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1987

*Document Version:*

Publisher's PDF, also known as Version of record

[Link to publication](#)

*Citation for published version (APA):*

Axelsson, J. P., Mandenius, C. F., Holst, O., Hagander, P., & Mattiasson, B. (1987). *Experience in Using an Ethanol Sensor to Control Molasses Feed-Rates in Baker's Yeast Production*. (Technical Reports TFRT-7349). Department of Automatic Control, Lund Institute of Technology (LTH).

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5

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Experience in Using an Ethanol Sensor  
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March 1987

<b>Department of Automatic Control</b> <b>Lund Institute of Technology</b> P.O. Box 118 S-221 00 Lund Sweden		<i>Document name</i> INTERNAL REPORT	
		<i>Date of issue</i> March 1987	
		<i>Document Number</i> CODEN: LUTFD2/(TFRT-7349)/1-016/(1987)	
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<i>Title and subtitle</i> Experience in Using an Ethanol Sensor to Control Molasses Feed-rates in Baker's Yeast Production			
<i>Abstract</i> <p>An ethanol sensor has been tested for feed-rate control of baker's yeast production. The yeast was grown on molasses in an 8 dm<sup>3</sup> fed-batch reactor up to a cell concentration of 60-70 kg/m<sup>3</sup>. Studies were made on three levels: reliability of the sensor system, characterisation of the control problem, and evaluation of ethanol-controlled cultivations in terms of yield and production rate. Arguments are given for the conceptual advantages of ethanol control compared to other methods of substrate control. It is also shown that ethanol control allows for a simple regulator structure. In fact a PID regulator, with constant parameters, was used around an exponential dosage scheme. Tuning of the regulator parameters was performed by using simulation on a simplified model of the process. Several cultivations have been carried out. Results from four comparable cultivations are given in detail, and the experience from many others is summarized.</p> <p>The paper is submitted for publication in: Bioprocess Engineering, Springer International.</p>			
<i>Key words</i>			
<i>Classification system and/or index terms (if any)</i>			
<i>Supplementary bibliographical information</i>			
<i>ISSN and key title</i>			<i>ISBN</i>
<i>Language</i> English	<i>Number of pages</i> 16	<i>Recipient's notes</i>	
<i>Security classification</i>			

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## 1 INTRODUCTION

Production of baker's yeast requires improvement in several ways. The yeast is very sensitive to small changes in their fermentor environment [1, 2]. Feedback is therefore needed in order to maintain precise cultivation conditions. The scarcity of appropriate sensors to monitor significant process variables has until recently limited the control of the process to e. g. pH and temperature. However, new specific sensors for different substances in the fermentor has made it possible to monitor and control variables more closely related to the activity of the cells.

Sensors for direct measurement of substrate, cell mass or byproduct formation of for example ethanol, could then supplement the molasses dosage schemes used in industry today, using feedback control. Variations in the molasses feed quality, the condition of the inocula, the aeration, or inconsistencies of other factors related to the equipment, induce variations in cell growth. Feedback could be used for continuous compensation of such variations and thereby facilitate high yield and growth rate as well as a more uniform yeast quality.

To date, several controllers have been presented for fed-batch cultivation of baker's yeast. Depending on measurement technique and knowledge of the process, the structures have varied in complexity. A few groups have used on-line exhaust gas analysis for control [3, 4]. These measurements give information about oxygen uptake rate, carbon dioxide production rate, and respiratory quotient. Basically PID-controllers were used. Recently adaptive regulators have been tested in order to improve the performance of the substrate control loop [5-7]. There is also a report on a simple on-off regulator used on an industrial scale [8].

In a number of studies [9-12], an ethanol sensor based on a permeable membrane, that provides a reliable signal with a short response time, was tried and showed to be at least as sensitive as RQ-measurements in indication of the metabolic state of the culture [10]. Another advantage is its simplicity and low price. Mainly PID-control has been used, and ways to change the regulator parameters as the cell concentration increases, have been discussed [11, 12].

Much of the control difficulty in previous work, based on RQ or ethanol signals, is due to a slow response of the measurement system. A sensor with short response time will facilitate control. In this paper, it is shown how such a sensor combined with a simple regulator structure, based on a well-tuned PID-regulator around an exponential dosage scheme, controls the process for 15-18 h. The paper is organized as follows. First the ethanol sensor is investigated and possible sources of disturbances are discussed. Then the control problem is considered. The variation in dynamics during a cultivation and likely disturbances in industrial scale use, are discussed. The third part of the paper deals with the results obtained from cultivations subject to ethanol control and suggestions are made for further research using ethanol control as a tool.

## 2 EXPERIMENTAL

### Cultivation conditions

Baker's yeast, *Saccharomyces cerevisiae*, was used in this study. The strain was a gift from Svenska Jästbolaget AB, Rotebro, Sweden. The yeast was kept on malt agar plates and was reinoculated every month. Sugar beet molasses with a sugar concentration of 50 % (w/v) was used as medium. This was also supplied by Svenska Jästbolaget AB.

Cultivations were performed in a fermentor (FLC-B-8 Chemoferm AB, Hågersten, Sweden) with a working volume of 6 dm<sup>3</sup>. Temperature was controlled at 30°C. Two molar NaOH was used to keep the pH constant at 5.0. Foam was controlled by addition of the antifoam Glanapon DG 111. Aeration rate was 1.0 volume/volume·min. Dissolved oxygen was measured using a galvanic oxygen electrode [13].

Cultivations have been carried out in three stages: growth of inoculum, batch and fed-batch cultivation. The growth of inoculum took 16 h, the batch cultivation 12-14 h, and after a two hour pause the fed-batch cultivation started and continued for about 18 h. One critical stage was the start-up of the fed-batch cultivation. The end of the batch cultivation is clearly indicated by the sudden rise of the dissolved oxygen signal and the level off of the signal from the ethanol sensor. The zero level of the ethanol signal was at this point recalibrated. The culture was starved for two hours, and then it was given a substrate pulse of 20 g of the fed-batch media. In this way the ethanol concentration of the broth rose quickly and came close to the set-point 0.4 kg·m<sup>-3</sup>, within 45 min. The regulator was then started. The fed-batch cultivation continued for 18 h.

The inoculum was cultivated in shaker flasks for 16 h at 30° on 50 kg·m<sup>-3</sup> molasses, 5 kg·m<sup>-3</sup> NH<sub>3</sub>, 5 kg·m<sup>-3</sup> H<sub>3</sub>PO<sub>4</sub> and 2.5 kg·m<sup>-3</sup> MgSO<sub>4</sub>·7H<sub>2</sub>O. The magnesium salt was sterilized separately. The pH was adjusted to 5.5 with H<sub>2</sub>SO<sub>4</sub>/NaOH.

The medium in the batch was composed as follows: 10 kg·m<sup>-3</sup> molasses, 9.38 kg·m<sup>-3</sup> NH<sub>3</sub>, 8.75 kg·m<sup>-3</sup> H<sub>3</sub>PO<sub>4</sub>, 4.75 kg·m<sup>-3</sup> MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 g·m<sup>-3</sup> ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.15 g·m<sup>-3</sup> biotin and 4.5 g·m<sup>-3</sup> thiamine. The pH was adjusted to 5.5 with H<sub>2</sub>SO<sub>4</sub>/NaOH. The molasses were diluted 1:1 and centrifuged at 8000 G for 10 min to remove suspended solids. The magnesium salt was sterilized separately and the vitamins were sterile filtered (Scheicher and Schuell, membranfilter 0.45 μm). The stirrer speed during the batch phase was set to 6.7 s<sup>-1</sup>.

The fed-batch feed contained 1.69 kg molasses with density 1.41 kg·m<sup>-3</sup> which was mixed with 1 dm<sup>3</sup> H<sub>2</sub>O and then centrifuged at 5000 G for 20 min to remove suspended solids. This gives a feed concentration of 0.628 g(molasses)/g(feed) or about 410 kg(sugar)·m<sup>-3</sup>(feed). The pH was adjusted to 5.5 with H<sub>2</sub>SO<sub>4</sub>/NaOH.

### Feed pump

A voltage controlled pump, Ismatec mp-4, was used with a silicone rubber tubing, outer/inner diam 3.3/1.3 mm, 2030-969, LKB products AB Sweden. The voltage to feed rate was linear over the feed rates 0-100·10<sup>-9</sup> m<sup>3</sup>·s<sup>-1</sup>. The actual feed rate was checked frequently during cultivations, using data from the load cell which the substrate vessel was placed on. In the time scale of minutes the pump showed good stability over feed rates used. A slight drift was noticed in the time-scale of

half an hour. Considerable drift was observed in cultivations in Figure 4. In cultivation A and C the actual feed rate was 20 % less than calibrated, and in B the feed rate was found 20 % higher than expected. Only cultivation D showed no pump drift. The reason for such long term drift was wearing of the tubing.

### Ethanol sensor

Ethanol was monitored using a semiconductor gas sensor (TGS 812, Figaro Engineering Inc, Osaka, Japan) in combination with a membrane sampling probe immersed in the cultivation medium. A device based on continuous dilution of the sample stream from this membrane probe was used. A detailed description of the device is given in [15, 16]. Pure nitrogen was used in the sample and dilution streams. The sampling probe membrane was a silicone tubing of 45  $\mu$ m (diameter, 2 mm; wall thickness, 0.2 mm). The nitrogen flow rate was 2 cm<sup>3</sup>/min and the dilution factor was 2. This set-up and parameter choice resulted in a linear response to ethanol up to 0.8 kg·m<sup>-3</sup> followed by a non-linear response to 2 kg·m<sup>-3</sup>. By changing the flow rate or tubing length this response characteristic could be changed easily. Here the response time was 6 min (transport delay of 2 min and a time constant of 2 min). The ethanol detector is also sensitive to other volatile hydrocarbons (e. g. methanol, propanol, acetic aldehyde) but in the experiments performed the only other compound besides ethanol that could interfere would be acetic aldehyde.

The membrane tubing was mounted on a stainless steel shaft connected to a fermentor port and autoclaved together with the fermentor. No significant changes of the membrane due to this treatment were observed. Some variations in response time were obtained depending on the flow rate and age of the membrane. Calibrations were performed before each cultivation and checked afterwards.

### Analysis

Dry weight measurements were taken to determine cell concentration. Samples of the broth were taken from an outlet at the bottom of the fermentor. From each sample two or more dry weight filters (0.45  $\mu$ m) were prepared. Distilled water was used to wash the filter. Filters were dried for 75 min at a temperature of 105° C and later the weight was measured. In the meantime the filter was kept dry.

The yield calculations in Table 2 are based on dry weight measurement of the cell concentration, volume of the broth, and measurement of the amount of feed consumed. The volume was calculated from the known initial volume, amount of substrate and sodium hydroxide fed, and subtraction of the volume of samples taken. The amount of feed consumed was monitored using a load cell on which the substrate vessel was placed.

### Control system

A PDP 11/03 microcomputer was used for control, monitoring and data logging. Programs were written in PASCAL extended with a realtime kernel [17]. All signals were prefiltered using analog and digital technique. The digital filter was a 2nd order Butterworth filter with a time constant of 60 s. Data were logged on disc every 30 s. The feed rate was determined from a precalculated exponential

dosage scheme. A priori data for this calculation were: initial cell mass, concentration of fermentable sugar in the feed, expected growth rate and yield of the cultivation. Actually, only yield times concentration of fermentable sugar was used. Adjustments of the feed rate around this dosage scheme, were made using feedback from the ethanol signal. A PID regulator was used, see Figure 1. The integrator of the PID regulator was equipped with anti windup in order to account for saturation of the feed pump.

Temperature, pH and stirrer speed were controlled conventionally using an analog control unit manufactured by Chemoferm AB, Sweden. The stirrer speed, the dissolved oxygen and the signal from the substrate vessel load cell were also logged by the computer.

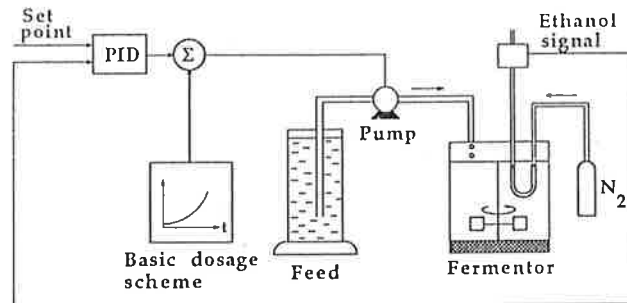
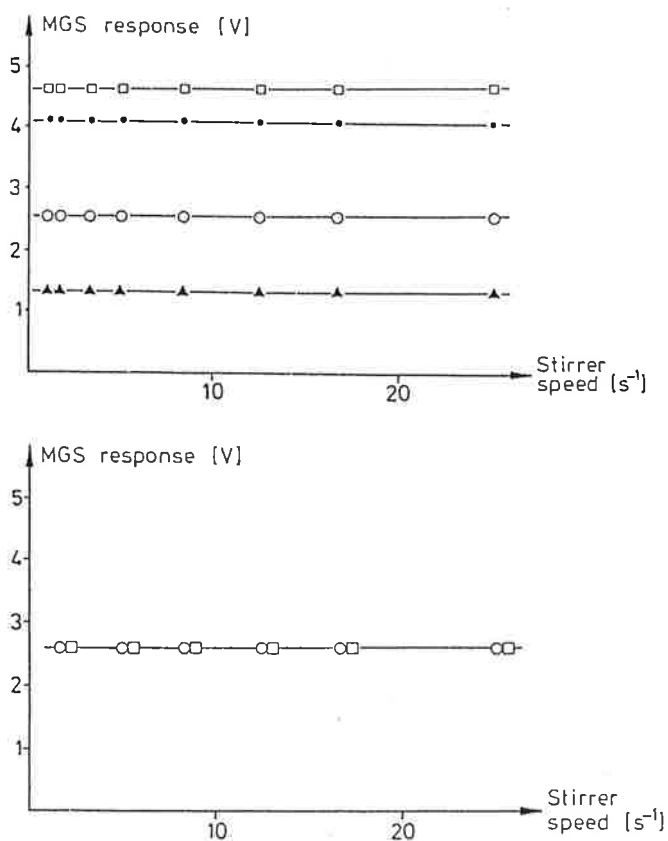


Fig. 1. Schematic diagram of the experimental set-up.

### 3 RESULTS AND DISCUSSION

#### Ethanol monitoring

To achieve good control of ethanol concentration it is necessary to have access to a reliable ethanol sensor. It must be unaffected by the agitation in the broth and/or stirrer speed, the concentration of cell mass, the addition of antifoam, aeration rate and other potentially influencing factors. Using the membrane gas sensor (MGS), a continuous analysis instrument based on the perfusion of volatile compounds through a gas permeable silicone membrane, disturbances of these kinds were reduced or completely eliminated. Figure 2:a shows how various ethanol concentrations respond to changes in stirrer speed of  $1-25 \text{ s}^{-1}$ . Similar results have previously been presented for the  $1.6-6.6 \text{ s}^{-1}$  range [9, 10, 14]. Influence of the density of cells may at least theoretically result in a reduced perfusion of ethanol as well as affecting the partial pressure in the solution. Nevertheless, the results indicate that this change is not of significance for the response of the ethanol sensor. Various concentrations of yeast cells gave the same response to ethanol concentrations of  $0.2-2.0 \text{ kg/m}^3$ , and were not influenced by stirrer speed in the  $1-25 \text{ s}^{-1}$  range.



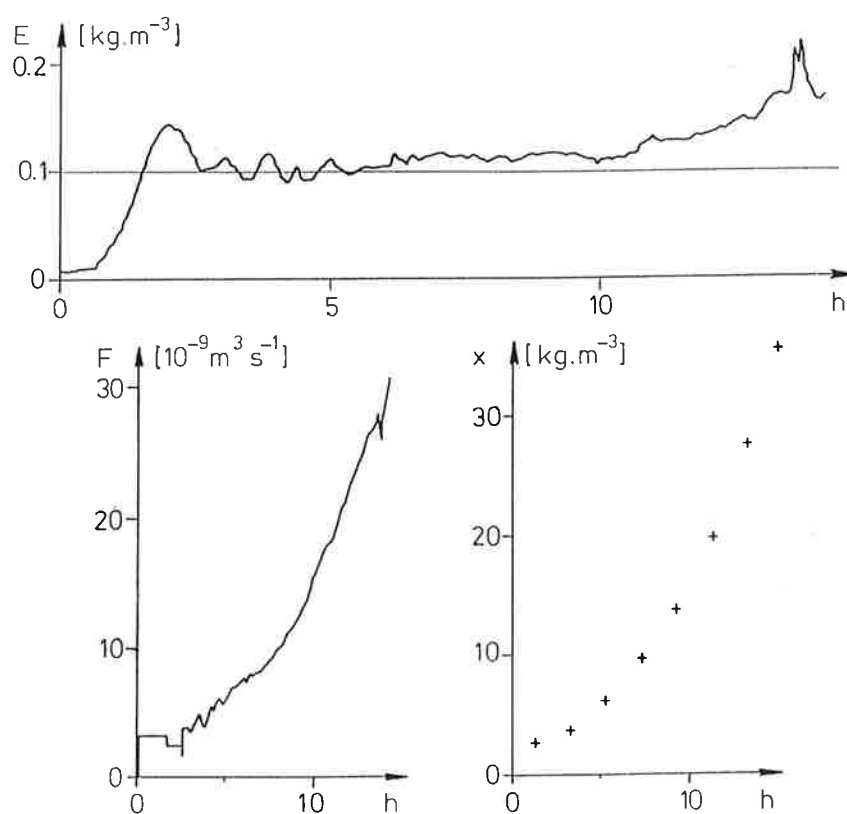
**Fig. 2.** The upper diagram shows the influence of the stirrer speed at different ethanol concentrations in water. The ethanol concentrations were:  $0.20$ ,  $0.40$ ,  $0.80$  and  $1.0 \text{ kg}\cdot\text{m}^{-3}$ . Influence of anti-foam on the ethanol signal is shown in the lower diagram. Antifoam concentration  $3 \text{ kg}\cdot\text{m}^{-3}$  was used in comparison with a solution without any anti-foam, at the same ethanol concentration.



Antifoam is normally applied in laboratory and production scale fermentations and is another factor possibly affecting the ethanol perfusion of the silicone membrane. In this study it was shown that the antifoam used had no observable effect on the ethanol perfusion at  $3 \text{ kg}\cdot\text{m}^{-3}$  antifoam, and variations in stirrer speed  $1\text{-}25 \text{ s}^{-1}$ , gave no synergetic effect, Figure 2:b. Thus the MGS gives a consistent signal under the varying conditions of a yeast cultivation from 3 to  $70 \text{ kg}\cdot\text{m}^{-3}$  at  $6.6\text{-}25 \text{ s}^{-1}$  as applied in this study. A test was made with  $1 \text{ kg}\cdot\text{m}^{-3}$  ethanol in water and stirrer speed set to  $6.7 \text{ s}^{-1}$ . During a 15 h period only 0.25 g ethanol left the fermentor through aeration. This was considered insignificant.

### Ethanol control

During a fed-batch cultivation of baker's yeast there is a dramatic increase in substrate and oxygen demand due to cell growth. An example of a cultivation is given in Figure 3. The graph shows the exponential growth of biomass which follows the increasing feed rate. Throughout, the ethanol concentration is kept at a low level. The ethanol signal is a very sensitive indicator of the substrate demand [1, 2]. The cells switch within a minute from ethanol production to consumption.



**Fig. 3.** Fed-batch cultivation of baker's yeast under ethanol control. The set-point of ethanol concentration ( $E$ ) was kept at  $0.1 \text{ kg}/\text{m}^3$  throughout. Molasses ( $F$ ) was controlled around a precalculated exponential dosage scheme, by a PI regulator. The PI regulator had fixed parameters. The cell growth was monitored by dry weight measurements ( $x$ ) every second hour.

A small deviation in the substrate feed rate from the actual substrate demand, gives either a rapid production or consumption of ethanol. Due to the fed-batch nature of the process, ethanol accumulates or vanishes. Since there are variations in the inoculum size and quality as well as in the feed composition, it is clearly not possible to use a precalculated dosages scheme only. To keep a constant ethanol concentration in the broth, feedback is needed.

During a cultivation from an initial cell concentration of  $3 \text{ kg}\cdot\text{m}^{-3}$  to a final concentration of  $65 \text{ kg}\cdot\text{m}^{-3}$  and a corresponding increase in the nominal feed rate from 3 to  $100 \cdot 10^{-9} \text{ m}^3\cdot\text{s}^{-1}$ , there is a considerable change in process conditions. A couple of conclusions can be drawn from simple experiments, about the possibilities of controlling ethanol concentration throughout a cultivation.

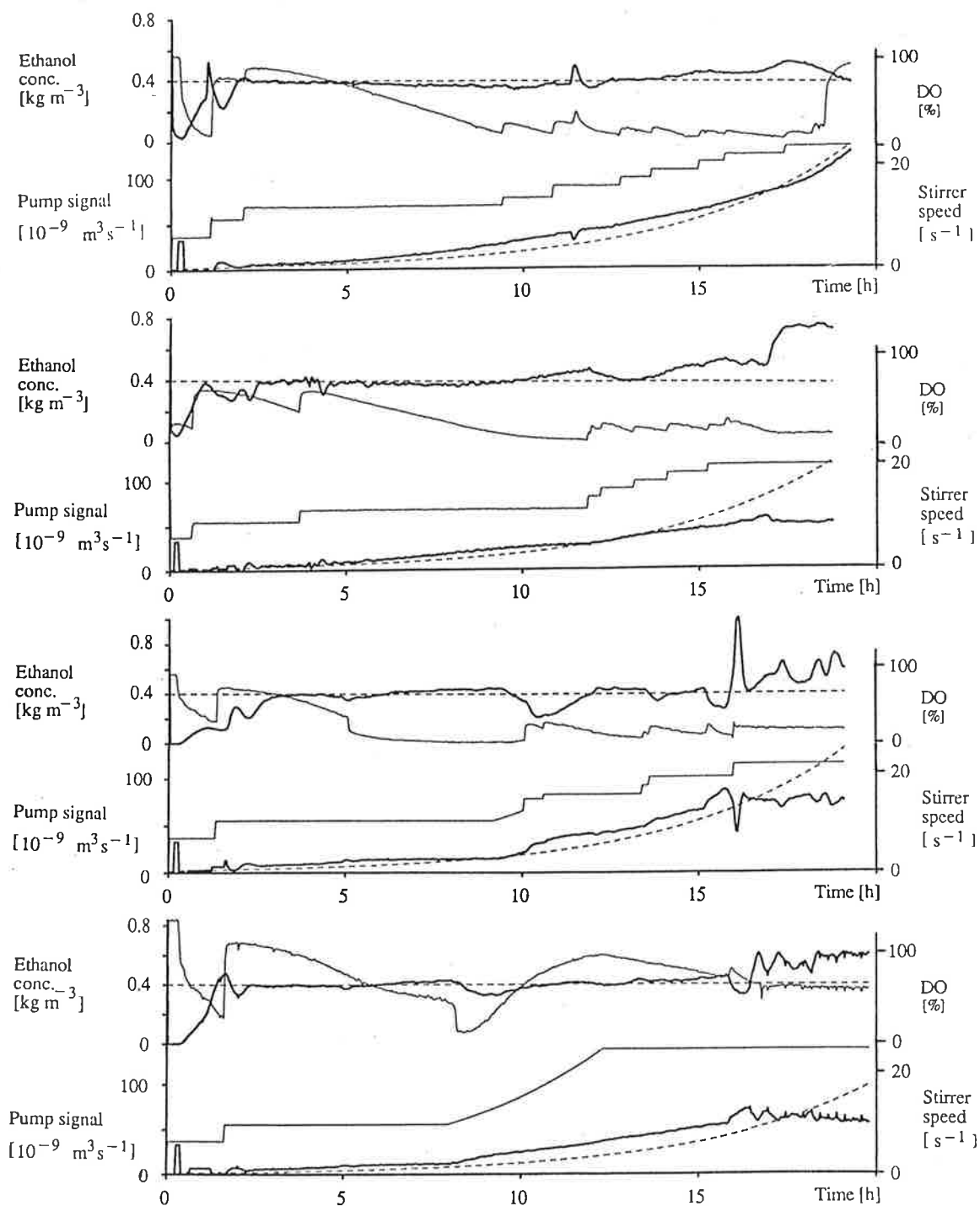
The sensitivity of ethanol concentration to deviations from the nominal feed rate at different cell concentrations, is shown in Table 1. From these figures it is seen, that there is considerable control authority throughout a cultivation. The main limitation on control authority is during the first few hours, when a zero feed rate results in a low rate of decrease in ethanol.

Table 1. The influence of feed rate changes ( $\Delta F$ ) on the ethanol production or consumption rate (EPR) in the reactor is shown. Figures are given for three different cell concentrations ( $x$ ) at corresponding nominal feed rates ( $F$ ). The time constant given reflects the time to reach steady state production or consumption rate after the change in the feed rate.

$x$	5	15	45	$[\text{kg}\cdot\text{m}^{-3}]$
Volume	4.0	4.3	5.5	$[10^{-3} \text{ m}^3]$
F nominal	7	20	67	$[10^{-9} \text{ m}^3\cdot\text{s}^{-1}]$
EPR ( $\Delta F = \pm 7$ )	$\pm 300$	$\pm 280$	$\pm 220$	$[10^{-6} \text{ kg}\cdot\text{m}^{-3}\cdot\text{s}^{-1}]$
EPR ( $\Delta F = -F$ )	-300	-850	-2500	$[10^{-6} \text{ kg}\cdot\text{m}^{-3}\cdot\text{s}^{-1}]$
Time constant	400	130	50	$[s]$

The decrease in the ethanol sensitivity to small changes in the feed rate, seen in the table, is approximately inversely proportional to the broth volume [12], while the sensitivity to relative feed rate changes increases as cell density, since feed rate grows as cell mass. The time constant in the rate of change of ethanol production, related to how cells convert substrate, is inversely proportional to cell density [12]. When taking the sensor dynamics into account it follows that the main control problem is the sensor delay and the high sensitivity late in the cultivation to pumping errors or deviations in cell substrate demand.

A PID regulator has been used to adjust the feed rate around an exponential dosage scheme, cf. Figure 1. The regulator parameters were chosen based on simulations at different cell concentrations using a simplified model of the process [12]. The regulator parameters were kept constant if not otherwise stated. Despite variations in the process the feedback system behaved reasonably, although there is room for improvements.



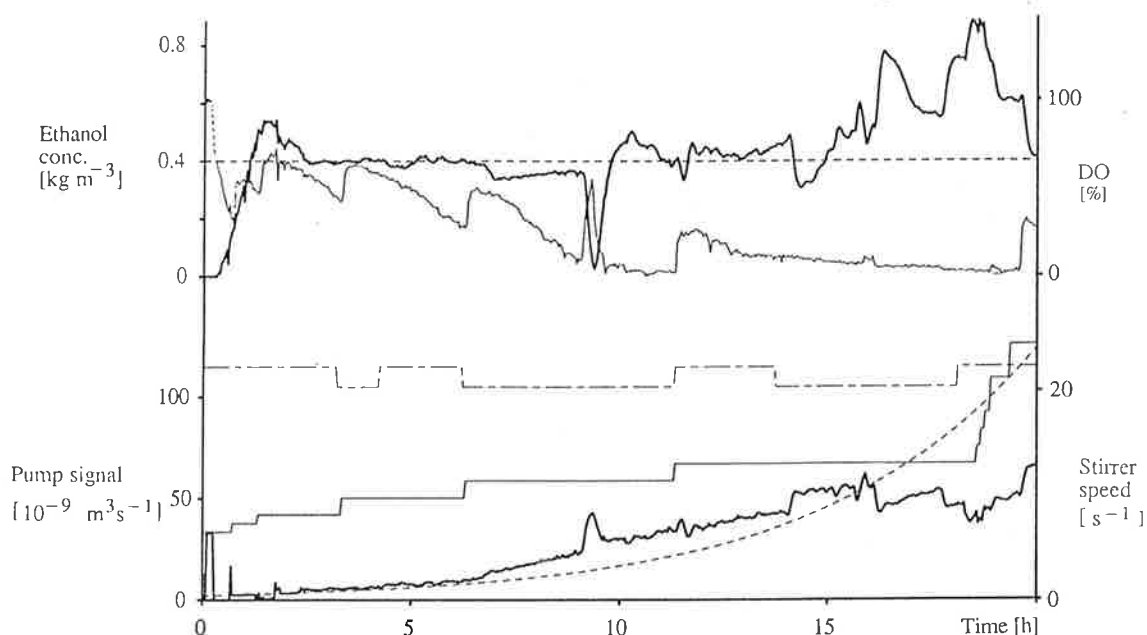
**Fig. 4.** Registrations from four almost identical cultivations. The main difference was variations in the DO profile due to different schemes of stirrer speed. **Remarks:** In cultivation A, there was after 11 h a peak disturbance in the ethanol signal for about 15 min, due to the measurement system. In cultivation C there was a peak in the ethanol signal after 16 h. This peak was likely due to the shift in stirrer speed immediately before. By accident the stirrer speed was zero for 45 s before the higher speed was obtained. Note the DO signal. The regulator parameters were changed in cultivations C and D after 13.5 h, as indicated by the arrow. **Lines:** thick line = ethanol concentration and pump signal, dashed line = ethanol set-point and exponential basic dosage scheme, thin line = dissolved oxygen concentration and stirrer speed.

Four cultivations are shown in Figure 4. The cultivation shown in Figure 3 was carried out with a PI-regulator, and the absence of the D-term implied a lower proportional gain parameter. That regulator is less robust, and the influence of the changing conditions on control is noticeable. Most striking is the drift to a higher ethanol concentration during the latter part of the cultivation. This shows the main control difficulty, i.e. to track the exponential increase in substrate demand. The drift is due to a miss-match between the approximate substrate demand, described by the precalculated dosage scheme, and the actual demand. This difference increases exponentially and is hard for a regulator to compensate for. Further, the performance of the feedback varies during a cultivation. At start-up the loop gain is high, and the regulator is almost oscillating. Later the regulator is not strong enough to keep the ethanol concentration down. This is an effect of the variation in the dynamics of the process as discussed above.

#### Main disturbances in the ethanol control loop

It is important to consider likely disturbances in the design of a control system. In this work the ethanol sensor has provided a signal which was easy to interpret, reliable, and with a low noise level. On a laboratory scale it is possible to use feed pumps with high precision. Therefore, few disturbances were introduced through the measurement system or through the feed pump. In larger reactors the measurement problem might be more difficult, and the problem of maintaining sufficient precision in the feeding during a cultivation is well understood. Another disturbance is introduced if the batch of feed is changed during a cultivation. This may result in an abrupt change in the substrate concentration. These two disturbances are similar from a control point of view. The control system was tested on a laboratory scale for this type of disturbances, by switching between two different sources of molasses with sugar content of 90 % and 110 % of the nominal concentration. See Figure 5. The regulator had no difficulties in eliminating such a disturbance. However, an increased sensitivity as the feed rate increased was observed. This behaviour is explained by the fact that the ethanol concentration in the reactor is affected by the absolute amount of over- or under-feeding, hence at higher nominal feed rates a variation in the substrate concentration makes a greater difference than at lower feed rates.

Another source of disturbances, also likely to occur on a production scale, is the influence of a low dissolved oxygen concentration. When there is a shortage of oxygen, the yeast will be more inclined to produce ethanol, the Pasteur effect. This effect increases the gain of the control system and might result in unstable behaviour. In Figure 4 and in Figure 5 there are a couple of examples of changes in the DO level. In the cultivation in Figure 5 there was an increase in the feed rate after the change in the stirrer speed after 7 hours. The same phenomenon was seen in cultivation 4 B after 12 h and in C and D after 10 h and 8 h, respectively. In Figure 5 the culture was grown at a very low DO level the last 5 hours. The last 2 hours of the cultivation the stirrer speed was increased rapidly and a pronounced effect was observed in the ethanol/feed rate loop. In the examples discussed above there was no indication of unstable control actions.



**Fig. 5.** The influence of changes in the substrate concentration in the feed on the ethanol control system was tested in this cultivation. The feed was changed between two vessels with 90 % and 110 %, respectively of the nominal concentration of molasses. Remarks: There was a delay due to transport in the pump tubing. At high feed rates this delay decreased to a few minutes. Note the increase in the sensitivity to the changes in the substrate concentration as the feed rate increases. After 9 hours of cultivation there was an accident with the feed pump and the molasses supply was zero for 10-15 min. The increase in the stirrer speed influences the ethanol/feedrate loop. Note the response at the end of the cultivation. Lines: thick line = ethanol conc and pump signal, dashed line = ethanol set-point and exponential basic dosage scheme, thin line = dissolved oxygen concentration and stirrer speed, dash-dotted line = changes in the feed concentration.

### Tuning of the regulator

The insight obtained, in variations in the dynamics of the fed-batch process and in characteristics of likely disturbances, give an idea of how the regulator parameter could be changed during a cultivation and what trade-offs are to be done. At start-up, it is important that the regulator is tuned so that an overshoot in the ethanol signal is avoided, because of the long time required for the cells to consume it. A few hours later, the cell mass has doubled and an overshoot in the ethanol concentration is consumed correspondingly faster. During the early hours it is also important that the regulator is able to adjust to variations in the inoculum size. During the latter half of the cultivation it is advisable to increase the gain in the regulator. There are several reasons for this. There may be an increase in the difference between nominal and actual substrate demand; there is

an increase in the relative sensitivity to deviations in the feeding pump or substrate concentration; there is also a slight decrease in the process gain due to the increase in broth volume. However, at higher cell concentrations there is also a risk of oxygen shortage. In this case the regulator gain should be decreased rather than increased. In cultivations C and D in Figure 4, the regulator parameters were changed after 13.5 h. The regulator gain was increased by 25% and 50% respectively, and the integral and derivative time were decreased by 40% in both cases.

#### Results from cultivations subject to ethanol control

Several cultivations were carried out using ethanol control and a certain degree of reproducibility was obtained. Here four cultivations are shown in detail, and experience from a number of other cultivations is summarized.

Cultivations were grown from  $3_3 \text{ kg}\cdot\text{m}^{-3}$  to  $66 \text{ kg}\cdot\text{m}^{-3}$  in 18 h and the volume of the broth increased from  $4.0 \text{ dm}^3$  to  $6.0 \text{ dm}^3$ . The ethanol concentration was kept at a constant value. Results from the four cultivations are shown in Figure 4.

The set-point of ethanol was kept constant through many cultivations at a value of  $0.4 \text{ kg}\cdot\text{m}^{-3}$ . This value was chosen somewhat arbitrarily. Our first cultivations were done with a set-point of  $0.1\text{-}0.2 \text{ kg}\cdot\text{m}^{-3}$ . During some of these cultivations a certain odour was present. This odour never occurred when the cultivations were grown with the high concentration set-point.

The four cultivations shown in Figure 4, were all carried out under almost identical procedures. The main difference was in the increase schemes for stirrer speed, resulting in different DO profiles. However, most of the time, the DO level was kept high, and variations should not have affected the cells. At certain moments, midway through cultivations B, C and D, there was an increase in the stirrer speed that led to an immediate increase in the DO level. These changes affected the feed rate/ethanol loop, and a pronounced increase in the feed rate was obtained.

Two overall characteristics of a cultivation are productivity and yield. Cell mass and the amount of consumed molasses at different times during the cultivations are shown in Table 2. The productivity was found to be remarkably reproducible, despite a variation in the DO level between the four cultivations. However, the yield varied substantially. It is not likely that the DO profile played a role in this. There was actually a considerable difference in the DO profile even between cultivations with the same yield.

A closer investigation of the dry weight measurement taken during these cultivations, shows that up to a cell concentration of  $35 \text{ kg}\cdot\text{m}^{-3}$  the variation in yield between the cultivations was well within measurement errors. The reproducibility was comparable with previous results on a medium of glucose, yeast extract, vitamins and minerals [10]. Therefore conditions during growth from  $35$  to  $65 \text{ kg}\cdot\text{m}^{-3}$ , are believed to be the main reason for the 20 % variation in the yield between the cultivations, cf data in Table 2. When longer time periods are considered, the same measurement errors give rise to less uncertainty in the calculation of the yield.

**Table 2.** Cell mass and consumed molasses at different times during the four cultivations A, B, C and D. The specific productivity  $\mu_p$ , was calculated from the initial and the final cell mass. The yield Y, was calculated as g(yeast)/g(molasses). The molasses were from the same batch in all four cultivations.

Comment	Time [h]	x [kg·m <sup>-3</sup> ]	V [m <sup>3</sup> ]	Feed [kg]	Y acc. [g/g]
A week: 8519	0.00	2.60	4.00·10 <sup>-3</sup>	0	
$\mu_p = 0.20 \text{ h}^{-1}$	10.00	24.1	4.49·10 <sup>-3</sup>	0.538	0.289
Y = 0.25 g/g	12.00	33.7	4.75·10 <sup>-3</sup>	0.855	0.279
	14.00	44.1	5.12·10 <sup>-3</sup>	1.270	0.270
	16.00	55.0	5.56·10 <sup>-3</sup>	1.794	0.262
	18.00	65.1	6.09·10 <sup>-3</sup>	2.407	0.256
B week: 8519	0.00	2.52	4.00·10 <sup>-3</sup>	0	
$\mu_p = 0.20 \text{ h}^{-1}$	11.35	34.5	4.81·10 <sup>-3</sup>	0.834	0.298
Y = 0.25 g/g	13.30	42.3	5.08·10 <sup>-3</sup>	1.181	0.276
	15.30	50.7	5.49·10 <sup>-3</sup>	1.643	0.260
	18.30	65.3	6.03·10 <sup>-3</sup>	2.445	0.250
C week: 8547	0.00	3.50	4.00·10 <sup>-3</sup>	0	
$\mu_p = 0.19 \text{ h}^{-1}$	11.05	24.5	4.66·10 <sup>-3</sup>	0.578	0.276
Y = 0.29 g/g	13.00	34.5	4.93·10 <sup>-3</sup>	0.892	0.279
	16.00	54.2	5.64·10 <sup>-3</sup>	1.619	0.287
	17.30	64.8	5.97·10 <sup>-3</sup>	2.027	0.293
D week: 8547	0.00	2.58	4.00·10 <sup>-3</sup>	0	
$\mu_p = 0.20 \text{ h}^{-1}$	10.00	21.4	4.49·10 <sup>-3</sup>	0.452	0.302
Y = 0.30 g/g	12.50	34.0	4.94·10 <sup>-3</sup>	0.867	0.290
	15.50	51.6	5.57·10 <sup>-3</sup>	1.507	0.292
	17.50	66.8	6.00·10 <sup>-3</sup>	2.070	0.300

From our data there are reasons to believe that factors other than variations in the ethanol concentration influence the yield. In the literature there have been some investigations concerning growth inhibitory substances. In a fed-batch process there is an accumulation of un-metabolized substances which might influence the growth of the culture [20]. Another possibility is the production of inhibitory substances during growth [18, 19, 21]. In the recent work by Pons et al [21], there are indications (for certain strains of *S. cerevisiae*) that the level of ethanol concentration in the broth affects the production of an inhibitory substance (acetic acid). This is another indication of the importance of ethanol control.

#### 4 GENERAL DISCUSSION

For a decade work has progressed at laboratory level to control the feed rate in baker's yeast production. A variety of controllers have been presented for the fed-batch cultivation of baker's yeast. Depending on the choice of measurement technique and knowledge of the process, the regulator structures vary in complexity.

On-line measurement of the ethanol concentration in the broth using the tubing method provides a useful signal for substrate control. It is at least as sensitive as RQ measurement as an indicator of the metabolic status of the culture, and also has some advantages as it is more direct [10, 21]. It is in fact also much cheaper than equipment for exhaust gas analysis. The ethanol sensor has been used in our laboratory for more than three years, and our experience is very good. The ethanol sensor system is not affected by different conditions in terms of molasses matrix, antifoam, yeast cell density or stirrer speed. Its response time is fast, implying good potential for control. A PID-regulator can actually provide an almost constant ethanol level. The tuning of its parameters must, however, be done with care.

Previous work on ethanol control [9-12] has also shown that a PID regulator around an exponential dosage scheme is usable. However, in work by Dairaku et al [11], difficulties with tuning of the regulator parameters were reported. This was solved by changing the parameters according to a rough estimate of the cell growth. The PID regulator presented in this paper succeeds in keeping the ethanol concentration close to the set-point, using constant parameters. It is remarkable because the cultures were here grown from a lower cell concentration to almost twice the concentration reported in [11]. Our tuning was done using simulation on a simple model and a trade off was made between performance at low and high cell concentrations.

The objective of control is normally vaguely stated, high yield and growth rate, and a good and uniform quality. It is however laborious to evaluate the results of control in these terms. Previous work on ethanol control [10] was done using a medium of glucose, yeast extract, vitamins and minerals. In that work there is support for the hypothesis that constant ethanol control gives the optimal growth rate under the constraint of maximal yield. From our work with molasses, it may be concluded that ethanol control permits cultivation with high exponential growth up to a cell concentration of of  $60-70 \text{ kg/m}^3$  dry weight, maintaining a high yield. However, one may suspect that factors other than the metabolic state, are important for the yield. Such studies are still lacking also in the case of RQ control or growth rate control, for example.

An ethanol control system gives a tool for a new type of investigations of growth of yeast in a fed-batch reactor. There are indications in our work that the choice of ethanol set point influences the yeast culture. It would be interesting to study in more detail how yield, growth and quality depend on different ethanol set points and perhaps also to allow for different profiles of ethanol concentration during a cultivation. In work by Pons et al [21] the level of ethanol concentration in the broth seems to affect the production of inhibiting acetic acid. Steady state experiments in a chemostat have shown that the ethanol production rate is related to the growth of a culture. Recently, a clear correspondance has been shown between ethanol production rate and growth rate also in a fed-batch reactor [22]. There are indications that feeding strategies which give different growth rates during various phases of the cultivation, might be important in obtaining good quality yeast [23, 24].



There are good reasons to believe that it is easier to evaluate experiments using ethanol control than using growth rate estimates based on optical density, exhaust gas analysis or pH. Each has its limitations. Direct measurement of the cell concentration using optical density is difficult. Methods based on exhaust gas analysis or pH require several model assumptions in order to interpret the signals. It is therefore less certain that cultivation conditions are reproduced using such feed rate control.

Several applications of adaptive techniques to control baker's yeast production have been reported recently [5-7]. However, reasons for introducing a more sophisticated regulator were only briefly discussed in these papers. The main argument seems to be the time variation of the dynamics due to cell growth. Uncertainties in the process model in general are also referred to, and more specifically in inoculum size and quality as well as in the sugar concentration of the feed. However, feedback itself has the property of reducing the effects of uncertainties in the process and a more quantitative discussion is called for in order to justify adaptive control. Our experience is, that the main control difficulty lies in tracking the exponential increase in substrate demand. Variations in the dynamics due to cell growth and increase of the reactor volume, are of less importance. However, a sensor system with a longer response time would accentuate the importance of accounting for variations in the dynamics in the design of the regulator. Actually, disturbances may cause larger variations in the process dynamics than the increase of cell mass and volume of the broth. Such effects can be seen in the cultivations in [5, 6]. As previously discussed insufficient oxygen supply and different disturbances in the substrate feeding change the process gain and might call for adaptive techniques in order to obtain a robust control system. A good idea is to incorporate some process knowledge also in the design of an adaptive regulator [25]. In this way stability can be ensured for a wider class of disturbances.

In summary, ethanol control is a promising technique to improve baker's yeast production. It has conceptual advantages compared to other methods of substrate control, and a simple regulator structure will give a reasonable performance at the laboratory level.

#### ACKNOWLEDGEMENT

The authors wish to thank Mr. Lennart Wallström, Jästbolaget, Sweden, for valuable discussions concerning industrial aspects of the process. The project was supported by the Swedish Board for Technical Development, contract 82-3359 and 82-3494.

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