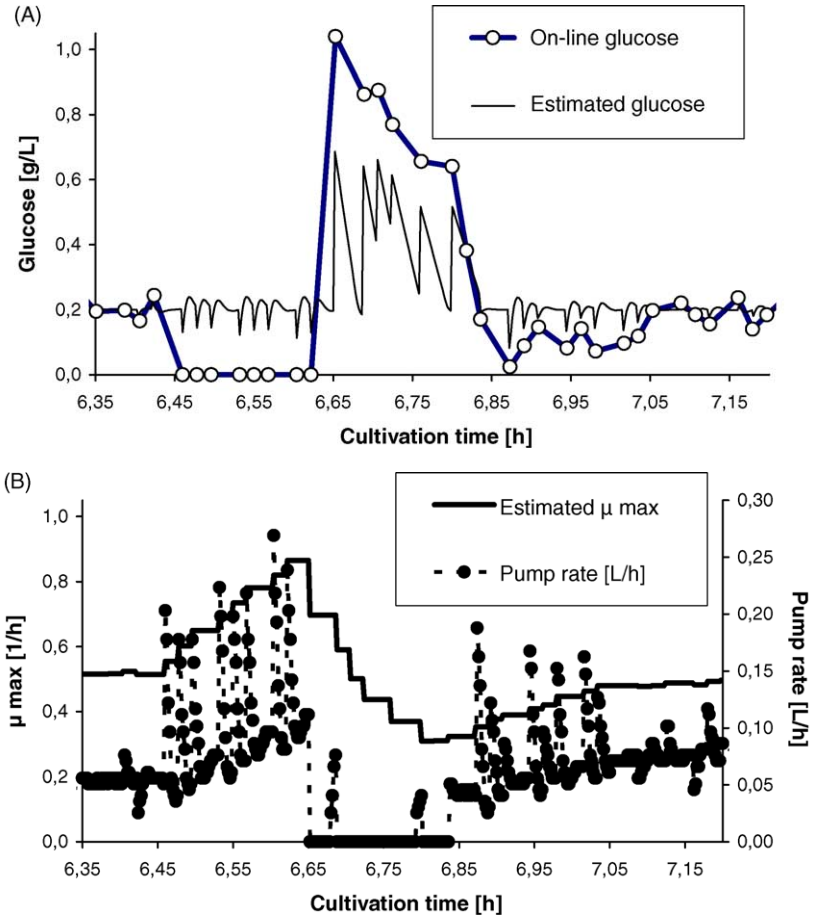

Fig. 3. (A) The on-line measured and estimated glucose concentration during the malfunction and shortly afterwards of the glucose measurement system. (B) The estimated specific maximal growth rate and the pump rate during the malfunction and shortly afterwards of the glucose measurement system.

After the fault had been recognised and corrected by the operator, the following on-line as well as off-line measurement of the glucose concentration gave almost 1 g/L (at 6.65 h cultivation time). The details following the malfunction are shown in Fig. 3A and B. Now, getting proper measurements, the Kalman filter lowered the estimated maximal specific growth rate and biomass and increased the estimated glucose concentration to 0.69 g/L. Because the filtered glucose value was now high, the PI-controller switched off the feeding pump until the estimated glucose concentration had reached the set point. However, because the maximal growth rate was estimated much too high (more than 0.8 1/h), which resulted in an overrated estimation of glucose uptake, the pump was activated too early. At 6.82 h cultivation time the estimated and the measured glucose concentration were very similar, both at 0.39 g/L; however, the estimated maximal growth rate was now too low and therefore the process dynamic was not well estimated. Roughly 0.2 h after the process analyser was fixed the on-line measured glucose concentrations returned to the set point, but again an overshoot can be seen resulting from the wrong estimation of process dynamics. After 0.4 h (at 7.05 h cultivation time) the control system reached its intended performance.

3.3. Overall process evolution

In Fig. 4 the estimated biomass and the off-line measured biomass is presented. Until 9 h cultivation time the predicted as well as the measured biomass values corresponded very well. The estimated growth rate as well as the CO_2 concentration in the exhaust gas are shown in Fig. 5. The values of the CO_2 increased almost steadily for 9 h; however, an effect of the malfunction can be seen. At 6.83 h cultivation time

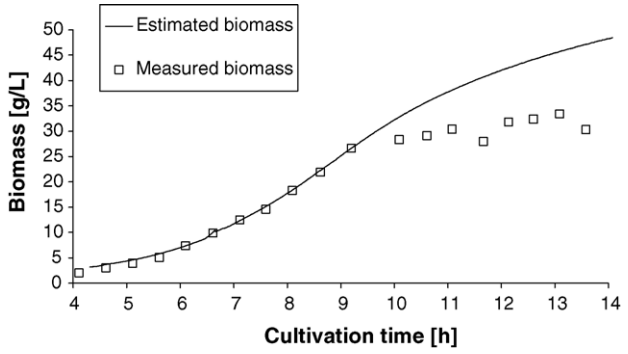

Fig. 4. The measured (squares) and estimated (solid line) biomass concentrations during the feeding phase of the cultivation.

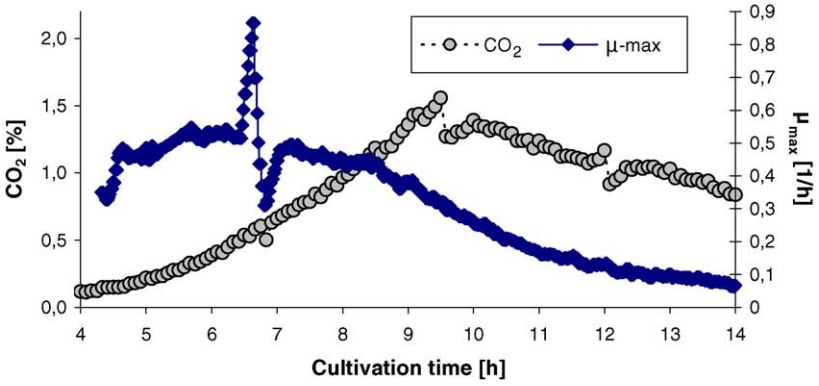

Fig. 5. The carbon dioxide concentration and the specific maximal growth rate during the feeding phase of the cultivation.

a little dump was produced, because the Kalman filter had reduced the estimated maximal growth rate too much with the consequence, that the feedforward part of the controller was not pumping enough glucose into the reactor. Due to this glucose limitation the formation of CO_2 was reduced for a very short time. Apart from this little obstruction the CO_2 values indicated that the process ran well until 9 h of cultivation time.

The estimated maximal specific growth rate was almost constant between 4.6 and 6.4 h at a value of 0.5 1/h. During the fault the values of μ_{max} first rose to 0.86 1/h and than fell to 0.31 1/h. In Fig. 4 it seems that its overall evolution was not affected very much by the malfunction of the analyser. Its value shortly before the fault was 0.51 1/h and after it was 0.48 1/h; but a steady decrease in its values can be seen.

After 9 h cultivation time almost no significant increase in biomass concentration occurred as can be seen in Fig. 4. The cells still grew, but the culture broth was diluted by the feeding solution ($\Delta V = 0.42$ L between 9 and 14 h cultivation time). However, as can be seen in the CO_2 concentration, the cells changed their metabolism shortly after 9 h cultivation time. This metabolic change is not considered by the theoretical model and, therefore, the estimated values of the biomass are wrong. However, although the model is no longer valid, the quality of the control system is not affected significantly. The sharp drop in the CO_2 concentrations at 9.5 and 12.0 h cultivation time are malfunctions of the exhaust gas analyser and have no biological reason.

The change of the behaviour of the cells at 9 h can also be seen in Fig. 6 where the acetate concentration as well as the total phytase activity (intracellular plus secreted phytase) are presented. During the batch phase just small amounts of acetate were produced; whereas during the malfunction a little acetate peak appeared. Significant amounts of acetate were produced after 9 h cultivation time. Here the concentration increased from 0.17 g/L at 9.2 h to 1.6 g/L at 11.7 h cultivation time. However, the acetate concentration was never higher than 2 g/L, which is assumed not to inhibit growth. But the yield of biomass changed and the overall estimation of biomass failed, as seen in Fig. 4. Furthermore, during the second part of the cultivation the maximal growth rate decreased more and more. Therefore the glucose consumption for the maintenance of the cells, which was not considered in the theoretical model, got a significant influence. This also disturbed the overall glucose balance. The phytase activity increased almost linearly with a slope of 13 U/mL h. Most of the phytase was secreted into the culture broth. Compared to other control strategies based on dissolved oxygen (Kleist

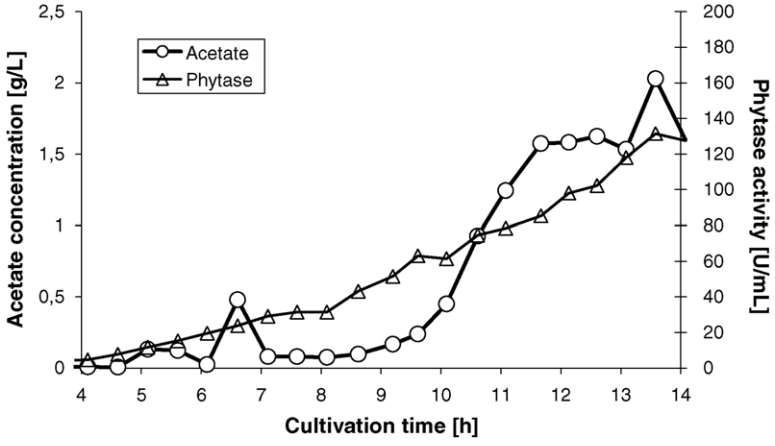

Fig. 6. Acetate concentration and phytase activity during the feeding phase of the cultivation.

et al., 2003), the yield obtained with the presented controller is the same as the best ones of the other strategies (DO-set point between 5 and 10% oxygen saturation).

4. Conclusion

In this contribution a control strategy has been presented and applied to an *E. coli* cultivation based on direct glucose measurements complemented with an extended Kalman filter and a feedforward–feedback controller. The same control system has been previously applied for the control of yeast cultivations (Arndt & Hitzmann, 2004). The main difference is the value of the yield coefficient, apart from minor practical optimizations of the measurement system such as a higher flow rate of the sample stream and a shorter tubing distance to the bioreactor. Therefore, without significant changes the controller system can easily be applied to cultivations of different microorganisms. Although the estimation of process variables was not accurate at the second half of the process, the control results are still satisfactory. It seems that the adaptation of the maximum specific growth rate by the Kalman filter is able to compensate this model inadequacy.

To improve the prediction capability of the system the theoretical model must be extended for acetate formation and the maintenance coefficient. Furthermore, other measurement variables should be incorporated into the dynamic process model, such as the oxygen and carbon dioxide concentration in the exhaust gas. But then the model is very specific to a special process and the application to other processes will require more effort. Furthermore, the observability of the state variables of very complex models might not be guaranteed by the available measurement variables.

The malfunction that happened can hardly be avoided. A clogging can always happen. But with the presented system it can be identified and analysed much earlier. A knowledge-based system, e.g. an expert system, would be very helpfull, such as the one proposed by Brandt and Hitzmann (1994) and Löhn and Hitzmann (1999). It can analyse the measurement values continuously and can inform the operator immediately if inconsistent measurements are identified. After the second time a glucose value of zero was measured, it should have been clear, that something was running wrong. It is not only the measurement system but also a malfunction of the feeding pump would give zero glucose concentration. Other then incorporated measurement signals like carbon dioxide or dissolved oxygen would indicate this immediately.

A disadvantage of the measurement system is the glucose consumption on the way from the reactor to the dissolved oxygen probe. If the biomass and the activity of the cells are high, their substrate uptake has to be considered. To prevent oxygen consumption by the cells a filtration module for sampling has to be used. The time delay, which will be caused by such a system, could then be compensated by the Kalman filter.

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