Bioautomation, 2004, vol. 1, pp. 16-29

Ultrasonic Measurements and its Evaluation for the Monitoring of *Saccharomyces cerevisiae* Cultivation

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Abstract: *The monitoring and supervision of batch Saccharomyces cerevisiae cultivations are presented by ultrasonic velocity measurements. The measurements are performed in a by-pass to reduce the influence of bubbles. Using these signals the typical phases of such cultivations can be identified. Applying a multi-linear regression model the ultrasonic velocity can be estimated by the biomass, the glucose and the ethanol concentration with a mean estimation error of 1.6 m/s. The multi-linear regression model has also been used to predict one of the three process variables by the other two and the ultrasonic velocity. Here the mean error of prediction is 0.6 g/L, 2.3 g/L and 1.5 g/L for biomass, glucose and ethanol concentration respectively. Using a Kalman filter theses variables have been estimated with mean errors of 0.6 g/L, 1.8 g/L and 1.6 g/L.*

Keywords: *Ultrasonic velocity, Bioprocess supervision, Observer, Multi-linear regression*

Introduction

In bioprocess engineering it is still a problem, that important variables can not be measured on-line [7]. For the analysis of the performance of bioreactors, measurements are fundamental [4]. Therefore, new measurement systems are still required. An alternative are software sensors, which can also be used for elucidation of a bioprocess state and behaviour. State observers are a type of such software sensors. With an observer information can be gained even about non measurable variables of a process. Observers predict these variables by using mathematical models as well as data from measured variables. The on-line calculation of the non measurable variables is performed by the integration of the general state observer equations. Due to different optimization criteria during calculation of the variables different observers like the (extended) Luenberger or Kalman observer have been realized successfully [1].

Another type of software sensors based more on black box models like artificial neuronal networks, multi-linear regression or principal component regression. Here, the model structure and equation has no inherently knowledge about the process. The data of on-line measured process variables and sometimes also their historic data form pattern, which are used by these models to determine the model structure as well as their parameter values. However, these data driven methods need a lot of measurement data from different process runs to build the model reliably. Most often the prediction ability can get lost, if process conditions are changed. If the measured values are highly correlated themselves, then principal component analysis is applied, for data transformation and reduction. The data transformation bases on a variance analysis and creates so called latent variables, which are orthogonal. The latent variables, whose significance has to be determined by special criteria, are then used as input pattern for artificial neuronal networks or for a multi-linear regression model to predict the non measurable variables.

These two different kinds of software sensors are also used together as a hybrid model or grey box model. The process knowledge is used to develop a mechanistic model whose unknown relations, such as how a kinetic expression depends on specific variables, are modelled by any black box model. These types of models gain more and more significance, which will still increase. Such a grey model for the evaluation of ultrasonic measurements is presented by Becker et al. [2]. The model uses the velocity measurements to determine the density of beer during fermentation. The measurement is performed outside the reactor tank. They pointed out, that for every sort of beer and every tank the geometry parameters have to be clarified. Resa et al. [5] presented an analysis of the density and the ultrasonic velocity changes during the alcoholic fermentation of several aqueous mixtures. They concluded that ultrasonic techniques are well suited to monitor fermentation processes, but the influence of carbon dioxide has to be investigated further. An overview of the application of ultrasonic sensors in the process industry is presented by Hauptmann et al. [3].

The goal of this investigation is the analysis of ultrasonic signals for the supervision and monitoring of *Saccharomyces cerevisiae* cultivations. Ultrasonic signals depend on several bioprocess variables, such as biomass, glucose and ethanol concentration but also on further variables such as bubbles, pressure and temperature. This multivariate dependence makes the evaluation of the measurement difficult. Here the supervision by ultrasonic velocity measurements of the yeast cultivation of *Saccharomyces cerevisiae* is investigated by the prediction of biomass, glucose and ethanol concentration.

Material and Methods

1. The reactor and measurements

The ultrasonic measurements have been carried out at a cultivation of *Saccharomyces cerevisiae* in a 1.5 L bioreactor. As medium the Schatzmann medium [6] supplemented with glucose was used. Four different cultivations have been performed in batch mode with different start glucose concentrations of 33 g/L, 32 g/L, 30 g/L and 9 g/L, which will be referenced as cultivation A, B, C, and D respectively. The temperature was controlled at 30°C, the pH at 5.5 and the stirrer speed at 1000 rpm. CO_2 concentrations were measured on-line; for off-line glucose measurements as well as for biomass and ethanol concentration determination samples of about 10 mL were taken roughly every 1.5 hours. To prevent metabolic activity of the cells in the sample taken, they were cooled down in ice water immediately. Glucose concentration was measured using the Yellow Springs Analyzer (Yellow Springs Instruments, USA). Ethanol has been determined by the gas chromatograph Shimadzu GC-14B (Shimadzu, Germany).

Fig. 1 presents the ultrasonic measurement system (US2100, IFAC, Barleben, Germany) used for supervision of the cultivation. As can be seen the measurement system is connected to the reactor in a bypass. A valve was used to stop the flow of culture broth in the measurement chamber to reduce the influence of bubbles on the ultrasonic velocity.

2. The software sensors

As observer the continuous-discrete extended Kalman filter was used to predict the concentrations of biomass, glucose and ethanol. The model (state equations) considers the cultivation of the yeast cells in an ideal stirred tank reactor in batch mode, where the typical diauxic growth is described by two Monod models with respect to glucose and ethanol as the limiting substrate. During the oxido-reductive growth on glucose ethanol is produced. If all glucose has been consumed, the second phase starts in which ethanol is consumed for biomass production. This behaviour can be described by the following equations:

$$\frac{dX}{dt} = \left(\frac{\mu_S^{\max} S}{K_S + S} + \frac{\mu_E^{\max} E}{K_E + E} \right) X \tag{1}$$

$$\frac{dS}{dt} = -\left(\frac{\mu_S^{\max} S}{K_S + S} \right) \frac{X}{Y_{XS}} \tag{2}$$

$$\frac{dE}{dt} = \left(\frac{\mu_S^{\max} S}{K_S + S} \right) X Y_{ES} - \left(\frac{\mu_E^{\max} E}{K_E + E} \right) \frac{X}{Y_{XE}} \qquad (3)$$

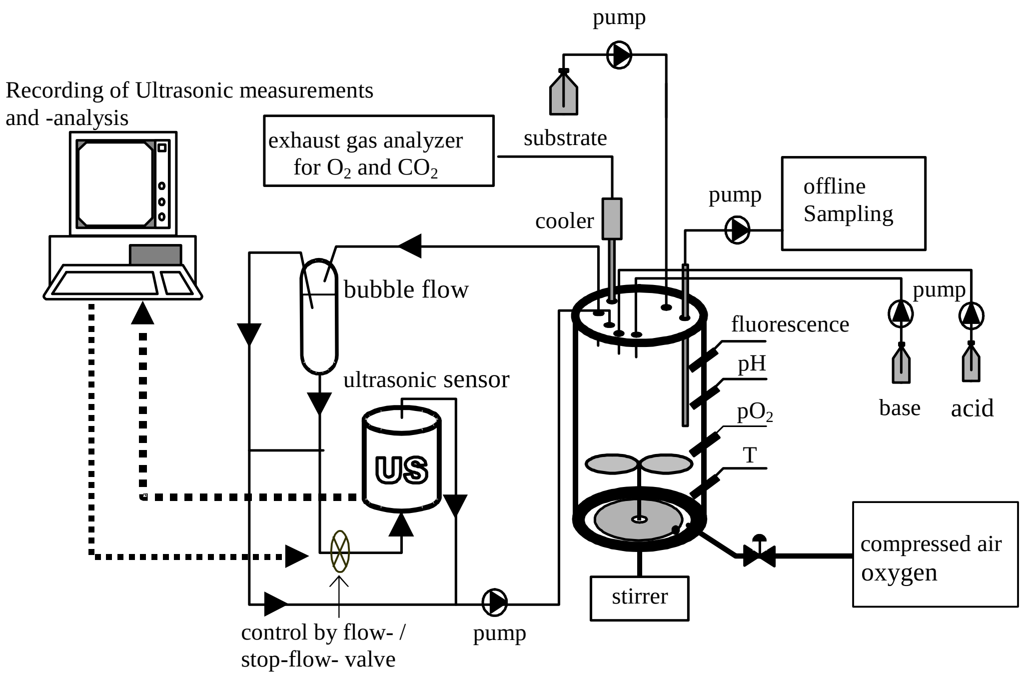

Fig. 1 The ultrasonic measurement system connected to the bioreactor by using a by-pass.

Here X is the biomass, t the time, $\mu_S^{\max}$ the maximal specific growth rate of the Monod model with respect to the substrate glucose S, K_S the corresponding Monod constant, $\mu_E^{\max}$ the maximal specific growth rate of the Monod model with respect to the substrate ethanol E, K_E is the corresponding Monod constant, Y_{XS} is the yield factor of biomass with respect to glucose, Y_{ES} is the yield factor of ethanol with respect to glucose and Y_{XE} is the yield factor of biomass with respect to ethanol.

For the numerical integration of the differential equations the 4th order Runge-Kutta method has been applied. As a measurement model for the Kalman filter the following equation has been applied:

$$v_{US} = v_0 + c_x X + c_s S + c_E E \qquad (4)$$

Here v_{US} is the ultrasonic velocity, v_0 is the constant factor of the multi-linear regression model, and c_X, c_S, and c_E are the regression coefficient with respect to biomass, the substrate glucose and ethanol, respectively. The continuous-discrete extended Kalman filter was implemented by using Borland Delphi (Version 4).

For the calculation of the mean error of prediction the following equation is used:

$$MEP_X = \sqrt{\frac{\sum_{i=1}^{N}(x_i^m - \hat{x}_i)^2}{N-1}} \tag{5}$$

Here MEP_x is the mean error of prediction with respect to the variable x, N the number of measurements of the variable x, x_i^m the ith measurement of x, and $\hat{x}_i$ is the corresponding estimated value.

Results

The ultrasonic measurement system has been applied during several cultivations. A typical signal of the cyclic measurement mode, which has been used throughout all experiments, can be seen in Fig. 2. After the flow in the measurement chamber has been stopped (marked by A in the Figure) the ultrasonic velocity decreases from v_{US}=1532 m/s to v_{US}=1520 m/s. At the same time also the temperature in the measurement chamber was decreasing for about ΔT=1 °C. This temperature change influences also the ultrasonic velocity. A temperature difference of 1 °C will cause a decrease in ultrasonic velocity by roughly Δv_{US}=2 m/s. However, the used ultrasonic measurement system compensates such temperature changes by itself automatically. Therefore the change in the ultrasonic velocity Δv_{US}=12 m/s is mainly caused by the bubbles, which are leaving the measurement regime after the flow has stopped. The stop of flow has been performed for just 40 s, so that the residence time of the cells in the by-pass was not too high and therefore the oxygen limitation not too long.

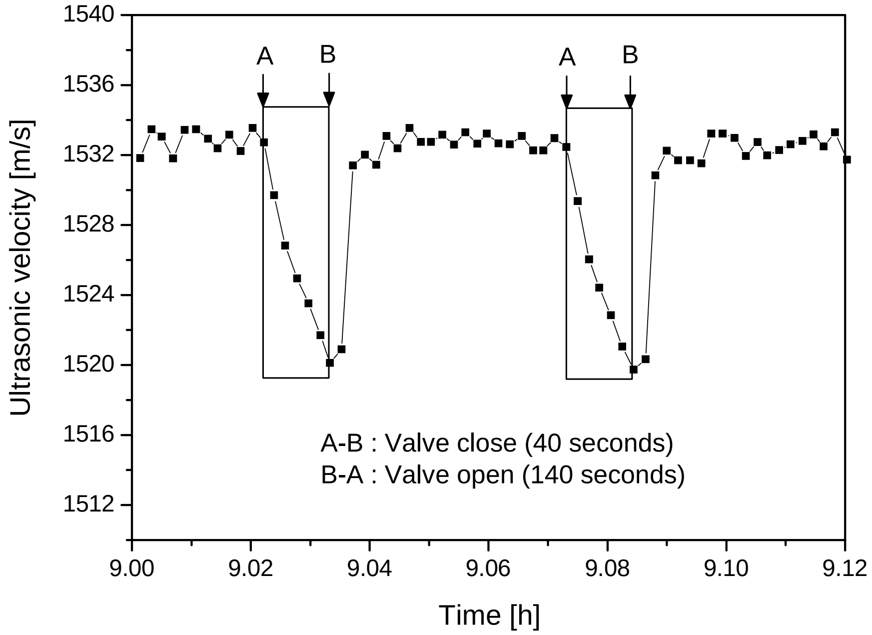

Fig. 2 The ultrasonic measurement signals during flow and stop-flow phases

In Fig. 3 all ultrasonic measurements of one batch cultivation are presented compared to the carbon dioxide concentration in the exhaust gas. One can clearly distinguish the ultrasonic measurements during the flow phase (higher values) and the stop-flow phase (lower values). The typical three phases of such a cultivation can be seen in the ultrasonic measurements. During the consumption of glucose and the ethanol production phase until 9 h cultivation time (Fig. 4), the ultrasonic velocity is almost constant. Here the decrease of the velocity due to decreasing glucose concentration is compensated by the increase of the velocity due to increasing biomass and ethanol concentration. During the ethanol consumption phase the decrease of the ultrasonic velocity can not be compensated by the biomass production and the values slowly decrease. Immediately after all ethanol is consumed the third phase starts and the cells begin to metabolize acetate, here the velocity rises sharply.

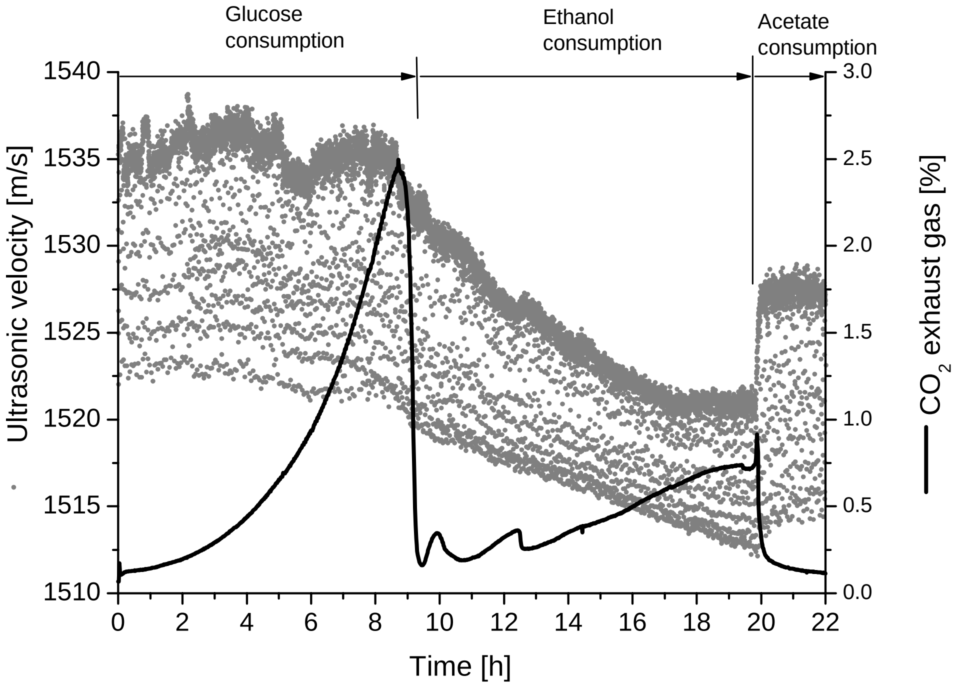

Fig. 3 The ultrasonic velocity and the carbon dioxide concentration during the *Saccharomyces cerevisiae* cultivation A

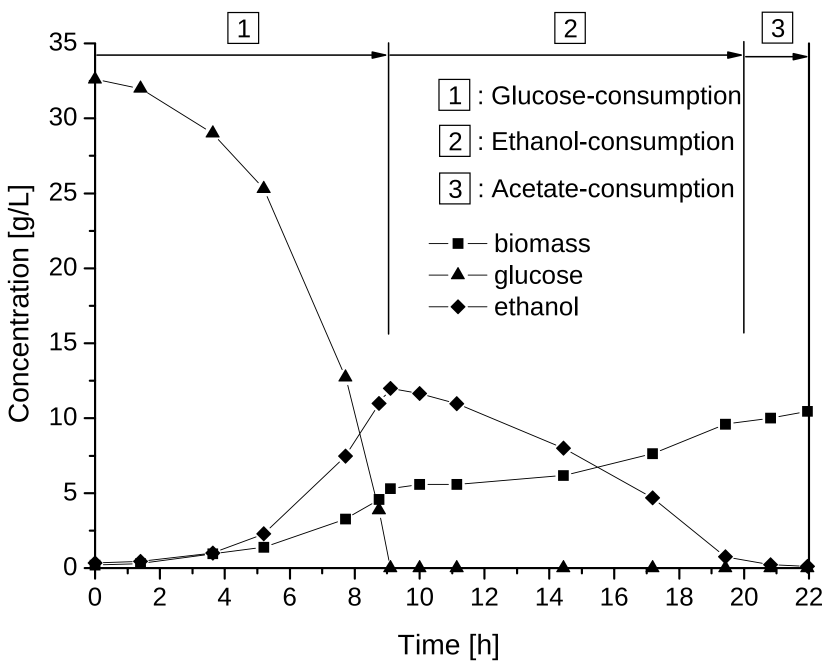

Fig. 4 Off-line measurements of biomass, glucose and ethanol during the cultivation of *Saccharomyces cerevisiae* B

To evaluate the ultrasonic measurements a multi-linear regression model was calculated from equation (4) by using the measurements from biomass, glucose and ethanol. However, due to the fact that not many off-line measurements have been available and to reduce the measurement noise the measurement values were not used directly. Instead simulated values have been applied, which were obtained from the bioprocess model (1) – (3), whose parameters were determined by the measurements of cultivation A. The higher number of values improved the regression model significantly. The simulated process variables as well as its corresponding measurements are presented in Fig. 5. The obtained regression coefficients are as follows: v_0=1457.6 m/s, c_X=5.7 mL/sg, c_S=2.0 mL/sg, c_E=2.7 mL/sg. The multi-linear regression model can be used to predict the ultrasonic measurements of new process runs. The results are presented in Fig. 6 for the cultivation B. Here the estimated velocity is calculated by using simulated values of the process variables. The mean error of prediction is MEP_{us}=1.6 m/s.

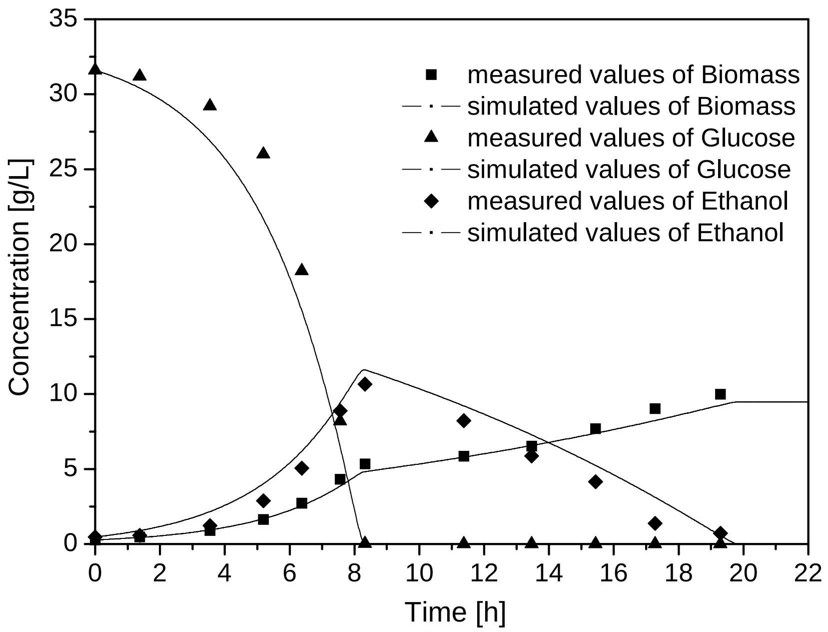

Fig. 5 Simulated and measured bioprocess variables of the *Saccharomyces cerevisiae* cultivation B (the parameter estimation of the model has been performed with measurements of process run A)

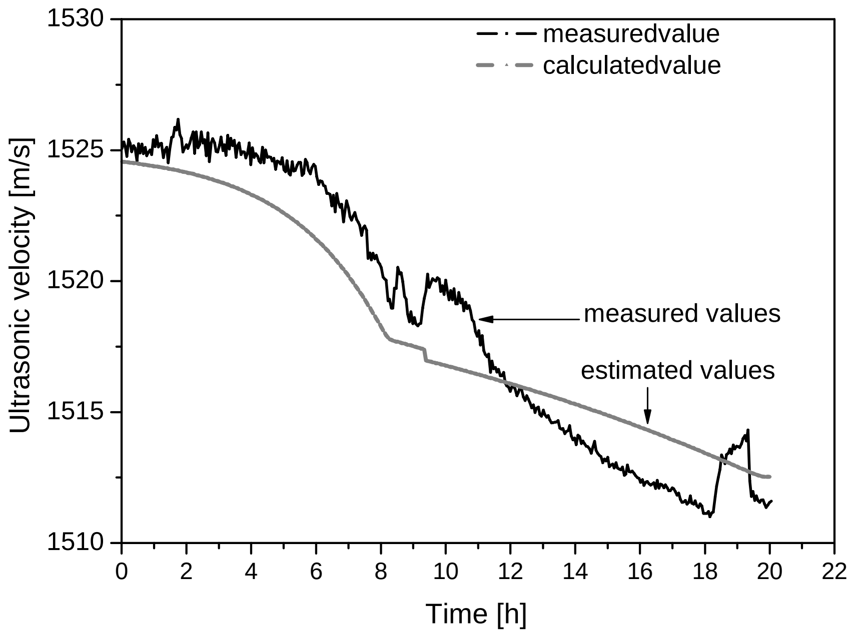

Fig. 6 Measured and predicted ultrasonic velocity of the *Saccharomyces cerevisiae* cultivation B

The multi-linear regression model can also be used to predict one of the three process variables by using the simulated values of the other two as well as the ultrasonic measurements. In Fig. 7 the predicted process variables as well as the corresponding simulated variables are presented. As one can see, the simulated as well as the estimated values fit quite well. The mean error of prediction with respect to the off-line measurements for the process variables are MEP_X=0.7 g/L for biomass, MEP_S=5.1 g/L for glucose and MEP_E=1.8 g/L for ethanol estimation. In Table 1 the mean errors of prediction of all cultivations are presented.

Table 1 Mean error of prediction off the bioprocess variables calculated with the multi-linear regression model, ultrasonic measurements and the corresponding process variables

Cultivation	Purpose	MEP_X [g/L]	MEP_S [g/L]	MEP_E [g/L]
A	model building	0.3	0.7	0.6
B	model validation	0.7	5.1	1.8
C	model validation	0.6	1.1	1.8
D	model validation	0.6	0.7	0.8
Mean value of model validation		0.6	2.3	1.5

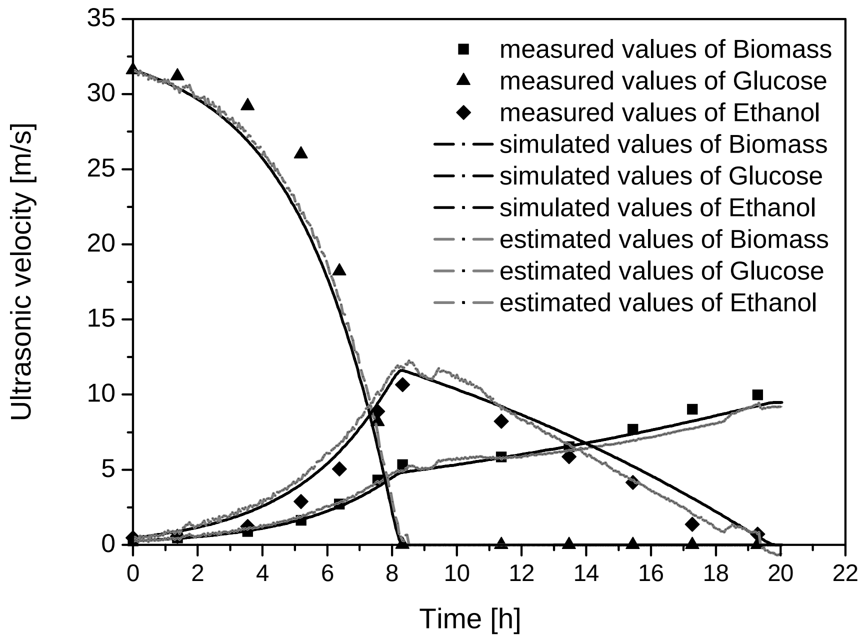

Fig. 7 Off-line measurement values of the process variables, its prediction using the multi-linear regression model as well as simulated values of a *Saccharomyces cerevisiae* cultivation B

To improve the estimation of the bioprocess variables by using the ultrasonic velocity measurements a Kalman filter was applied. As process model the equation (1) – (3) are used, as measurement model the equation (4) was applied. The measurement data for the cultivation A have been used to calculate the parameters of the process model. During simulations the parameters of the Kalman filter were determined. The parameter values are presented in Table 2.

The off-line measurements and the simulated data of cultivation A can be seen in Fig. 8. Fig. 9 presents off-line measurement and estimated values from the process variables of cultivation B. The mean errors of estimation for the process variables of all cultivations are presented in Table 3.

Table 2 The determined parameters of the process model and the Kalman filter

Parameter	Value
$\mu_{\max}^{S}$ [1/h]	0.3
$\mu_{\max}^{E}$ [1/h]	0.06
K_m^S [g/L]	0.4
K_m^E [1/h]	0.005
Y_{XS}	0.12
Y_{ES}	0.4
Y_{XE}	3.6
R [m²/s²]	0.17
Q[1,1] [g²/L²s]	0.00001
Q[2,2] [g²/L²s]	0.000085
Q[3,3] [g²/L²s]	0.0001
$Q\underset{i\neq j}{[i,j]}$ [g²/L²s]	0

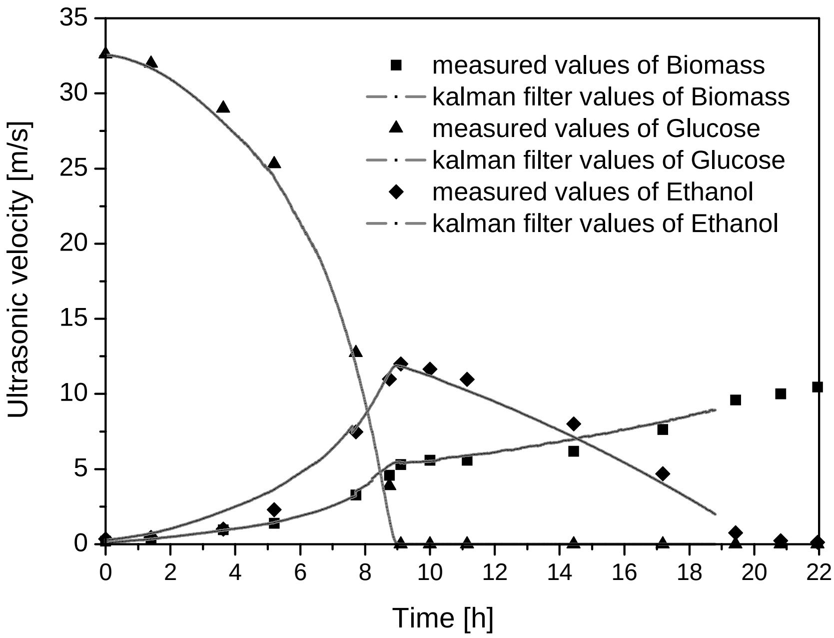

Fig. 8 Measurement data and predicted data by the Kalman filter of cultivation A

Table 3 The mean errors of prediction by the Kalman filter

Cultivation	Purpose	MEP_X [g/L]	MEP_S [g/L]	MEP_E [g/L]
A	model building	0.3	0.5	0.6
B	model validation	0.6	1.5	2.0
C	model validation	0.7	3.8	2.0
D	model validation	0.6	0.2	0.8
Mean value of model validation		0.6	1.8	1.6

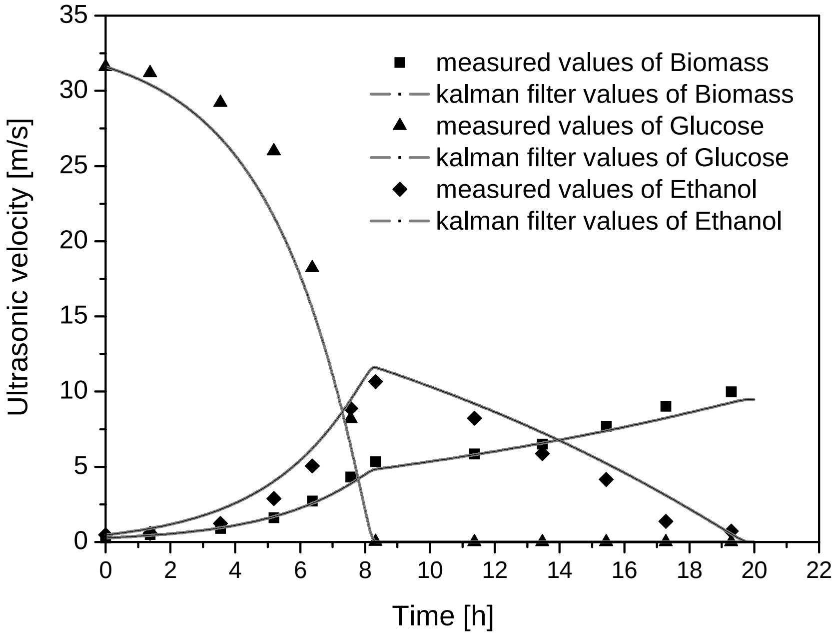

Fig. 9 Measurement data and predicted data by the Kalman filter of cultivation B

Discussion

The measurements presented show, that the ultrasonic measurements can be used to identify the different phases of the *Saccharomyces cerevisiae* cultivation. During the glucose phase the velocity signals were almost constant, after glucose depletion the signals began to decrease. About 13 m/s the velocity was reduced during ethanol consumption. When all the ethanol is consumed, a skip in the measurements can be recognised, which indicate the start of using acetate as substrate. Therefore, by the ultrasonic measurements themselves the different phases of the cultivation can be identified.

The evaluation of ultrasonic velocity measurements is presented by a multi-linear regression model. Here the ultrasonic signals can be used to predict one of the three bioprocess variables biomass, glucose and ethanol concentration, if the other two variables are known. The errors are acceptable; however measurement errors of the two process variables will directly influence the prediction quality.

If no further measurements are available, then extra process information must be used such as process information in form of a Kalman filter. Here all three variables can be predicted. But as typical for the Kalman filter, it depends heavily on the quality of the mathematical model behind the Kalman filter. If the model is not valid, the estimation will be wrong. Therefore, its application is restricted to cultivations, for which a precise model is available.

Conclusion

The investigation shows that valuable information about the yeast cultivation can be obtained by the ultrasonic velocity measurements. Using the multi-linear regression model by itself important bioprocess variables can be determined; however other measurements are required. A Kalman filter enables the prediction of biomass, glucose and ethanol concentration simultaneously, without any further measurement and is, therefore, top quality.

Acknowledgements

The authors acknowledge the financial support of the AIF. They thank P.-C. Daur and Prof. Dr. B. Henning providing the ultrasonic measurement system.

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