Characterizing the Substrate Control Problem of Ethanol Monitored Fed-Batch Yeast Production

Axelsson, Jan Peter

1988

Document Version:
Publisher's PDF, also known as Version of record

CODEN: LUTFD2/(TFRT-7390)/1–006/(1988)

Characterizing the Substrate Control Problem of Ethanol Monitored Fed-batch Yeast Production

Jan Peter Axelsson

Department of Automatic Control
Lund Institute of Technology
May 1988

Department of Automatic Control
Lund Institute of Technology
P.O. Box 118
S-221 00 Lund Sweden

Document name
INTERNAL REPORT

Date of issue
May 1988

Document Number
CODEN: LUTFD2/(TFRT-7390)/1-006/(1988)

Author(s)
Jan Peter Axelsson

Supervisor
Per Hagander

Sponsoring organisation
STU

Title and subtitle
Characterizing the Substrate Control Problem of Ethanol Monitored Fed-batch Yeast Production

Abstract

The substrate control problem of fed-batch yeast production is investigated. The control principle is based on the fact that the yeast produces ethanol in case of over-feeding and consumes ethanol in case of under-feeding. A mathematical model is derived for the ethanol dynamics in a fed-batch reactor. The transfer function has one fix and one time-varying part. Several closed-loop identification experiments were done on laboratory scale, to validate the model at different cell concentrations. Interpretation of the data from these experiments are discussed in some detail. Data was fitted to time invariant linear models of different orders. It was possible to follow process variations under a cultivation. A wider experience from many ethanol controlled cultivations are also summarized. The most apparent difference between ethanol-controlled cultivations are deviations in the exponential feed rate profile. This is considered the main reason for control. The sensor time delay and dynamics impose a limitation on the control performance. However, the results from identification promise good possibilities for dead time compensation. Further, the structure of the time-variable, more uncertain part, can be described by a first order system, and this fact might be useful in control design. Differences in process dynamics between cultivations are also reported and the implications for tuning are discussed.

The paper is submitted to the IFAC symposium on Adaptive Control of Chemical Processes, ADCHEM' 88, Copenhagen, August 17-19, 1988.

Key words
Baker's yeast, fed-batch production, ethanol sensor, closed-loop identification, model reduction, structured uncertainity.

Classification system and/or index terms (if any)

Supplementary bibliographical information

ISSN and key title

ISBN

Language
English

Number of pages
6

Recipient's notes

Security classification

Characterizing the substrate control problem of ethanol monitored fed-batch yeast production

Jan Peter Axelsson

Department of Automatic Control, Lund Institute of Technology
P.O. Box 118, S-221 00 Lund, Sweden

Abstract. The substrate control problem of fed-batch yeast production is investigated. The control principle is based on the fact that the yeast produces ethanol in case of over-feeding and consumes ethanol in case of under-feeding. A mathematical model is derived for the ethanol dynamics in a fed-batch reactor. The transfer function has one fix and one time-varying part. Several closed-loop identification experiments were done on laboratory scale, to validate the model at different cell concentrations. Interpretation of the data from these experiments are discussed in some detail. Data was fitted to time invariant linear models of different orders. It was possible to follow process variations under a cultivation. A wider experience from many ethanol controlled cultivations are also summarized. The most apparent difference between ethanol-controlled cultivations are deviations in the exponential feed rate profile. This is considered the main reason for control. The sensor time delay and dynamics impose a limitation on the control performance. However, the results from identification promise good possibilities for dead time compensation. Further, the structure of the time-variable, more uncertain part, can be described by a first order system, and this fact might be useful in control design. Differences in process dynamics between cultivations are also reported and the implications for tuning are discussed.

Keywords: Baker's yeast, fed-batch production, ethanol sensor, closed-loop identification, model reduction, structured uncertainity.

Introduction

There are several studies on substrate control of baker's yeast production. The yeast changes metabolism within a minute in case of over-feeding (Käppeli and Sonnleitner, 1986). Different measurement techniques have been presented to monitor the yeast metabolism. Exhaust gas analysis of carbondioxide and oxygen is a general, widely spread technique. Fluoroscence measurement, related to the internal cell concentration of NADH, is a more speculative method but has been tested for process control (Meyer and Beyeler, 1984). Ethanol measurement in the broth using a semipermeable membrane (Dairaku et al, 1981), is a promising technique that is gaining acceptance. It is motivated from the fact that the yeast excretes ethanol in case of over-feeding and takes up ethanol in case of under-feeding.

Modern control concepts have been applied to this process in different ways. Observers have been designed based on stoichiometric models developed primarily for stationary conditions (Wang, Cooney and Wang, 1977) (Dekkers, 1983). There is a lack in understanding the dynamical behaviour of yeast cultures. This have been a motive for a few groups to test the feasability of adaptive control (Dekkers and Voetter, 1985) (Verbruggen et al, 1985) (Montgomery et al, 1985). On the other hand, there is an emerging interest in cell culture dynamics, not only for control purpose but also motivated purely biotechnically (Kätterer et al, 1985).

Here is presented a dynamical study of baker's yeast in a fed-batch reactor. The broth ethanol concentration was measured continuously using a membrane gas sensor (Mandenius and Mattiasson, 1983). Experimental results are compared with a theoretical model. The disturbances motivating control are also evaluated, as well as the process uncertainty. General implications are discussed for control design and tuning.

Experimental

A description of the cultivation conditions is briefly given below. For details see (Axelsson et al, 1988).

Cultivation conditions

Baker's yeast, *Saccharomyces cerevisiae*, was grown on sugar beet molasses with a sugar concentration of 50% (w/v). The feed contained 1.69 kg molasses, which was mixed with 1 litre H_2O, and the resulting sugar concentration was about 410 g/L. The yeast strain and the molasses were supplied by Svenska Jästbolaget AB. Cultivations were performed in a fermentor (FLC-B-8 Chemoferm AB, Hägersten, Sweden) with a working volume of 6 litre. The dissolved oxygen concentration was monitored using a galvanic oxygen electrode. The aeration was controlled by the stirrer speed. It was kept constant during the test periods if not otherwise stated. Temperature was kept at 30° C and pH at 5.0 with $NaOH$, using conventional control.

Ethanol sensor, pump and control system

Ethanol was monitored using a semiconducter gas sensor (TGS 812, Figaro Engineering Inc, Osaka, Japan) in combination with a silicone membrane sampling probe immersed in the cultivation medium (Mandenius, 1983). The substrate feed rate was controlled by a peristaltic pump (Ismatec mp-4). The actual feed rate was checked using data from a load cell (Sartorius mp-8), which the substrate vessel was placed on. A PDP 11/03 microcomputer was used for control, monitoring and data logging. The ethanol signal was prefiltered with a second order Butterworth filter with a time constant of 60 sec. Data was logged on disc every 30 sec. The feed rate was controlled around an exponential basic dosage scheme. Adjustments were made using feedback from the ethanol signal. A PID regulator was used with a sampling interval of 30 sec. The experimental set-up is shown in Figure 1.

Process model

The yeast was grown in a well-mixed fed-batch reactor under substrate limited conditions near the critical growth rate where the yeast metabolism switches. The oxygen supply was assumed to be sufficient.

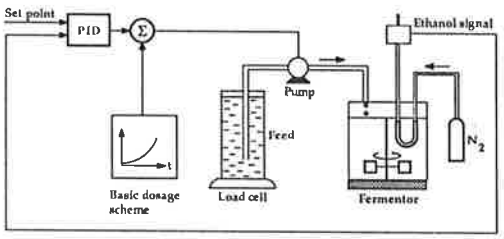

Figure 1. The experimental set-up.

Ethanol dynamics in a fed-batch reactor

Mass balance equations are given below. Note that the cell concentration X, as well as the volume of the broth V, increase during a cultivation. Let S denote the substrate and E the ethanol concentration in the broth.

$$\begin{aligned} \frac{dV}{dt} &= F \\ \frac{d(VX)}{dt} &= \mu(S,E)\cdot VX \\ \frac{d(VS)}{dt} &= -q_S(S)\cdot VX + S_{in}\cdot F \\ \frac{d(VE)}{dt} &= q_E(S,E)\cdot VX \end{aligned} \tag{1}$$

The relation between substrate uptake rates, cell growth and ethanol formation remains to be characterized. Here critical growth rate is assumed and only small variations in the substrate concentrations are considered. Further, the ethanol concentration is not allowed to reach inhibiting concentrations. The following approximations are then appropriate.

$$\begin{aligned} \mu(S,E) &= \mu_c \\ q_S(S) &= q_S^\circ + \alpha S \\ q_E(S,E) &= -q_E^\circ + \beta S,\ E>0 \end{aligned} \tag{2}$$

Note that under these assumptions the differential equations become linear.

$$\begin{aligned} \frac{d}{dt}\begin{pmatrix} VS \\ VE \end{pmatrix} &= \begin{pmatrix} -\alpha X & 0 \\ \beta X & 0 \end{pmatrix}\begin{pmatrix} VS \\ VE \end{pmatrix} \\ &+ \begin{pmatrix} S_{in} \\ 0 \end{pmatrix} F - \begin{pmatrix} q_S^\circ \\ q_E^\circ \end{pmatrix} VX \end{aligned} \tag{3}$$

The critical feed rate $F^0(t)$ to obtain stationarity in the ethanol concentration is proportional to cell mass.

$$S_{in}F^\circ = (q_S^\circ + \frac{\alpha}{\beta}q_E^\circ)VX \tag{4}$$

Metabolism operates in the time scale of a few minutes while cell growth is in hours. Over shorter time periods the ethanol dynamics can be approximated with a time invariant model.

$$T\frac{d\Delta S}{dt} + \Delta S = K_1\Delta F \tag{5a}$$

$$\frac{d\Delta E}{dt} = K_2\Delta S \tag{5b}$$

The parameters depend on volume and cell concentration as

$$T = \frac{1}{\alpha}\cdot\frac{1}{X} \tag{6a}$$

$$K = K_1\cdot K_2 = \frac{S_{in}}{\alpha}\frac{1}{VX}\cdot\beta X = \frac{\beta}{\alpha}\cdot\frac{S_{in}}{V} \tag{6b}$$

The ethanol dynamics change during a cultivation according to (6) as cell concentration and volume increase. There are some factors in the reactor conditions that are not modeled in (2). For instance, the oxygen transfer is often a limiting factor at high cell concentrations. Further, unmetabolizable products of the feed and certain byproducts of cell metabolism, may accumulate and reduce growth.

Ethanol sensor and substrate feeding system

On a laboratory scale the measurement and the feed distribution become much easier than in a larger reactor. Since a homogeneous fermentor is assumed the placement of the sensor is not critical. The feed can also be assumed to be immediately mixed. Controllable high precision feed pumps are available at a reasonable cost. During a typical cultivation the flow increases from 0.01 L/h to 0.35 L/h.

Ethanol was monitored using a semiconducter gas sensor in combination with a membrane sampling probe immersed in the cultivation medium. The dynamics of the sensor system is dependent of the ethanol diffusion over the membrane, the transport to the gas sensor, and the subsequent adsorbtion on to the semiconducter surface. The dynamics is well described by

$$T_s\frac{dy}{dt} + y = E(t-T_d) \tag{7}$$

Typical parameters were $T_s = 2$ min and $T_d = 2$ min. The noise level of the sensor is low, however the nonlinear characteristic may give severe problems in case of drift. It is further discussed in the section on modelling the disturbances.

The substrate feed rate was controlled by a peristaltic pump. Measures were taken to make the feed rate respond within seconds to a change in the pump signal. The feed tubing between substrate vessel, pump and fermentor was kept short, and any air-bubbles in the tubing were eliminated. The feed dropped into the broth from the top of the fermentor and it was easy to see that the drop frequency changed immediately when the pump signal changed. The actual feed rate was checked using data from the load cell.

Structure of the process transfer function

Insight into the control difficulties can be obtained by studying the process model in the frequency domain. A transfer function can be derived from the linear differential equations for the reactor (3) and the sensor (7). It has one fix and one time-varying part. The parameters K and T then depend on the volume and the cell concentration according to (6).

$$G(s) = G_{var}(s)\cdot G_{fix}(s) = \frac{K}{Ts+1}\cdot\frac{e^{-sT_d}}{s(T_ss+1)} \tag{8}$$

For control design it is important to consider the process transfer function around the bandwidth. A typical value lies in the range 5 – 20 rad/h. At start-up there is a considerable phase lag. During the first few hours the phase increases for all frequencies. The gain variation is more complex. In a mid frequency band, around the typical bandwidth, the parameter variations in K and in T interact and make the gain first increase and then decrease.

Process identification

Dynamical experiments were performed to validate the model.

Motivation for experiments in closed loop

The process model show that it would be difficult to make reproducible dynamical experiments in open loop. The process contains an integrator and is thus not stable. The cell mass grows and the critical feed rate increases. Further, the process gain is high, and accentuates the difficulties to maintain constant ethanol concentration without feedback.

Methods

The system was excited with a precalculated PRBS signal through the regulator ethanol set-point. The regulator was a PID regulator and the feed rate was controlled around an exponential basic dosage scheme. The PRBS signal amplitude was chosen to ± 0.15 g/L but at lower cell concentrations the amplitude was reduced to ± 0.05 g/L in order not to saturate the feed rate signal. The basic period was 4 min in data from cultivation 1 and 2, and 6 min in data 3a, 3b and 3c.

Statistical data analysis was done using the interactive program package MATLAB extended with the System Identification Toolbox (MathWorks, Inc). Maximum likelihood methods were used.

Inherent difficulties of the identification problem

There are several choices to make in order to obtain an informative experiment. Simulation studies are a good help.

In order to simplify the analysis of the data series, the length of the test period is chosen to be no longer than that the variation in the parameters (6) has a negligible influence. On the other hand, a short test period sets a limit to the maximal time constant that can be distinguished from a pure integrator. Here a test period of about three hours was used.

Minor differences between the dosage scheme and the critical feed rate could be reduced by the PID-regulator. However, larger differences may give rise to biases or trends in the measurement signal. Trends in both input and output signal are important to remove before process model identification.

The amplitude and frequency of excitation are crucial experiment parameters. It is important that the variation in the feed rate is no larger than that the model (5) is valid. This means that the feed rate should not be so low that the substrate vanishes and the ethanol consumption capacity saturates. Neither should the feed rate be too high so that the substrate concentration increases beyond the valid range of (2). The frequency of excitation was chosen so that the shortest peaks in the ethanol set-point should be noticeable in the ethanol signal.

The dynamics of ethanol production and consumption changes according to (6). The time constant T decreases typically from about 10 min at the start to 0.5 min at the end of a fed-batch cultivation. In this work the ethanol sensor had a time constant of about 2 min. This fact implies that it will be hard to determine T at high cell concentrations.

Results from experiments

Several identification experiments were done. In Figure 2. is shown raw data from three test periods during cultivation 3. The measured ethanol signal is compared with the output from the identified model in closed loop simulation. Note that the cell concentration increases considerable. See also Table 2. During the last test period the regulator was not able to keep the ethanol concentration down. The experiment was terminated before the PRBS sequence was finished. Note that the scales are changed between the test periods. To make comparison easy, the relation between the ethanol and feed rate scales are kept constant.

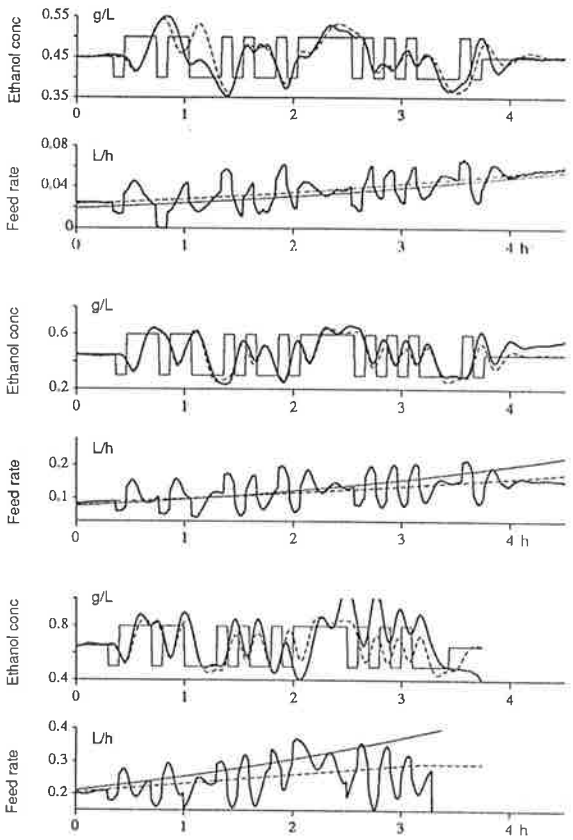

Figure 2. Three identification experiments: a, b and c, during cultivation 3. Thick lines are ethanol concentration and feed rate (upper, lower), thin lines are ethanol set-point and basic dosage scheme (upper, lower), and dashed line is the output from the identified model (upper) and estimated critical feed rate $\hat{F}^0(t)$ (lower).

Removing trends in the data

During some test periods the difference between the basic dosage scheme and the critical feed rate became substantial. The critical feed rate was estimated by fitting an exponential curve to the actual feed rate signal. However, in cultivation 3c a linear trend was more appropriate. By subtracting the estimated critical feed from the feed rate signal, the trend in the input signal was almost removed. Due to insufficient control a trend appeared also in the ethanol signal during some test periods. However, the trend in the measurement signal was close to a ramp and easy to remove.

The difficulties with removing trends in the feed rate signal is eliminated if identification is done between the set-point and the ethanol signal instead. However, it is difficult to interpret the closed-loop parameters in terms of process parameters.

Identified ARMAX models

The data was fitted to ARMAX models of different orders. The model order, delay and number of parameters were determined using Akaike test criterion and comparison of the models in the frequency domain (Ljung, 1987). Although a third order model is expected, only a second order model was motivated from the identification. The identified third order model gave the same frequency response as the second order model. The data fit was good and the residuals were white and not significantly correlated with the input. The obtained parameters showed the process integrator and one time constant.

Exploit the known integrator

In order to reduce the uncertainty in the estimate of process gain and time constant, the structure of the process integrator was included in the model by differencing the ethanol signal. Taking differences of the ethanol signal also reduces the effect of drifts due to poor control, on the identification. The data was fitted to a first order model, where $y(t) = E(t) - E(t-h)$, $u(t) = \Delta F(t)$, and $e(t)$ is a white noise residual. The sampling interval is denoted by h and the time delay by k.

$$\begin{aligned} y(t) = &- a_1 y(t-h) \\ &+ b_1 u(t-kh-h) + b_2 u(t-kh-2h) \\ &+ e(t) + c_1 e(t-h) \end{aligned} \qquad (9)$$

Data from six experiments are summarized in Table 1. One example is shown in Figure 3., where data is from experiment 3b. Note the low noise level.

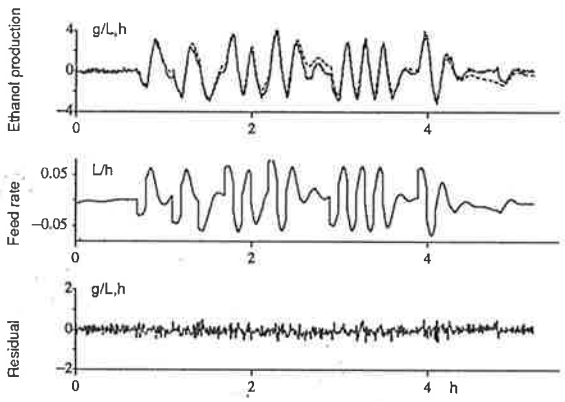

Figure 3. An example of differenced ethanol signal fitted to a first order model (9).. The upper diagram shows the differenced ethanol signal divided by the sampling interval, solid line. The dashed line is the output from the model. The variation of the feed rate from the estimated critical feed rate $\hat{F}^0(t)$ is shown in the middle and the lower diagram shows the residual $e(t)$ after identification. Data is from experiment 3b.

Table 1. Identified parameters in a first order ARMAX model.

Data	k	a_1	b_1	b_2	c_1
1	5	−0.79	0.034	0.058	−0.27
2*a*	8	−0.94	0.044	−0.010	−0.83
2*b*	4	−0.93	0.027	0.025	−0.06
3*a*	7	−0.89	0.074	−0.018	−0.43
3*b*	4	−0.87	0.030	0.050	−0.04
3*c*	4	−0.84	0.031	0.067	−0.82

The data in Table 1. needs a comment. The identified pole a_1 can be interpreted as a combination of the process and sensor time constants. At higher cell concentrations the sensor time constant dominates. The variation in the estimated time delay k with dynamics, is due to the fact that the underlying process dynamics has higher order and introduces extra phase lag. This fast dynamics was not sufficiently excited and could not be identified more than as an extra time delay. The noise level is generally low but it changes character between the test periods. In 2a and 3a the C-parameter is large and it is believed to be due to the lower signal levels and correspondingly higher quantization error.

Identified process parameters

The continuous time parameters $\hat{K}$ and $\hat{T}$ in (5) can be calculated from the identified discrete time parameters in Table 1.

$$K = \frac{1}{h}\frac{b_1 + b_2}{1 + a_1}, \quad T = -\frac{h}{\ln(-a_1)} \qquad (10)$$

provided the sensor time constant is negligible compared to the ethanol dynamics. The identified time delays $\hat{T}_d$ are also given. Dry weight measurements of the cell concentrations were done before and after each experiment except for experiment 1, where the cell concentrations were estimated from the amount of feed consumed.

Table 2. Interpretation of the data in terms of process parameters.

Data	V $[L]$	X $[g/L]$	$\hat{K}$ $[g/L^2]$	$\hat{T}$ $[min]$	$\hat{T}_d$ $[min]$
1	4.2 − 4.6	10 − 21	58	2.2	2.5
2*a*	4.0 − 4.1	4.6 − 9.3	70	7.8	4.0
2*b*	4.8 − 5.4	30 − 48	88	6.6	2.0
3*a*	4.0 − 4.3	4.0 − 13	60	4.2	3.5
3*b*	4.4 − 5.2	19 − 40	72	3.5	2.0
3*c*	5.8 − 6.3	50 − 63	74	2.9	2.0

Comments on the model

The results are in general very good. The ethanol dynamics show a deterministic behaviour and the noise level is very low.

A comparison between results from the same cultivation show an interesting result. The process time constant decreased towards the sensor time constant as expected. However, the process gain increased slightly while a slight decrease was expected from the model (6b). A natural explanation would be that the limiting oxygen transfer at high cell concentrations, also had an influence on the ethanol gain. However, the most immediate stoichiometric analysis does not reveal any relation between the availability of oxygen and the gain in the ethanol loop (Axelsson, 1988). Lack of oxygen would only decrease the critical feed rate.

Disturbances and process uncertainty

It is important to consider likely disturbances in the design of a control system. Here is discussed variations in the fermentation as well as disturbances introduced through the pump and the sensor. There is also differences in the process from cultivation to cultivation. Especially the critical feed rate profile differs. The disturbances could be divided into two time scales, and one should distinguish between differences between cultivations and variation during a typical cultivation. The discussion in this section leads up to a background for evaluating the robustness of a control system.

Variations in the exponential feed demand

The most apparent disturbance during the cultivations was the deviation in the feed demand from the exponential dosage scheme. The decrease in the feed rate growth was related to a decrease in the cell growth rate at high cell concentrations. The oxygen transfer might be a limiting factor, and certain products may accumulate and reduce the growth rate as well. Typical examples can be seen in the data series shown in Figure 2.

The feed rate profile also differs considerably between cultivations. The size and quality of the inocculum vary. These differences have a drastic influence on the feed demand during the latter half of the cultivation.

Sensitivity to errors in the feeding system

An observation from the process model is that the relative sensitivity to variations in the feed rate increases considerable during a fed-batch cultivation. It increases with cell density, since the critical feed rate grows as cell mass (4), and the process gain K remains almost constant, (6b) and Table 1.

This means that the pump accuracy should be measured in absolute terms. Here an high precision peristaltic pump was used. There was no problem to maintain good precision throughout a cultivation. The actual feed rate was checked afterwards using the load cell data. The pump signal was compared with differences in the weight measurement signal, and the pump gain and the pump bias were estimated. Our experience is that the bias was close to zero while the gain usually varied about 10% between cultivations.

On an industrial scale it is common to use several pumps to achieve accuracy over a wide range of feed rates. A typical installation could use combinations of pumps with different capacities which are turned on and off according to the feed rate demand. Then, on top of that, a special pump with variable feed rate could be used for control purpose. The on-off control of the larger pumps is likely to influence the ethanol control loop. Step disturbances at the feed rate is a reasonable model to account for this difficulty, when a control system is designed.

Deterioration of the ethanol sensor

The ethanol sensor was calibrated before and after each cultivation. The fact that the sensor has a pronounced non-linear characteristic makes it sensitive to parameter variations. An observed bias in the calibration may also result in a gain variation.

The dynamics of the sensor did not change. Only extraordinary circumstances made the time constant and the time delay increase. However, fouling of the membrane was never a problem. The noise level of the sensor was negligible.

Gain variations due to reactor phenomena

There are several factors that may influence the process gain. Drifts in the sensor and pump calibrations are already mentioned. The substrate concentration of the feed is another source of uncertainty. Molasses is a complex substrate and contains many differents sugars. The industrial experience is that concentration of fermentable sugars may vary with 15 %. In the identification experiments all these uncertainities were eliminated. Despite these precautions there is a deviation of the identified process gain from what was expected. The process model implies that the gain should decrease as the volume increases (6b). However, from the identification studies the tendency was that the gain increased slightly during the cultivation.

In our ethanol controlled cultivations (Axelsson et al, 1988) there are several examples of how a low dissolved oxygen concentration have decreased the feed rate increase. When the dissolved oxygen concentration increased due to change of stirrer speed, the feed rate increased. These examples did not show any tendency to unstable control actions. There is no evidence so far of an increased gain in the ethanol loop due to low dissolved oxygen concentrations.

Under extraordinary conditions there are of course many factors that might influence the process gain in the substrate control loop. During one cultivation for example, low pH caused unstable control actions (Axelsson, 1987). Such disturbances should be detected and cause an alarm, rather than be compensated for by adapting the regulator.

Differences between cultivations

The accumulated experience over a two year period with ethanol controlled yeast cultivations is that the process differs considerable in some respects, (Axelsson, 1987) and (Axelsson et al, 1988). The differences in the feed rate profile is striking, see Figure. 4. This difference in the feed rate demand from cultivation to cultivation, shows that feed-back is valuable to maintain a precise respiratory state of the yeast culture throughout a cultivation.

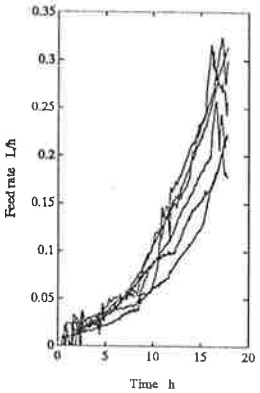

Figure 4. Feed profiles in six cultivations when the broth ethanol concentration was kept constant.

The identification results from different cultivations show some variations in the process gain. Part of these differences were due to drift in the calibration of the pump and this have been compensated for before presenting the data. There were also differences in the identified process time constant.

Background for a robustness discussion

The main reason for control is to track the exponential increase in the feed rate demand. The uncertainty in growth rate and in initial cell mass makes it of limited value to use only a precalculated dosage scheme. It is hard to avoid an exponentially growing load.

The time delay and dynamics of the sensor set a constraint on the magnitude of the control gain that can be used with maintained stability. In other words, there is a relation between the response time of the sensor and the deviation in the exponential feed demand that can be compensated.

The limitation imposed by the sensor delay on the control system, can be diminished if the process is predictable in the time scale of the sensor response. A good process model is therefore of great interest. Part of the dynamics is well-known. The time-varying, more uncertain

part can be described in terms of a first order system. The structure of the uncertainty may be exploited in control design.

The results from the identification experiments promise good possibilities for short term prediction. If on-line estimation of the process model were to be implemented, the level of excitation could only justify a reduced order model. However, a reduced order model means that the estimated time delay varies considerably and it should be accounted for in the estimation algorithm. The change in the process time constant is quite predictable and could be exploited in the algorithm. A quantitative robustness analysis of the closed loop performance is called for, in order to determine how accurate the short term prediction ought to be. The control system must also be designed to manage reasonable differences in the equipment gains between cultivations. An estimate of the process gain with an uncertainty of a factor two should be safe.

The demands on the control change character during the cultivation. The exponential load on the system is stressed here. It becomes pronounced mainly during the latter half of the cultivation. Further, on an industrial scale, step disturbances in the feed rate may be more frequent during this period. At low cell concentrations, during the start-up of the cultivation, the feed rate growth is small. The main difficulty is then the slow dynamics in the reactor combined with the fact that the control authority is low. It is important that an overshoot in the ethanol concentration is avoided, because of the long time required for the cells to consume it. The different character of the control difficulties at start-up and late in the cultivation may call for different regulators.

Conclusion

The possibilities for good substrate control in baker's yeast production has been investigated for the case when the broth ethanol concentration is monitored. A process model was developed, and identification experiments confirmed the model. The ethanol signal is well predictable in the time scale of minutes using a second order model. Comparing the identified models at different cell concentrations reveals an interesting fact. The experimentally found process gain increases slightly during the cultivation while the theory predicts a slight decrease.

The main reason for feedback is to track the exponential increase in the feed demand. The sensor response time imposes a limitation on the deviation in the exponential feed demand that can be compensated. The process time constant decreases with the cell concentration and an approximate value can be precalculated. A longer sensor response time demands a more precise model to predict the ethanol concentration and adaptive techniques may be justified. Then it is important to allow for a variation in the time delay which was observed in the identification. The fact that the process uncertainty can be described in terms of a first order system can be used in robustness analysis of such an adaptive system.

Differences in the ethanol dynamics between cultivations may call for re-tuning of the regulator. Automatic procedures are interesting but there is not much time for tuning experiments during a fed-batch cultivation. An auto-tuner that incorporates part of the known process dynamics may reduce the tuning experiment time.

In fact, a well-tuned PID-regulator have been used for dozens of cultivations in our laboratory (Axelsson et al, 1988). Tuning was done occasionally, but not without difficulties. There is room for improvements. Especially, a process situation with longer sensor response time would call for precise tuning and may be a more complex regulator to ensure a robust performance.

Acknowledgements

The author wish to thank Ms Lena Nielsen for excellent technical assistance and docent Per Hagander for reading the manuscript and giving fruitful criticism. Dr Carl Fredrik Mandenius and Dr Olle Holst had the responsibility for the laboratory and Carl Fredrik also developed the ethanol sensor. The yeast strain and the molasses were gifts from Svenska Jästbolaget AB. The work was part of a joint project between the departments of Biotechnology and Automatic Control at LTH, and it was supported by the Swedish Board for Technical Development, contract 82-3359 and 82-3494.

References

AXELSSON, J. P. (1987): "Ethanol-controlled fed-batch cultivation of baker's yeast on molasses - experimental experience," CODEN: LUTFD2/TFRT-7316, Department of Automatic Control, Lund Institute of Technology, Lund, Sweden.

AXELSSON, J. P. (1988): "Experimental techniques and data analysis to determine baker's yeast ethanol dynamics," *Anal. Chim. Acta*, submitted for publication.

AXELSSON, J. P., C. F. MANDENIUS, O. HOLST, P. HAGANDER and B. MATTIASSON (1988): "Experience in using an ethanol sensor to control molasses feed-rates in baker's yeast production," *Bioprocess Engineering*, **3**, 1–9.

DAIRAKU, K., Y. YAMASAKI, K. KUKI, S. SHIOYA and T. TAKAMATSU (1981): "Maximum production in a baker's yeast fed-batch culture by the tubing method," *Biotechnol. Bioeng.*, **23**, 2069–2081.

DEKKERS, R. M. (1983): "State estimation of a fed-batch baker's yeast fermentation," in A. Halme: *Modelling and control of biotechnological processes - 1st IFAC workshop, preprints.*, IFAC, Pergamon Press, pp. 201–211.

DEKKERS, R. M. and M. VOETTER (1985): "Adaptive control of fed-batch baker's yeast fermentation," in A. Johnson: *Modelling and control of biotechnological processes - 1st IFAC symposium, preprints.*, IFAC, Pergamon Press, pp. 73–80.

KÄPPELI, O. and B. SONNLEITNER (1986): "Regulation of sugar metabolism in *Saccharomyces*-type yeast: experimental and conceptual considerations.," *Critical Reviews in Biotechnology.*, **4**, 299–325.

KÄTTERER, L., H, ALLEMANN, O. KÄPPELI and A. FIECHTER (1986): "Transient responses of continuously growing yeast cultures to dilution rate shifts: a sensitive means to analyze biology and the performance of equipment," *Biotechnol. Bioeng.*, **28**, 146–150.

LJUNG, L (1987): *System identification*, Prentice Hall.

MANDENIUS, C. F and B. MATTIASSON (1983): "Improved membrane gas sensor systems for on-line analysis of ethanol and other volatile organic compounds in fermentation media," *Eur. J. Appl Microbiol Biotechnol*, **18**, 197–200.

MEYER, C. and W. BEYELER (1984): "Control strategies for continuous bioprocesses based on biological activities," *Biotechnol. Bioeng.*, **26**, 916–925.

MONTGOMERY, P. A., D. WILLIAMS and B. H. SWANICK (1985): in A. Johnson: *Modelling and control of biotechnological processes - 1st IFAC symposium, preprints.*, IFAC, Pergamon Press, pp. 81–89.

VERBRUGGEN, H. B., G. H. B. EELDERINK and P. M. VAN DEN BROECKE (1985): in A. Johnson: *Modelling and control of biotechnological processes - 1st IFAC symposium, preprints.*, IFAC, Pergamon Press, pp. 91–96.

WANG, H. Y., C. L. COONEY and D. I. C. WANG (1977): "Computer-aided baker's yeast fermentation," *Biotechnol. Bioeng*, **19**, 69–86.