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Abstract

This report summarizes experimental experience from our work on fed-batch cultivation of baker's yeast at laboratory level. The cultivations were grown subject to substrate limitation. The feed rate was controlled based on ethanol measurements in the broth. The objective of control was to maintain a constant low level of the ethanol concentration. Two different set-points have been used.

The work so far has been concentrated on gathering knowledge about the process under well controlled conditions, rather than on evaluating different control schemes. The main problem so far has been large variations in the growth rate at cell concentrations above 30 g/L.

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INTRODUCTION

This report summarize experimental experience from fed-batch cultivation of baker's yeast on molasses. The cultivations have been carried out between july 84 - july 86. The work so far was concentrated on gathering knowledge about the process under well controlled conditions, rather than on evaluating different control schemes. The main question was variations in the growth rate at cell concentrations above 30 g/L. A guide to the experimental data is given in appendix. Equipment was refined and routines and protocols were sharpened during the work. The equipment is described in the appendix. No apparent problems with infections occured. Three different batches of molasses were used.

The yeast was grown subject to substrate limitation. All nutrients, minerals and vitamins were present at start of the fed-batch phase of the cultivation. Only molasses, oxygen, NaOH and anti-foam were supplied during the fed-batch. A detailed description of the cultivation conditions are given in appendix. The ethanol concentration is a good indicator of the substrate demand, [Dairaku 81], [Sonnleitner 86]. In order to obtain substrate limitation, the substrate feed rate was adjusted so that the ethanol concentration in the broth remained at a constant low level. Two different set-points have been used. The dissolved oxygen concentration varied, but was kept high. The oxygen supply was increased stepwise by increasing the stirrer speed as the cultivation proceeded. At higher cell concentrations there might be local oxygen limitation as well. In Figure 1 an example of a cultivation is shown.

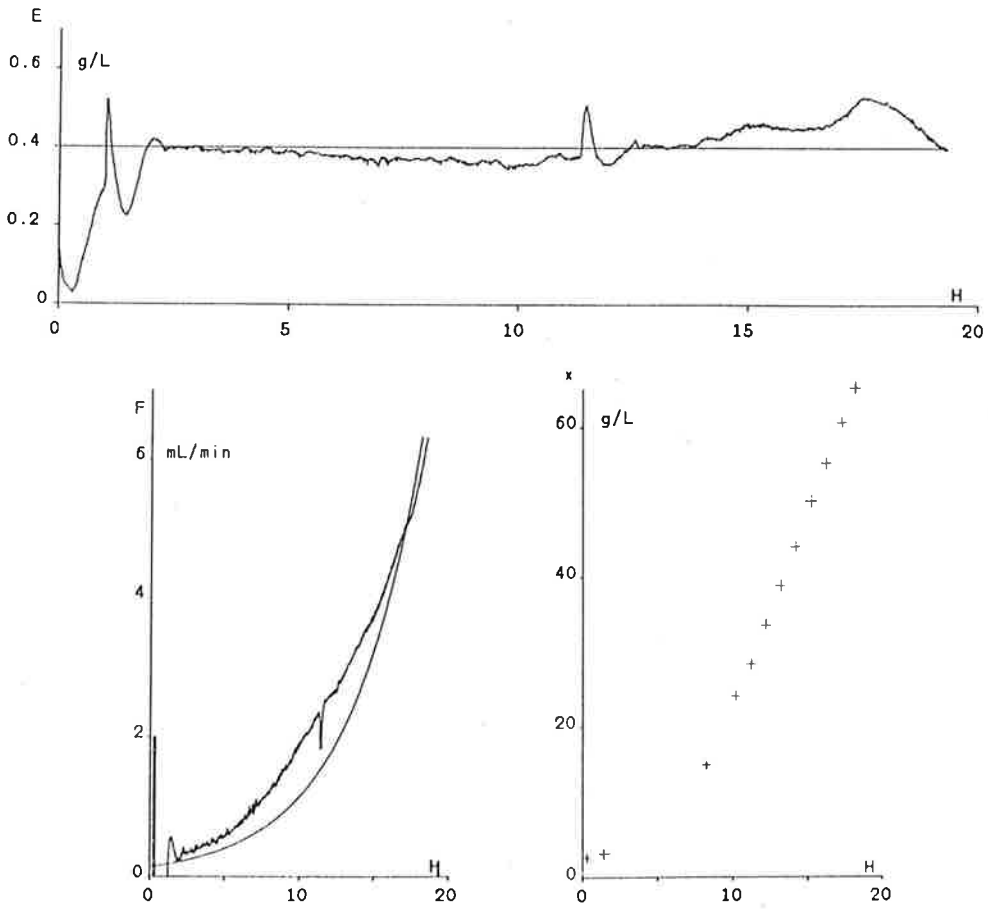

Fig. 1. Fed-batch cultivation of baker's yeast subject to substrate control of the ethanol concentration. Symbols: E = ethanol conc, F = feed rate, x = dry weight cell conc. Experimental data from week 8519 A.

RESULTS FROM CULTIVATIONS SUBJECT TO ETHANOL CONTROL

Several cultivations have been 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.

From four almost identical cultivations, carried out during week 8519 and 8547, an idea of the parameter variation was obtained. The cultivation grew from 3.0±0.5 g/L to 66±1 g/L in 18 h and the volume of the broth increased from 4.0 L to 6.0 L. The production rate has been possible to reproduce with high accuracy, while the yield varied more between cultivations. During these four cultivations the production rate during 18 h was 0.20±0.01 h^{-1} and the yield 0.27±0.03 g(yeast dry weight)/g(molasses). Results from the four cultivations are shown in Figure 2 and in Table 1.

The set-point of ethanol was kept constant through many cultivations at a value of 0.4 g/L. This value was chosen somewhat arbitrarily. Our first cultivations were done with a set-point of 0.1-0.2 g/L. 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 2, 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 1. 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 g/L 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, [Dairaku 81]. Therefore conditions during growth from 35 to 65 g/L, are believed to be the main reason for the 20 % variation in the yield between the cultivations, cf data in Table 1. When longer time periods are considered, the same measurement errors give rise to less uncertainity in the calculation of the yield. A thorough error propagation analysis is done in the appendix.

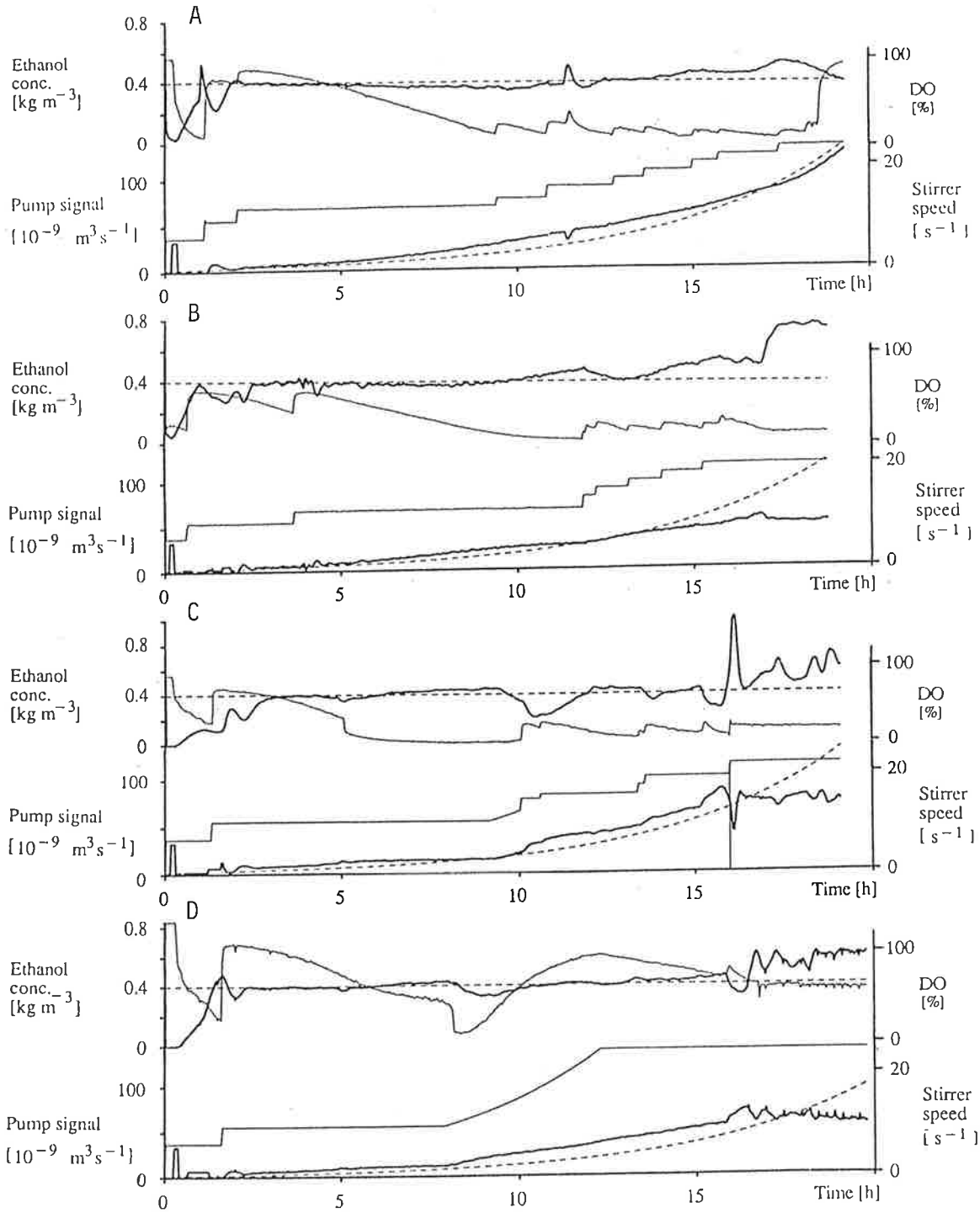

Fig. 2. Results from four almost identical cultivations are shown. The main difference was variations in the DO profile due to different schemes of stirrer speed. Remarks: In cultivation A, there was after 11 h a peak disturbance in the ethanol signal for about 15 min, due to the measurement system. 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.

Table 1. Cell mass and consumed molasses at different times during the four cultivations A, B, C and D. The productivity μ_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 [g/L]	V [L]	Feed [g]	Y acc. [g/g]
A week: 8519	0.00	2.60	4.00	0	
$\mu_p = 0.20\ h^{-1}$	10.00	24.1	4.49	538	0.289
Y = 0.25 g/g	12.00	33.7	4.75	855	0.279
	14.00	44.1	5.12	1270	0.270
	16.00	55.0	5.56	1794	0.262
	18.00	65.1	6.09	2407	0.256
B week: 8519	0.00	2.52	4.00	0	
$\mu_p = 0.20\ h^{-1}$	11.35	34.5	4.81	834	0.298
Y = 0.25 g/g	13.30	42.3	5.08	1181	0.276
	15.30	50.7	5.49	1643	0.260
	18.30	65.3	6.03	2445	0.250
C week: 8547	0.00	3.50	4.00	0	
$\mu_p = 0.19\ h^{-1}$	11.05	24.5	4.66	578	0.276
Y = 0.29 g/g	13.00	34.5	4.93	892	0.279
	16.00	54.2	5.64	1619	0.287
	17.30	64.8	5.97	2027	0.293
D week: 8547	0.00	2.58	4.00	0	
$\mu_p = 0.20\ h^{-1}$	10.00	21.4	4.49	452	0.302
Y = 0.30 g/g	12.50	34.0	4.94	867	0.290
	15.50	51.6	5.57	1507	0.292
	17.50	66.8	6.00	2070	0.300

Variations in the growth rate

The growth rate obtained varied also during the cultivation. A characteristic phenomena was that the growth rate started at $\mu \approx 0.23\ h^{-1}$, and when the cell concentration reached a level of 25-35 g/L the growth rate decreased within one or two hours to $\mu \approx 0.10\text{-}0.15\ h^{-1}$. Some cultivatons did not show this decrease in the growth rate. The physical or biological background to this phenomena is not clear. One observation was that during the cultivations with high growth rates through out, the feed rate was changed in large steps around a basic dosage scheme. These abrupt changes in the feed rate made the cells switch from ethanol production to rapid consumption.

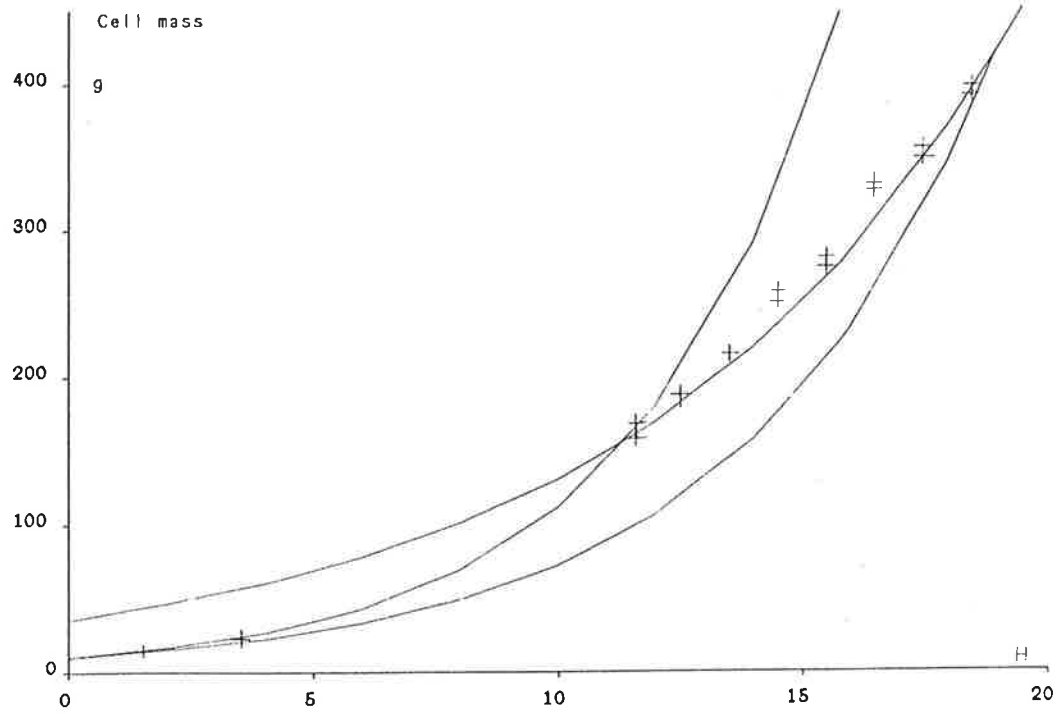

Fig. 3. In most of the cultivations the growth rate decreases at the higher cell concentrations. The diagram below shows exponential curves fitted to the data of week 8519 B. The growth rates were 0.24 resp 0.13. The production rate was 0.20. These values were from data at 0, 11.5 and 18.5 h.

Preliminary results on yield variations

The yield was calculated from the dry weight measurements at the start and at the end of the fed-batch cultivation. For a discussion of precision and error propagation in these calculations, see appendix. The yield is given in g(yeast)/g(molasses), i.e. molasses as supplied from Jästbolaget AB.

The aim of our work is not to study variations in the yield, but some interesting data was recorded. A typical characteristic of a fed-batch cultivation was that the yield and growth rate were higher during the first 10 hours, i.e. up to a cell concentration of about 30 g/L. Then the growth rate and also the yield dropped within a couple of hours. The yield dropped from about 0.32 to 0.24 g(yeast)/g(molass). However, measurement errors are large and it is difficult to say something specific from these data, cf error calculations in appendix. When

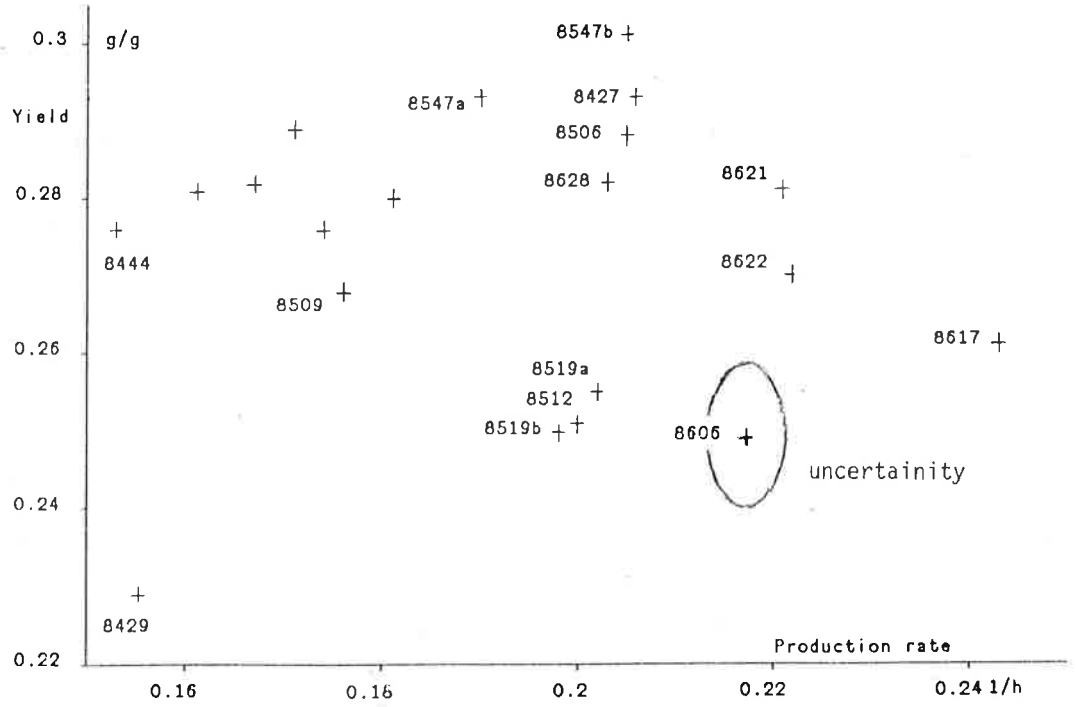

Fig. 4. Yield versus production rate over about 18 h are shown in the diagram. The uncertainity ellipse due to measurement errors is shown, according to appendix 6.

longer time periods are considered, the same measurement errors give rise to less uncertainity in the calculation of the yield. Comparing the overall yield and growth rate between cultivations, shows that cultivations with a high overall growth rate had a lower yield compared to those with a low overall growth rate, cf fig. 3. From this rule there are three exceptions: week 8506, 8547 A and B, 8606 where high growth rate was obtained with high yield.

Characteristic smells

The yeast produces a wide spectrum of organic compounds that have characteristic smells. Some observations in changes of the smell were done. The inoculum and batch had a characteristic smell of plum. During the cultivation the smell changes towards a more acid flavour. The overall impression is a fresh flavour. During some of the cultivations, the smell changed to an acrid organic sulphur odour. This is believed to be related to the low ethanol concentration in the broth [personal communication 850130, Wallström, technical manager, Jästbolaget AB]. The ethanol set point was increased from 0.2-0.4 g/L during the last three cultivation and the sulphur odour never occured.

The sulphur odour was not related to changes in the growth rate. The onset of the odour was observed after 8 h of fed-batch and cell concentration x=17 g/L during experimental week 8429. Other observations are done at the end of the cultivations but the time of onset of the odour was not clear.

Growth inhibiting factors

The changes of growth rate discussed above have led us to formulate a hypothesis of the production and consumption of a growth inhibiting substance. The production and consumption of this hypothetical substance is thought to be correlated to the production and consumption of ethanol. If the ethanol level is kept constant the growth inhibiting factor is thought to accumulate. However, if abrupt dips in the feed rate are done the ethanol consumption starts and the hypothetical substance is consumed as well.

There is some literature on the subject: Fukuda 78, Maiorella 83, 84 and Pons 86. Pons discusses inhibiting effects of acetic acid. Acetic acid is produced by the cells under certain circumstances.

A variation in the growth rate was found, as mentioned above, and also a variation in the maximal feed rate. However, there is not a good correlation between these two variables, see Figure 5. Cultivations with high production rate show a substantial variation in the maximal feed rate. This can be partly understood from the fact that the production rate is not much dependent on the actual growth rate during the last couple of hours while the maximal feed rate is determined during this period. Another important factor is how the yield differs from cultivation to cultivation during the last hours.

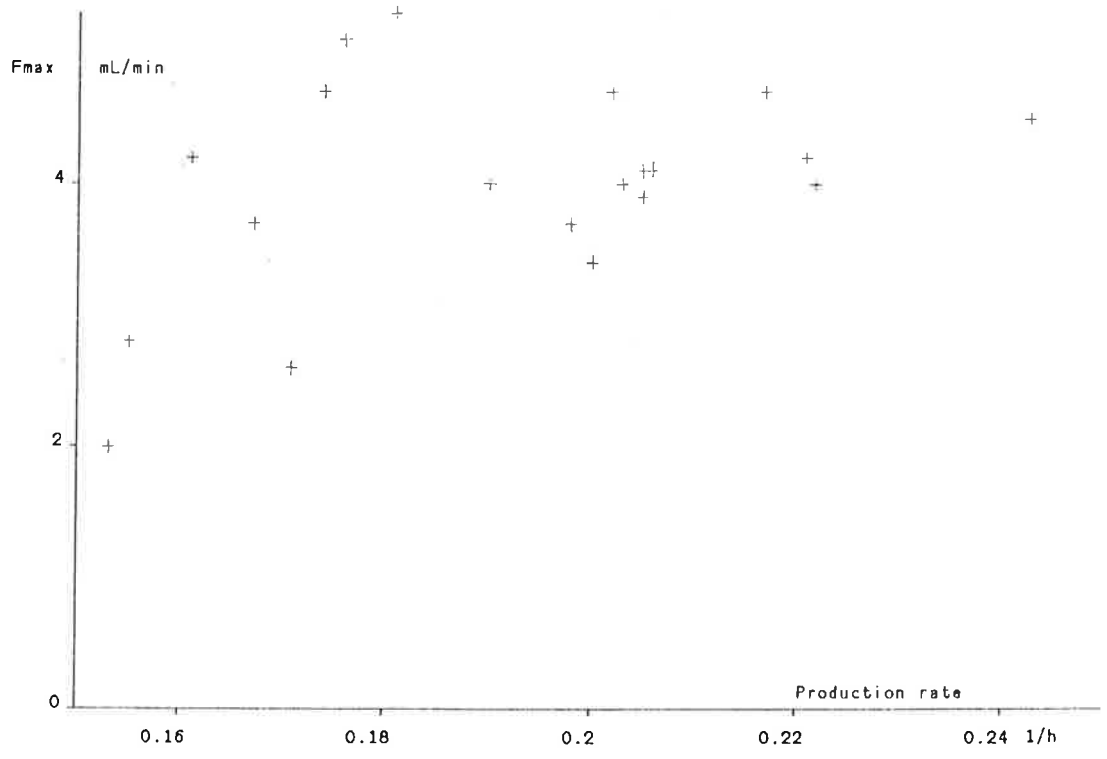

Fig. 5. Maximal feed rate versus production rate is shown.

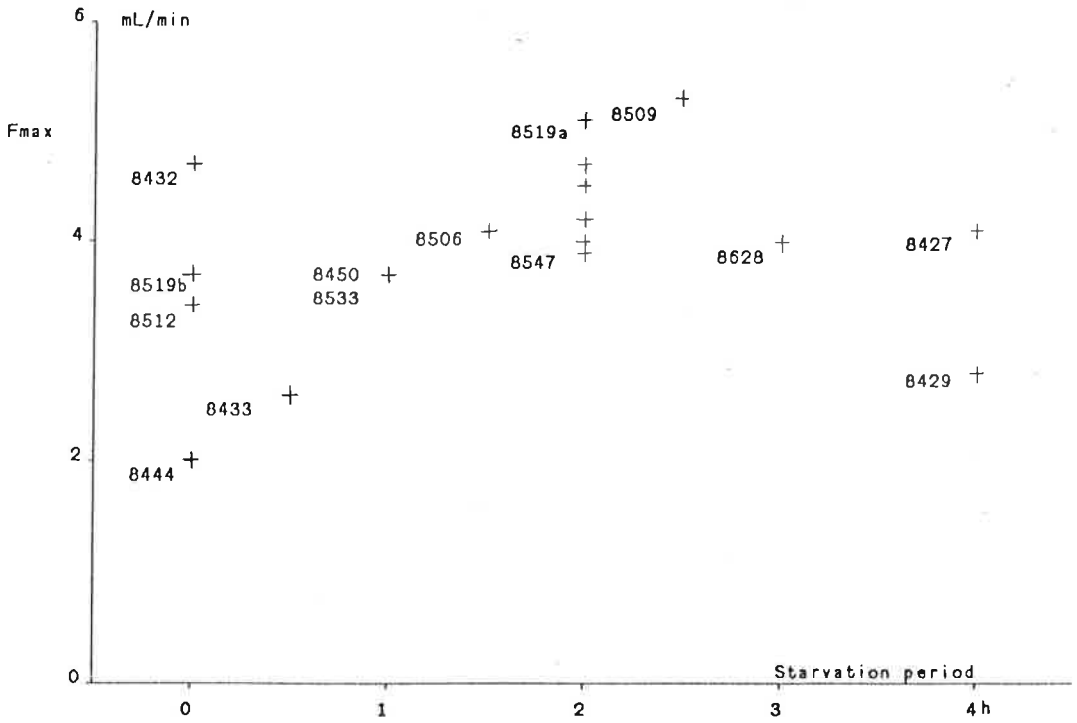

Fig. 6. Here is shown the maximal feed rate versus length of the starvation period, i.e. the pause between end of batch phase and start of fed-batch.

During the cultivations 8427, 8432 and 8509 remarkably high maximal feed rates and growth rates were recorded, compared to the results of week 8429, 8433 and 8444. (Data form week 8450 is not comparable because the broth was diluted twice with water in order to increase growth rate. Data from experiments during 1985 were obtained at higher set-point of the ethanol concentration and on another batch of molasses, and therefore comparison is difficult.) Typical for these cultivations were, that the feed rate oscillated and the cultivations switched between ethanol production and consumption. During 8427 and 8432 the feed rate was changed stepwise manually and during 8509 the regulator went unstable and gave an oscillating feed rate. Actually, there were two cultivations on the glucose media (8413 and 8414), where the feed rate oscillated and a high growth rate was obtained.

Investigation of the data reveals another surprising relation. Before the fed-batch phase, the culture has been grown batch wise from a prepared inoculum. There seems to be a linear relation between the maximal feed rate and the length of the pause between the end of the batch phase and the start of the fed-batch cultivation, see Figure 6. A longer pause, up to about three hours, gave a higher maximal feed rate. There were a couple of exceptions from this rule, but during those cultivations the feed rate has been oscillating and a higher than expected maximal feed rate had been obtained. These observationns are in agreement with the hypothesis of a growth inhibiting factor that the cell consumes when glucose is lacking.

A few remarks on the reproducibility in the lab

The reproducibility of the inoculum is not checked. The amount of cells that the inoculum is started with is not very precise. However, the amount of given substrate has been well defined and limits the cell growth. A varying parameter has been the shaking rate of the flask. These variances are likely to be of minor importance.

The reproducibility of the batch phase has been checked in different ways, especially during 85. An obvious parameter to compare is the final cell concentration and the time for the batch to complete, i.e. all substrate is consumed and the DO signal rises. Another variable that might give some information is the pH profile or rather the NaOH dosage. Two different batch concentrations of molasses have been given, 40 g/L and 110 g/L, and they resulted in a duration of the batch phase of 13.5-16.5 h and 18-20 h respectively.

The pump works very well in the time range 1 to 30 min, where the feed rate follows the control signal linearly over a wide range. Looking at the very short time interval, one notes that the feed enters the fermentor in droplets of about 30 μL. At very low feed rates, at the start of the fed-batch, the drop frequency is about 10 drops a minute. No indications of "hot spots" have been seen. Over hours, a drift can be found from experiment to experiment. This drift is automatically compensated for by the regulator in most cases. The weight from the remaining feed in the substrate vessel was monitored by a load cell and logged by the computer since week 8529. The pump is further discussed in the appendix.

Off-line measurements of the ethanol concentration were done with refined techniques in four cultivations during weeks: 8519 and 8547. They indicate a drift in the ethanol signal. During the later half of the cultivation the off-line analysis showed a 50% lower ethanol concentration than what the sensor signal gave. The sensor is sensitive to a spectrum of volatile compounds. One likely candidate is acetaldehyd, which might accumulate during the fed-batch phase of the cultivation, [Pons et al, 86], as discussed above under growth inhibiting factors.

The cell concentration (TS) was measured by the dry weight method, and these numbers are used when calculating the growth rate and the yield. The TS measurements taken are in the range 2.5-65 g/L. Usually two measurements are done on one sample. During week 8519 a lot of multipel TS values were taken in order to check the spread. Our results show that the spread is usually between 3-5%. At cell concentrations below 7 g/L, the spread can be up to 10%.

Routines at start-up of the fed-batch phase

During our work the start-up routine of the fed-batch phase has changed. At first we thought it was best to start the fed-batch phase just before the end of the batch phase, i.e. the ethanol signal was not allowed to come down to zero. However, it was found practical to let the ethanol signal come down to zero, in order to check and adjust the calibration. For different reasons this pause has some times been several hours long. Now it is believed that a pause might be good for the cells. Inhibiting substances might be consumed during the starvation. Recall Figure 6 showing the maximal feed rate versus pause before the start. A pause of two hours has been standardized.

The substrate dosage is started, since january 85, by a pulse of 20.0 g of feed, fed during 5-7 min. In this way ethanol is produced up to a concentration of about 0.3 g/L. The peak has been reached after about 45 min.

After the substrate pulse has been given, the feed rate has been set to zero for about 15 min. Then the feed pump has been controlled manually in order to reach an ethanol level near the set-point 0.4 g/L and come in steady state. It has taken about one hour. Now the PID regulator has been turned on. The PID regulator has been tuned for good performance during the middle part of the cultivation and that means that stability is poor at start-up. Therefore it has been important to turn on the regulator with great care.

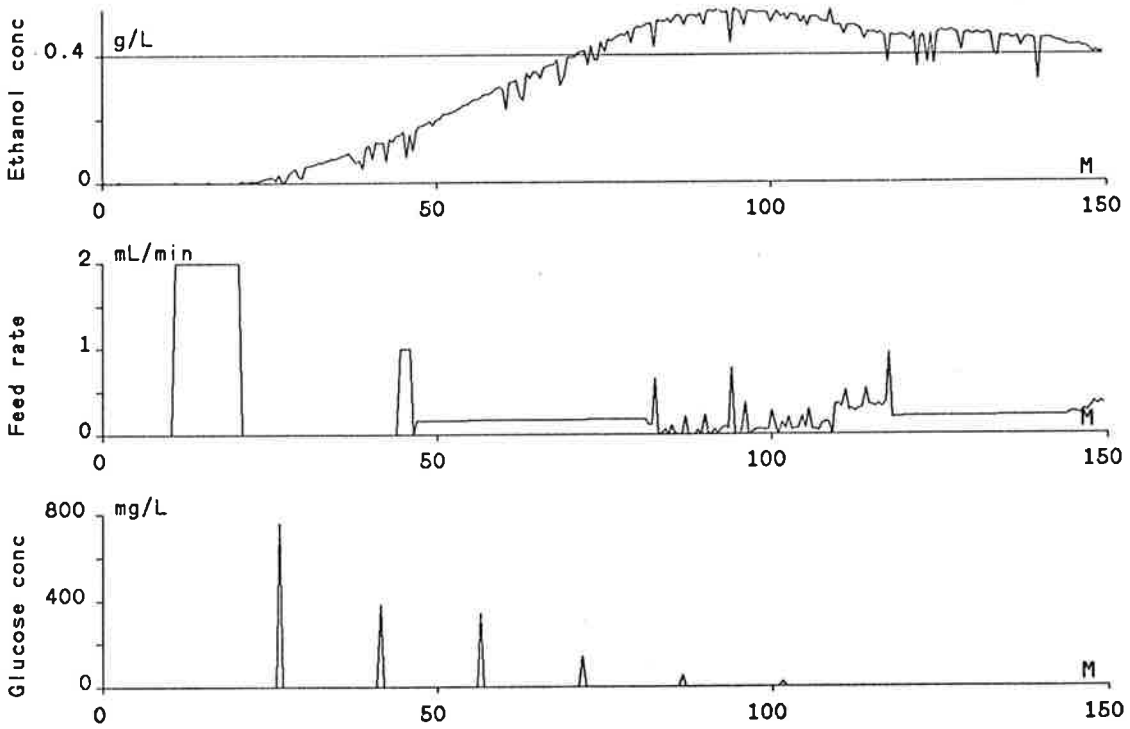

Fig. 7. Glucose concentration in the fermentor during the start-up. At this start-up two substrate pulses were given. The high noise level in the ethanol signal was due to a severe electrical disturbance that was eliminated later. Data from cultivation week 8629.

CHARACTERISTICS OF THE DIFFERENT CONTROL LOOPS

In our work emphazise has been on the substrate control loop, but it is only briefly discussed below. Aeration was mostly manually controlled using the stirrer speed. In some cultivations the stirrer speed was controlled by the computer and a precalculated scheme was followed. Temperature and pH were controlled conventionally using standard on/off regulator. The antifoam dosage was controlled by the computer according to a scheme.

Substrate control strategy

An approximate feed rate was determined from an exponential dosage scheme. A PID regulator with the ethanol signal as input, adjusted the feed rate in order to keep the ethanol concentration at the set-point value. The regulator had fixed parameters. The sampling interval was chosen to match the fast dynamics at the end of the cultivation.

The dynamics of the process changes during the cultivation. The time constant decreases from 7 min to less than 1 min. The process gain decreases slightly. Although a reasonable performance can be obtained using fixed parameters in the PID-regulator, it would have been better to change them during the cultivation.

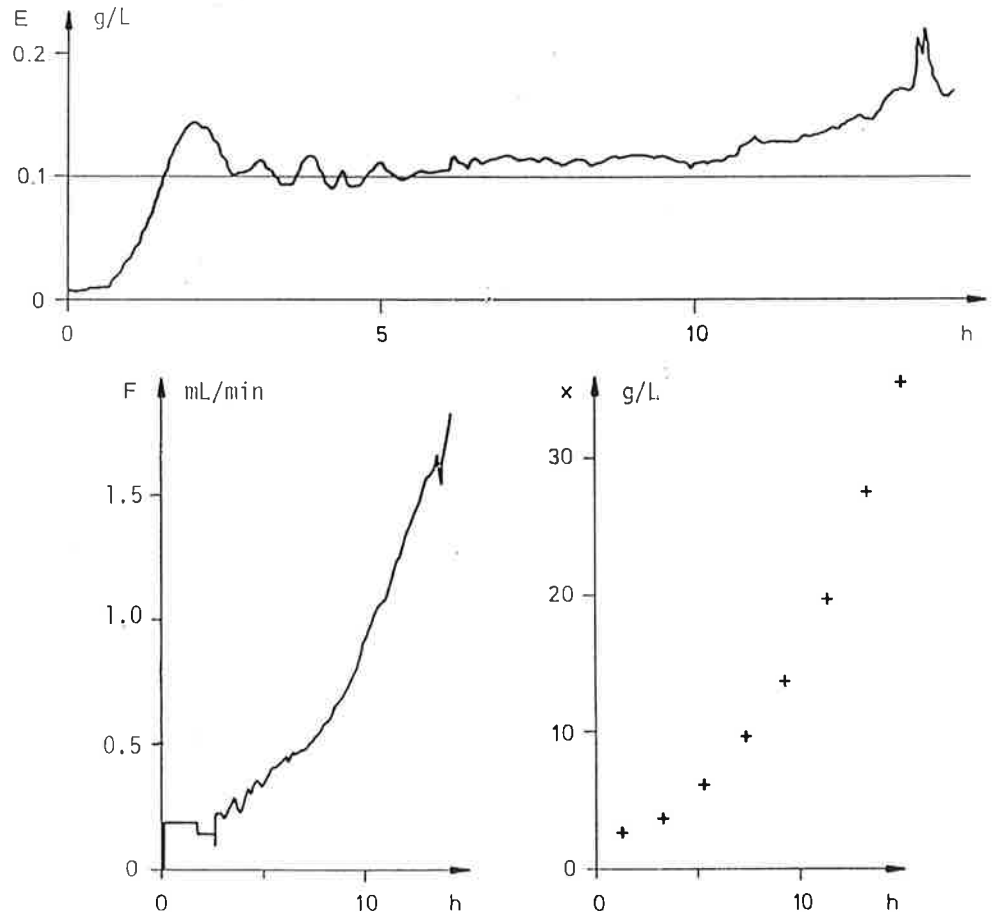

Fig. 8. Here is shown the performance of PI control during 15 hours of an 18 hours cultivation. Note the poor stability during the first hours of cultivation and that later the regulator is not strong enough to keep the ethanol concentration at the set-point. Data from cultivation 8433.

The main control difficulty is to track the exponential increase in substrate demand. Here an exponential dosage scheme is a priori assumed. Even minor deviations from the nominal demand give difficulties for the PID regulator. This can be seen in many cultivation as a "drift" in the ethanol signal to higher values at the end of the cultivation. See Figure 8.

The regulator parameters have been tuned using simulation on a simplified model of the process and some minor adjustments were done at the process. In order to track the exponential increase in substrate demand, it is a good idea to decrease the integral time of the regulator. It is also possible to increase the gain by about 50% after half of the cultivation, see week 8547 A and B. However, it is important to have a well tuned derivative part, in order to make these changes without loosing stability.

Oxygen supply

The cultivations have been grown with the intention to feed oxygen in abundance. Therefore the DO level has been controlled to be well above 20%. The stirrer speed was used for control. Sometimes there were oxygen limitation, especially at high cell concentrations. In a few cultivations there were an oxygen limitation in the middle of the cultivations. The reason to believe there is oxygen limitation is that, when the stirrer speed is increased, not only the DO level rises but also the feed rate (subject to ethanol control), see data from week 8519 B, 8547 A and 8547 B. There are indications that even variations in the DO level above 20% influence the yeast, see cultivation 8628. In this cultivation the DO level was kept at 20% most of the time during the first eight hours. The growth rate was 0.20 h^{-1} instead of 0.24 h^{-1} as it usually is during this period. Furhter, the ethanol control was almost unstable, even though the regulator gain was halfed. This indicates an increased sensitivity to overfeeding due to the Pasteur effect, [Sonnleitner 86].

During most experiments the stirrer speed has been changed manually. The stirrer speed was controlled by the computer during cultivation 8532, 8547 B, 8606 and 8617. The DO concentration is very sensitive to small variation in the stirrer speed and a fixed scheme cannot guarantee a stable DO level. Therefore, it would be a good idea to close the loop from the DO signal to the stirrer speed. However, variations in the stirrer speed might change the mixing properties of the substrate in a crucial way, and make it difficult to interpret data from a cultivation and to compare with data from other cultivations. Therefore long periods of constant stirrer speed have been used especially in cultivations done in order to investigate substrate/ethanol dynamics. In the early work, fast changes of stirrer speed seemed to influence the ethanol signal, see data from week 8429, 8432 and 8444. From january 85 on, an improved ethanol sensor sytem has been used which has the nitrogen gas flow is regulated, and that made the system less sensitive to changes in the stirrer speed.

There are reasons for having good control of the oxygen supply to the yeast. At high cell concentrations aeration is becoming expensive on an industrial scale (it amounts to about 10% of the final product). Even at low cell concentrations it its interesting to maintain a low but sufficient oxygen supply, because it influences the nitrogen uptake of the cells [P. M. van den Broecke, Gist Brocades coordinating manager automation and production services, personal communication, july 85].

pH control

The pH level has been controlled to around pH=5.0 using an on/off Chemoferm controller. No problems were observed with this control loop. The accumulated NaOH dosage was read out regularly. Since week 8509 it was continuously recorded by the computer. During a cultivation there is an increase in the demand for NaOH to keep the pH level constant, due to the cell growth. The pH of the molasses feed is 5.5 so the feed itself changes the pH in the broth slightly. After 15-18 h of fed-batch cultivation, i.e. near the end, the NaOH dosage ceased. During the cultivation 8509 there was a malfunction in the pH control. The pH drifted away to 3.7 and the ethanol control became almost unstable. The NaOH dosage varies between cultivations. The NaOH dosage is 1-2 mL/g(yeast). This variation is not understood. The pH sensor has not been checked for drift regularly, but a couple of checks during cultivations 8547 A and B, indicate no drift.

Antifoam dosage

The antifoam dosage was controlled according to a scheme, by the computer. A couple of drops every 30 min was enough to keep the foam down during the first half of the cultivation. Later the dosage interval was 15 min and sometimes 7 min near the end of the cultivation. The drop interval was set manually.

Too much antifoam decreased the dissolved oxygen concentration. During some cultivations a drop in the DO signal was recorded when the antifoam was added. The ethanol signal was not observed to be directly influenced by the antifoam, cf. appendix. However, during certain circumstances the control of the ethanol

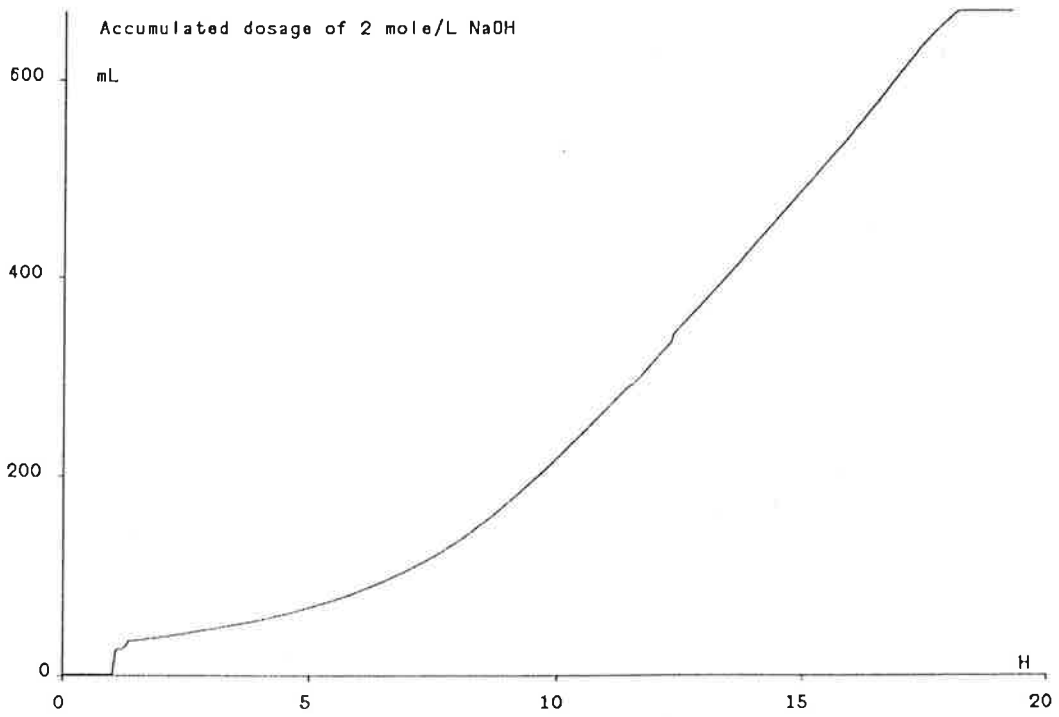

Fig. 9. Here the accumulated NaOH dosage during cultivation 8519 A is shown.

concentration might be disturbed by the antifoam dosage, due to the changes in the DO concentration. During cultivations 8528, 29 and 32 a lot of antifoam dropped into the fermentor due to a little leakage in the tubing. This caused a grey precipitation on the fermentor walls. The amount of antifoam might have decreased the growth rate during these weeks. Especially during week 8529 when a lot of antifoam entered the fermentor during the batch phase, and the growth rate was only 0.05-0.10 h^{-1}. The same thing possible happened week 8613. One explanation could be that a layer was formed around the cells and decreased the mass transfer.

On an industrial scale, the antifoam control is not satisfactorily solved [personal communication van den Broecke, Gist Brocades, july 85]. The foam gives problems at the air outlet from the reactors. There are sensors for detecting the foam height but they work only in an on/off manner.

Temperature control of the fermentor

A Chemoferm controller has been used for controlling the temperature of the fermentor. During the work, we observed that during the first hours of fed-batch cultivation, the fermentor was heated to remain at 30.0° C. Later, the fermentor was cooled. During some cultivations, the cooling was not sufficient and the temperature increased up to 30.3° C.

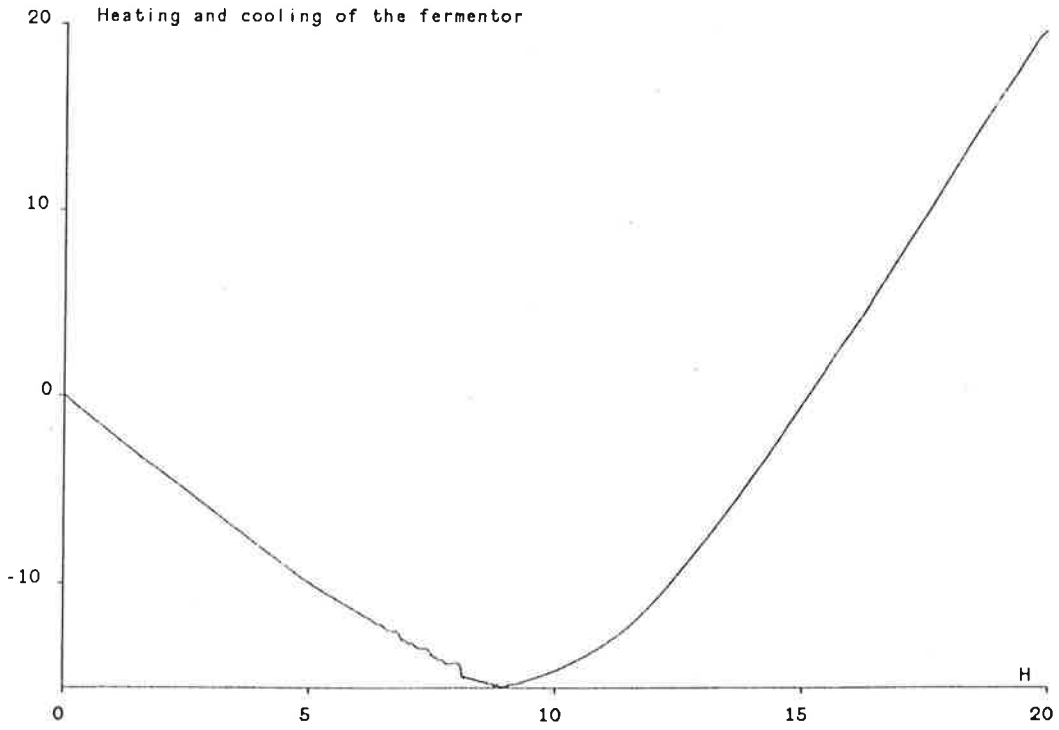

Fig. 10. Here is shown the integrated time the cool- and warm-water relay have been on. In this way an approximate data was obtained about the heat produced by the cells. Data from cultivation 8547 B.

A crude way to estimate the heat generated from the cells is to integrate the time the cold- and warm-water relays are turned on. Facilities for logging of the cold- and warm-water relays were included since experimental week 8529. A better estimation have to take into account the temperature of the cold- and warm-water and also the heat leakage from the fermentor to the surroundings which depends on the room temperature.

From the data it can be seen, that during the first 8 hours the fermentor was slightly heated and after that increasingly cooled. The cold water relay was more often on than the warm water relay, indicating less cooling than heating capacity.

CONCLUSION

Results and general experience from ethanol-controlled cultivations over a two year period have been summarized. During the period, 26 cultivations were carried out. Routines and procedures were sharpened and the equipment refined. The ethanol sensor system was further developed, and its third version is now in use.

From the yeast producers point of view, variations in growth rate and yield are very important to understand and if possible, be able to control. Our working hypothesis was that ethanol control facilitate the reproducibility of cultivation conditions. From our work with molasses, it may be concluded that ethanol control permits cultivation with high exponential growth up to a cell concentration of 60-70 g/L dry weight, maintaining a high yield. However, one may suspect that factors other than the ethanol profile, or rather the respiratory state, are important for the yield. The quality of the molasses is one crucial factor. However, it has been taken from the same batch for series of cultivations and this factor must be excluded. Another factor is accumulation of substances that may vary critically between cultivations and influence the yield. Both substances from the molasses and compounds produced by the yeast may influence. Finally, it is not clear to what extent these variations in yield can be controlled away or they reflect a fundamental variation inherrent in the complex growth process. Systematic studies that evaluate RQ control in terms of enhanced yield on molasses is also lacking.

The emphasize in our work is substrate control based on the ethanol sensor. The major control difficulty is to track the rapid increase in substrate demand as the culture grows. Internal models for the disturbances might be an idea, but then variations in growth rate and yield are important to consider also in design of the substrate control system.

The other control loops: dissolved oxygen, pH, antifoam and temperature are briefly discussed. The dissolved oxygen/stirrer speed loop has some influence on the ethanol/feed rate loop and needs further attention, while the remaining loops influence it only in case of extraordinary conditions. Low pH caused unstable control actions during one cultivation and too much antifoam hampered growth rate of the yeast during a few cultivations

ACKNOWLEDGEMENT

The work is a cooperative effort between the Department of Biotechnology, Chemical Center and the Department of Automatic control at LTH. Several people is involved. Carl Fredrik Mandenius and Olle Holst have the responsibility for the laboratory. Carl Fredrik developed the ethanol sensor system and Olle is the microbiology specialist and designed the media. He also helped us understand some of the complexities that may be involved growing yeast on molasses. Docent Per Hagander and professor Bo Mattiasson supervised the work and clarified our thoughts. Lennart Wallström, Jästbolaget AB, Sweden, gave us valuable insight into the industrial aspects of the process. The yeast strain and the molasses were gifts from Jästbolaget.

During the second year a lab assistant helped us with the practical work with the cultivations. Finngal Morén made a contribution during the summer 85 and Lena Nielsen took over the responsibility for the cultivations during 86. Throughout Gunter Held made technical service of the equipment, often with short notice.

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APPENDIX 1 - CULTIVATION CONDITIONS

Baker's yeast, Saccharomyces cerevisiae, was used in this study. The strain was a gift from Svenska Jästbolaget, Rotebro, Sweden. The yeast was grown under substrate limitation. Oxygen was given in excess if not otherwise stated.

Cultivations were performed in a fermentor (FLC-B8 Chemoferm AB, Hägersten, Sweden) with a working volume of 8 L. Temperature was controlled at 30°C. Two molar NaOH was used to keep the pH constant at 5.0. Foam was controlled by adding the antifoam Adekanol, when needed. Aeration rate was 1.0 volume/volume·min. The stirrer speed was changed manually.

After the batch culture was completed the feed was started after a pause. The pause between batch and fed-batch varied between zero and four hours. During later work a specified pause of two hours has been standard, cf discussion about start-up routines.

The feed and also NaOH and anti-foam drops into the fermentor. The droplets were assumed to be immediately mixed in the fermentor. There has been no sign of "hot spots".

Inoculum preparation

The strain was maintained on malt agar and reinoculated every month. Inoculum for batch cultivation was grown in shake flasks witih four intendations. The temperature was 30° C and pH was 5.5 at the beginning. The substrate (200 mL) was made up of: molasses 50 g/L; NH_3 5 g/L; H_3PO_4 5 g/L and $MgSO_4 \cdot 7H_2O$ 2.5 g/L. The inoculum was grown for 16 hours.

Batch cultivation on molasses

Molasses (beet) with a sugar content of 50 % w/v was used. In batch cultures (4L) molass (40g) was supplemented with NH_3 (25%) 50 mL; H_3PO_4 30g; $MgSO_4 \cdot 7H_2O$ 4.75 g; $ZnSO_4 \cdot 7H_2O$ 2 mg; biotin 0.6 mg and thiamin 18 mg. The pH was adjusted to 5.5 with H_2SO_4. The inoculum volume was 200 mL.

Media for the fed-batch cultivation

The feed was made up of 1.69 kg molasses and 1 L water. The concentration of fermentable sugar in the feed was about 410 g/L. All other nutrients were given during the batch phase.

Substrate pulse

The fed-batch phase was usually started by a substrate pulse in order to reach appropriate ethanol concentration before the regulator was turned on. The amount of feed in the substrate pulse was calculated as follows: Initial volume of the broth = 4.0 L; Initial cell mass = 10 g; Critical glucose concentration = 0.1 g/L glucose; Fill up the fermentor to this concentration: 4·0.1 = 0.4 g; Yield glucose to ethanol: 2·(46/342) = 0.27; Production of ethanol to 0.4 g/L: 4·0.4/0.27 = 6.0 g; Maintennace and storage of glucose in the cells: 10·0.2 = 2 g; Altogether 8.4 g glucose or 20 g feed with glucose content 410 g/L.

APPENDIX 2 - 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 [Elmqvist 1982]. 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.

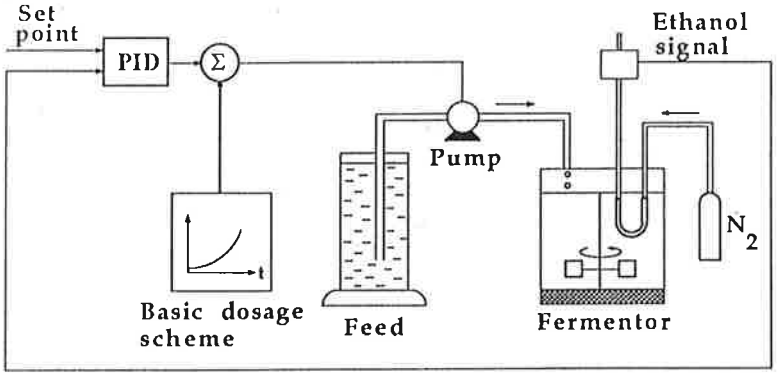


Fig. A2:1. The experimental set-up.

APPENDIX 3 - MEASUREMENTS AND PROTOCOLS

During the cultivations the following signals have been registered continuously: ethanol (E), dissolved oxygen (DO) and feed pump-signal (F). In the last experiments the stirrer speed (RPM) and the accumulated NaOH dosage have also been registered continuously.

At regular intervals: TS (dry weight), pH, accumulated NaOH dosage and accumulated substrate dosage were recorded. Further, samples of the broth were taken for analysis of different substances, for example glucose, sucrose and ethanol concentrations. Microscope studies were usually taken before and after a cultivation and sometimes during a cultivation, in order to look for infections in the culture.

During a cultivation checks of the status of the process were made at certain important times, like when sample are taken for off-line analysis. Checks were also made irregularly inbetween these samples. These checks were made according to protocol I. During the fed-batch phase the pump feed rate was checked several times using the weight measurement. Results were noted in protocol II. Double dry weight measurements were done in order to measure the cell concentration. Data were written in protocol III. From the dry weight measurements: cell mass, growth rate and yield were derived. These and some other numbers were written in protocol IV.

In a work like this, unexpected events take place and observations are done that might be important in later interpretation of the data. Such information were here written in a logg book and each piece of information was numbered sequentially and the actual time was also noted. Sometimes, it is important that it is possible to correlate notes in the logg book with events in the recorded signals. Therefore, a certain data file (in parallel with the data file for the ethanol signal, the feed rate etc) was used for marking events noted in the logg book. A command was simply given at the computer terminal and a mark was done in the data file.

After each cultivation a summary was done of unexpected events and other important information. In this summary characteristic numbers of a cultivation, like yield and growth rate are also given. These summaries are given in appendix 9.

FEDBATCHODLING Datum:

Datum
Tid (h)
Luftflöde (vvm)
Temp (C)
pH
Lutvolym
Löst syre (%)
Feedflöde (ml/min)
Feedvolym (ml)
Feedvikt (gram)
Varvtal
Etanolsignal
Ackumulerad inpumpad feed
Anm.

Test av feed pump

Tid	Mät tid	givet F	Spänning	erh.g/min	ml/min

TORRVIKT .....

Filter nr.	Tid	Filter före	Invägt	Filter efter	dw	X	X	Anm.

Utbyte och tillväxt under fedbatch vecka:

ρ(feed) =

C(g.molass/g.feed) =

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	$\rho\int$ Fdt	$\int$NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	

APPENDIX 4 - THE ETHANOL SENSOR SYSTEM

The overall impression is that the ethanol membrane gas sensor system is reliable. An improvement was done in January 1985 when the gas flow regulator system was installed. The working range of the ethanol sensor is 0.0-1.0 g/L. From dynamical point of view, the ethanol signal seems to follow the concentration in the broth very well. The ethanol signal gives a proper response to changes in the feed rate. However, the off-line analysis is performed, using enzyme kits, is in this case relatively inaccurate, due to the low concentration, volatility and rapid consumption of the ethanol.

A brief description of the membrane gas sensor system

The sensor probe was immersed in the fermentation medium where the volatile ethanol diffused through a silicone membrane tube (45x2 mm, wall thickness = 0.2 mm) into a flowing stream of dried nitrogen gas. The gas flow was regulated at 3 mL/min using Brooks flow meter. This ethanol containing flow was diluted threefold by continuous mixing with pure nitrogen gas and was subsequently directed to a semiconductor detector (Figaro gas sensor 812, Figaro Eng. Inc. Osaka, Japan).

The semiconductor changes its conductivity proportionally to the ethanol concentration in the ambient flow stream due to chemisorption of ethanol onto the surface. The ethanol concentration in the fermentor medium is proportionally related to the conductivity, since the ethanol transport across the silicone membrane is directly dependent upon the ethanol concentration. Interferences of oxygen and carbon dioxide as well as of non-volatile compounds showed negligible influence on the signal amplitude.

Dynamical properties

The time delay is due to the transport of the sample through the dilution system to the detector. At a nitrogen flow rate of 180 ml/h the time delay is 2 min and the time constant is 2 min.

The dynamic response and the reproducibility of the sensor were tested in a 'gradient mixer'. The reproducibility of the gradient profile was found accurate at a varity of matrix conditions. A relevant antifoam concentration of 3 g/L did not change static or dynamic properties of the sensor system. Influences from the molasses matrix was found neglible.

Calibration

The sensor is calibrated at suitable ethanol concentrations, e.g. 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 g/L. This is done before sterilizatiton of the tubing (and the fermentor) and usually after the end of the fed-batch cultivation. Calibration should be done at 30±1° C because of the sensitivity to temp changes.

Between the end of the batch phase and start of the fed-batch phase there is a pause and the ethanol is consumed. This gives a reference of the zero point, which is very useful. A considerable drift in the baseline have sometimes been found. However, the relative calibration has been correct.

Off-line analysis of the ethanol concentration in the broth

The off-line analysis is performed, using enzyme kits, is in this case relatively inaccurate, due to the low concentration, volatility and rapid concentration of the ethanol. During week 8519 and 8547 reproducible off-line meassurement were done. These showed, however, an ethanol concentraion only half of what was given by the ethanol signal. One hypothesis is, that there is an accumulation of volatile compounds in the broth that contribute to the ethanol signal.

Influence of abrupt changes of the stirrer speed

During the period 8427-8450 several, times the ethanol signal was disturbed by abrupt increases in the stirrer speed. Since the installation of the gas flow regulator, no such phenomenon was observed. Changes in the stirrer speed were also made slower.

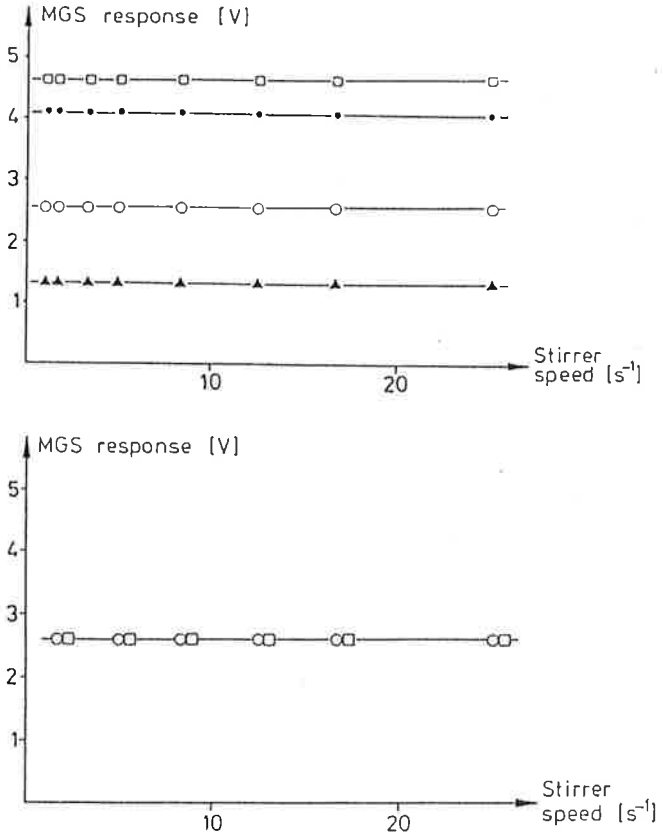


Fig. A4:1. The upper diagram shows the influence of the stirrer speed at different ethanol concentrations in water. The ethanol concentrations were: 0.20, 0.40, 0.80 and 1.0 g/L. Influence of anti-foam on the ethanol signal is shown in the lower diagram. Antifoam concentration 3 g/L was used in comparison with a solution without any anti-foam, at the same ethanol concentration.

APPENDIX 5 - THE PRECISON OF THE FEED PUMP

The working range is 0.0-6.0 mL/min and requirements of the precision is very high. The feed entered the fermentor from the top and the size of the droplets were approximately 30 μL. The pump was calibrated using continuous weight measurement of the substrate vessel and knowledge of the density of the molasses substrate. However, the calibration has been found to be sensitive to the condition of the tubing. The influence of the aging is not negligble. Therefore new tubing was used for every cultivation since may 85.

The pump has a linear characterisic: voltage-feed rate. The interface to the computer was via a D/A converter with a resolution of 5 mV. During the first hours of cultivation the quantization error was not neglible. At start-up the feed rate was 0.2 mL/min which corresponds to a voltage of 40 mV. However, at this low feed rate other disturbances were of the same magnitude.

The continuous weight measurement was logged by the computer since 8529. Previously the feed rate was checked manually for a few minutes, now and then. Now, off-line analysis of the whole substrate scheme was possible. Results show that most ofthe the pump gain decreases gradually to about 80% of its nominal value. There are also fluctuations in the pump bias with up to 1 mL/min at the higher feed rates. There were also examples of cultivaition when the pump was incredible stable throughout, cf (8519B), 8547B, 8606 and 8622. A larger decrease of the pump gain was found in cultivation 8506, 8532 and 8621. In cultivation 8532 the pump gain changed abruptly, compared to an otherwise gradual process.

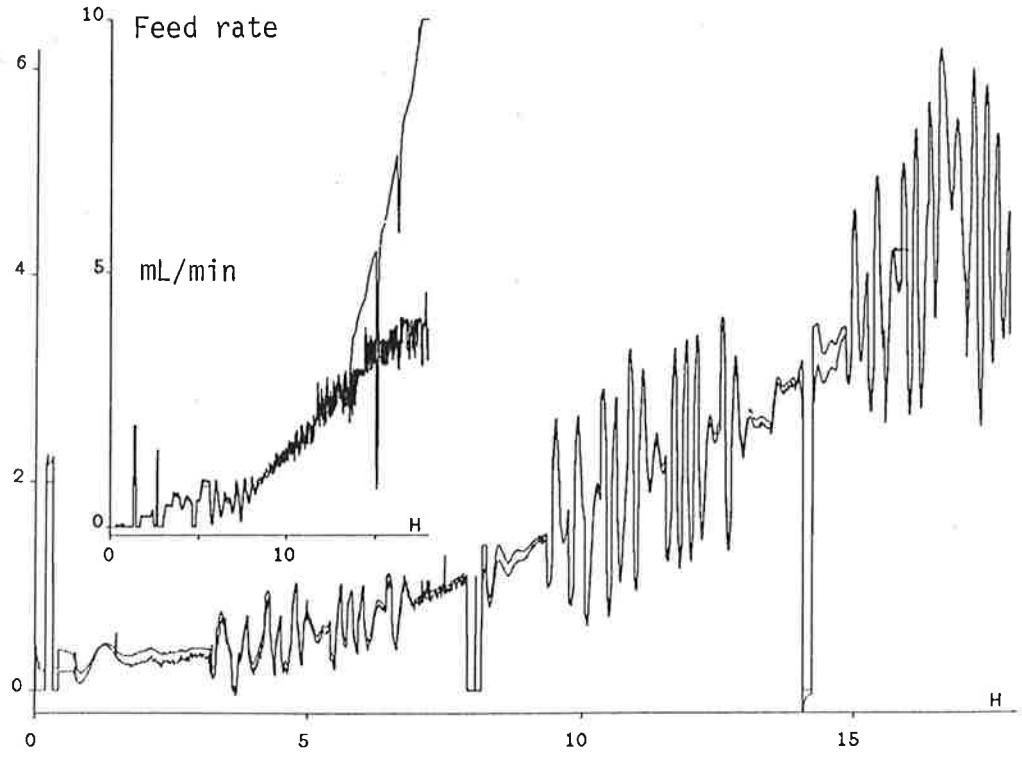


Fig. A5:1. The figure shows pump signal and actual feed rate during whole fed-batch cultivations. The actual feed rate was estimated from continuous weight measurement of the feed vessel. The large diagram shows result from week 8622. Note how well the feed pump follows the transients. The small digram show results from week 8532. During this cultivation the pump gain decreased abruptly. Note also the increased absolute noise level.

APPENDIX 6 - ERRORS IN YIELD AND PRODUCTIVITY CALCULATIONS

Two variables yield and productivity of a cultivation are among the interesting variables from industrial point of view. They have in our work been one measure of degree of reproducibilty between cultivations. It is important to make clear how much of the variation in these numbers is due to various measurement errors and how much is real.

Definition

Yield [Y] — Amount of cell mass produced from a given amount of molasses (as delivered from Jästbolaget).

Productivity [μ_p] — An exponential growth function is fitted to final and initial cell mass.

Calculations

$$(VX)_f = (VX)_i \cdot e^{\mu_p \cdot \Delta t} \qquad \text{(A6: 1)}$$

$$\mu_p = \frac{\ln\left(\frac{(VX)_f}{(VX)_i}\right)}{\Delta t} \qquad \text{(A6: 2)}$$

$$Y = \frac{(VX)_f - (VX)_i}{\Delta \text{ Feed}} \cdot C \qquad \text{(A6: 3)}$$

where

X	Cell concentration from dry weight measurement
V	Volume of the broth in the fermentor. The volume is calculated from knowledege of the volume at start-up of the cultivation, amount of feed fed, density of the feed, amount of NaOH fed and probe volumes removed from the fermentor.
$(VX)_f$	Final f (or initial i) cell mass calculated from resp. dry weight measurement and volume calculation.
C	Concentration of molasses in the feed.
Δt	Time period considered.
ΔFeed	Weight of feed fed to the fermentor during the period.

Discussion and estimation of measurement errors

Dry weight. Standard techniques have been used. Usually two measurements have been done on the same sample. During experimental week 8519 A and B, many multiple measurements have been done, in order to check the reproducibility. The result is that there is a spread in values of only about 4%. Measurement of low cell concentrations is more difficult and here is a spread of roughly 10%. The fact that two dry weight measurements are taken and the average value is used, halves the relative error.

Volume. It is very difficult to measure the volume of the broth during a cultivation, because aeration and impeller rotation increases the level in the fermentor. Therefore the volume was estimated. The volume is only measured once and that is when the batch media is prepared. Then the fermentor is autoclaved, then used for growth in batch phase (16 h) and then the fed-batch phase is started and the volume of the fermentor is used in calculations for the first time. There is a mark on the fermentor wall were the broth level should be and the resolution is 3-4 mm which corresponds to about 40 mL, i.e. 1% error in the initial volume.
During the fed-batch phase the volume increases. Molasses is fed and also NaOH. These volumes are carefully added in the estimate the volume. The volume of the molasses is actually calculated from the weight added and the density and is a very accurate estimate. There is also a volume check of the amount of feed fed, but this is of less precision and only noted for comparison. Maximally an error of 10 mL during a cultivation.
Between 5 and 15 samples are usually taken during the fed-batch for dry weight measuremet (2·20 mL each), and this corresponds to a removal of 200 to 600 mL broth. This removal is also accounted for but with less precision. In some cultivations samples are also taken for ethanol or glucose analysis (5 mL each) and these are not accounted for. Maximally an error of 20 mL during a cultivation.
There is also a number of factors that is not accounted for. The cells produce a little water when they completely oxidize sugar. An approximate calculation gives 200 mL water from the molasses (2 kg) during a cultivation. (2 kg feed $\approx$ 630 g glucose; 1 mole glucose = 6 mole water, in respiration; 50% of glucose to respiration, the rest to biomass; glucose mole weight = 180 g and mole weight water = 18 g; gives (630/180)·6·0.50·18 = 190 g.)
Another source of error in the volume estimate is how much water is carried away through aeration. An approximate calculation gives 200 mL during a cultivation. The exhaust gas is discussed in more detail below.
The production of water and the amount of water carried away by aeration cancel approximately during a cultivation. However, production of water is correlated with cell mass and therefore exponential and the amount of water carried away increases linearly with time (air flow is constant and influence of stirrer speed is neglected).
Uncertainity, in production of water and amoumt of water carried away by the aeration, contributes to the error in the volume estimate by say 30 mL during a cultivation.

To sum up, the initial volume has an error of 1%. The increase in the volume of the broth during a cultivation has 3% error. Adding the error in the initial volume and the added volume gives the error of the final volume of 2%.

Exhaust gas. There has been a condensor at the outlet of the exhaust gas. It has been chilled to 10-15° C. (Broth temperature 30.0° C). There has usually been water droplets in the tubing after the condensor and the air filter has usually

been changed once during a cultivation because it has been wet. An approximate calculation gives 200 g water carried away by aeration. (20 h aeration is 7200 L air; 20 g steem/kg air; 1 mole gas = 22.4 L; 1 mole air = 30 g; gives (7200/22.4)·0.030·20 = 192 g.)

Time. The samples for dry weight measurement have usually been taken on the hour and by the same person. Incidents have sometimes made it impossible to keep the time, but then the actual sample time is noted in the protocols. The error is estimated to be less than 3 min.

Concentration of molasses. The feed is mixture of water 1.00 L water and 1.69 kg molasses (as delivered from Jästbolaget). The laboratory procedure is precise and the error is far below 1%. The content of fermentable sugar in the molasses is believed to be constant when the molasses is taken from the same batch. Three different batches have been delivered from Jästbolaget and no difference has been observed in any way. The feed is autoclaved and centrifuged and this might change the concentration, see below.

Influence of autoclaving. This is not investigated much. Fixed routines and times ensures reproducibility. The spread in autoclaving time has been about 15 min.

Calculation of error propagation

The calculations of productivity and yield are monotonous in each measurement and it is therefore straight forward to calculate the error in Y and μ_p from the errors in the measurements. The worst case is easily calculated by inserting a combination of minimal and maximal values of resp measurement. An analytical expression for propagation of relative errors is also derived. The equations are logarithmed and differentiated and an approximate upper bound is found. This bound is slightly higher than the bounds obtained by inserting minimal and maximal values in the original equations.

$$\left|\frac{d\mu_p}{\mu_p}\right| \leq \frac{1}{\mu_p \cdot \Delta t}\left(\left|\frac{dV_f}{V_f}\right| + \left|\frac{dX_f}{X_f}\right| + \left|\frac{dV_i}{V_i}\right| + \left|\frac{dX_i}{X_i}\right| \right) + \left|\frac{d\Delta t}{\Delta t}\right| \qquad \text{(A6: 4)}$$

$$\left|\frac{dY}{Y}\right| \leq \left|\frac{X_f dV_f + V_f dX_f + X_i dV_i + V_i dX_i}{(VX)_f - (VX)_i}\right| + \left|\frac{d\ \Delta Feed}{\Delta Feed}\right| + \left|\frac{dC}{C}\right| \approx \qquad \text{(A6: 5)}$$

assume large difference between the cell masses

$$\approx \left|\frac{dV_f}{V_f}\right| + \left|\frac{dX_f}{X_f}\right| + \left|\frac{d\ \Delta Feed}{\Delta Feed}\right| + \left|\frac{dC}{C}\right| \qquad \text{(A6: 6)}$$

The relative error in both productivity and yield decreases the longer the time interval for observation is taken. In the formula for the relative error in the productivity, the time interval is explicitly there, and the relative error decreases linearly with time. The dominatig term, in the yield relative error, is the relative error when initial and final cell masses are subtracted. If the time interval is long the initial cell mass is neglible compared to the final cell mass and the simplified formula A5:6 is valid. However, calculating yield over only a couple of hours is more sensitive to measurement errors, because two large numbers (the cell masses) are subtracted. In formula A5:5 is seen that the relative error (due to the dominating term) increases when the difference in cell masses decreases.

A typical example

The following errors are motivated in the previous discussion.

$\left|\frac{dX_i}{X_i}\right| = 5\%$ $\left|\frac{dX_f}{X_f}\right| = 2\%$ $\left|\frac{dC}{C}\right| = 1\%$ $\left|\frac{d\ \Delta Feed}{\Delta Feed}\right| = 0\%$

$dV_i = \pm 0.020$ L $dV_f = \pm 0.050$ L $d\Delta t = \pm 3$ min

Data from cultivation 8547 B:

nominal	minimal	maximal
VX_0 = 10.3 g	9.75	10.9
V_f = 6.003 L	5.950	6.050
X_f = 66.8 g/L	65.8	67.9
ΔFeed = 2070 g(molasses)	2070	2070
C = 0.628 g(molasses)/g(feed)	0.622	0.634
Δt = 17.83 h	17.78	17.88
μ_p = 0.205 h^{-1}	0.200	0.210
Y = 0.300 g(yeast)/g(molasses)	0.290	0.311

This gives a productivity and a yield with 2.5% and 3.3% relative error resp. Using formulas A5:4-6 gives 2.8% and 5.0% resp

Calculating productivity and yield over only 3 hours are done below.

nominal	minimal	maximal
V_i = 4.937 L	4.887	4.987
X_i = 34.0 g/L	33.0	35.0
V_f = 5.571 L	5.521	5.621
X_f = 51.6 g/L	50.6	52.6
ΔFeed = 640 g(molasses)	640	640
C = 0.628 g(molasses)/g(feed)	0.622	0.634
Δt = 3.00 h	2.95	3.05
μ_p = 0.179 h^{-1}	0.154	0.205
Y = 0.298 g(yeast)/g(molasses)	0.258	0.338

This gives a productivity and a yield with 14% and 13% relative error resp. Using formulas A5:4-5 gives 16% and 14% resp.

APPENDIX 7 - A COMPARISON OF EXPIMENTAL RESULTS: WEEK 8427 - 8450

Pioneer work with molasses were done during this period. The substrate molasses did not seem to disturb the ethanol sensor. The first fed-batch was run using manual control of the feed rate, based on the ethanol signal. Later, a PI control around an exponential basic dosage scheme was used. Good results were obtained during the first 15 h, week 8433. These data were presented at the conference in Munich, [Axelsson, 84]. During this period it was found that the growth rate at cell concentrations above 30 g/L varied considerably from cultivation to cultivation. That meant that the maximal feed rate varied a factor 2. Further, attention was made to a strange sulphur odouer in the lab. Microscope studies did not show any apparent infection.

A comparison between the cultivations:

Week	8427	8429	8432	8433	8444	8450
Batch [h]	15	16.5	16.5	15	20	18
Starvation period [h]	4	4	0	0.5	0	1
Substrate pulse [g]	yes	24.1	no	no	no	no
X initial [g/L]	3.05	3.50	2.68	2.35	4.75	5.30
X final [g/L]	60.5	60.1	64.8	76	71	41.9
pH at start of batch	5.4	5.3	6.0	6.3	5.9	5.8
pH at start of fb	5.2	5.3	5.9	6.2	5.35	5.0
Dosage of NaOH batch [L]	0	0.022	0	0	0	0.010
NaOH dosage [mL/g(yeast)]	1.32	1.75	1.03	1.05	0.51	1.15
Time of fed-batch [h]	16	20	20.5	21	19.5	16
Yield Y [g(y)/g(molasses)]	0.293	0.229	0.276	0.289	0.276	0.282
Growth: μ [h^{-1}]	0.206	0.155	0.174	0.171	0.153	0.167
μ_p	0.211	0.215	0.198	0.195	0.220	0.169
μ_1	0.202	0.095	0.153	0.144	0.090	0.164
μ_2						
Fmax [mL/min]	4.1	2.8	>4.7	2.6	≈2.0	3.7
E peak [h]	15-16	1-4	6.5-11	-	18-22	-
E set-point [g/L]	0.2	0.1	0.2	0.1	0.2	0.3
Control	manual	PI	manual	PI	PI	PID
Dips in the feed rate	no	no	no	1	no	many
Sulphur smell	?	yes	no	yes	yes	?

- A COMPARISON OF EXPERIMENTAL RESULTS: WEEK 8506 - 8519

This was a very good period. The three last cultivations, 8512 and the two during 8519, went through almost without any troubles. The stability of the ethanol measurement system was improved at the beginning of the period, when the gas flow regulator system was installed. The stirrer speed was now also logged by the computer. Further, the set-point of the ethanol concentration was increased to 0.4 g/L, after recomendation, (mr Wallström, personal communication). The characteristic sulphur smell was thought of as an indication of starving. The smell was not observed any more. At the start of this period, new molasses in plastic vessels were delivered. Before the molasses came in metal vessels.

A comparison between the cultivations:

Week	8506	8509	8512	8519a	8519b
Batch [h]	15	13.5	14.5	14	13
Starvation period [h]	1.5	2.5	-	2	-
Substrate puls [g]	24	25	-	20	20
Start period [min]	40	90	65	100	150
X initial [g/L]	2.15	2.37	2.08	2.60	2.52
X final [g/L]	68.4	61.5	48.5	65.1	65.3
pH at start of fb	5.6	5.1	5.9	5.3	5.3
NaOH dosage [mL/g(yeast)]	1.15	1.71	1.37	1.68	1.25
Time of fed-batch [h]	18.5	20	18	18	18.5
Yield: Y [g(yeast)/g(molases)]	0.29	0.28	0.25	0.25	0.25
Growth: μ_p [h^{-1}]	0.205	0.181	0.200	0.202	0.198
μ_1	0.22	0.18	0.23	0.23	0.24
μ_2	0.18	0.18	0.15	0.16	0.13
F_{max} [mL/min]	4.1	5.3	3.4	4.7	3.7

- A COMPARISON OF EXPERIMENTAL RESULTS: WEEK 8528 - 8547

During this period lab assistant Fingal Morén was engaged in the practical work. However, the last two cultivations, week 8547, were done by ourselves. The feed weight meassurement signal and the signal for cooling and heating were incorporated. The stirrer speed was also controlled from the computer and exponential increase in the stirrer speed were tried. A lot of practical problems, made the work during these weeks of less value. The results from week 8547 were very good. During the first three cultivations a lot of anti-foam came into the fementor by accident. Special effects were observed. The dynamic precision of the pump was documented for the first time during these experiments.

A comparison between the cultivations:

Week	8528	8529	8532	8533	8547a	8547b
Batch [h]	17.5	21	18	15	12.5	13
Batch substrate [g]	110	110	110	40	40	40
Starvation period [h]	2.0	2.5	2.0	1.0	2.0	2.0
Substrate pulse [g]	20	20	20	20	20	20
Start period [min]	150	120	240	?	120	60
X initial [g/L]	4.14	5.85	5.50	2.10	3.50	2.58
X final [g/L]	64.2	17.6	65.7	29.6	64.8	66.8
pH at start of fb	5.1	5.1	5.0	5.1	5.2	5.1
NaOH dosage [mL/g(yeast)]	1.64	1.58	1.71	2.07	0.985	1.17
Time of fed-batch [h]	18	15.5	18	12	17.5	18
Yield [g(y)/g(molasses)]	0.268	0.253	0.281	0.283	0.293	0.301
Growth μ_p [h^{-1}]	0.176	0.070	0.161	0.127	0.190	0.205
μ_1	0.177	-	0.159	-	0.191	0.223
μ_2	0.173	-	0.164	-	0.188	0.183
F_{max} [mL/min]	5.1	0.8	4.2	?	4.0	3.9

- A COMPARISON OF EXPERIMENTAL RESULTS: WEEK 8606 - 8629

During this period lab assistant Lena Nielsen was engaged in the practical work. The very first cultivation Lena did, 8606, went through after initial trouble with the ethanol sensor. This cultivation is almost comparable with 8519 and 8547. Otherwise this period was devoted to dynamical experiments in closed loop. A new improved version of the ethanol sensor, MGS-2, was used from week 8617. A PID regulator was used as usually and the reference value followed a PRBS sequence during 3-4 hours. Preliminary results were obtained week 8609. Various technical problems made cultivations week 8607, 8613 and 8617 of less value. Good results were obtained week 8621 and 8622. During week 8628 a cultivation with low DO level was made and a definite increase in the gain was found in the ethanol loop. Finally, in week 8629, a ±10% variation was done in the feed concentration in order to test the robustness of the regulator.

A comparison between the cultivations:

Week	8606	8607	8609	8613	8617
Remark	rep	error	PRBS	error	error
Batch [h]	13	12.5	14	17	14
Starvation period [h]	2.0	4.0	4.0	9	2.0
Start period [min]	70	80	60	80	80
X initial [g/L]	2.29	2.09	-	1.98	1.96
X final [g/L]	58.1	-	-	-	42.8
pH at start of fb	5.4	5.1	-	5.1	5.1
NaOH dosage [mL/g(yeast)]	1.69	-	-	-	2.75
Time of fed-batch [h]	17	3	13	8	14
Yield [g(y)/g(molasses)]	0.249	-	-	-	0.261
Growth μ_p [h^{-1}]	0.217	-	-	-	0.243
μ_1	0.239	-	-	-	0.275
μ_2	0.175	-	-	-	0.176
F_{max} [mL/min]	4.7	-	-	-	4.5

Week	8621	8622	8628	8629
Remark	PRBS	PRBS	low DO	feed var
Batch [h]	13	13	12	15
Starvation period [h]	2.0	2.0	3.0	2.0
Start period [min]	80	100	80	100
X intial [g/L]	2.51	2.04	2.41	2.19
X final [g/L]	67.2	62.5	46.7	59.5
pH at start of fb	5.2	5.1	4.8	5.1
NaOH dosage [mL/g(yeast)]	1.15	1.63	1.88	1.92
Time of fed-batch [h]	17	17.5	16	21
Yield [g(y)/g(molasses)]	0.281	0.270	0.282	-
Growth μ_p [h^{-1}]	0.221	0.222	0.203	0.185
μ_1	0.241	0.244	0.206	0.210
μ_2	0.184	0.183	0.196	0.133
F_{max} [mL/min]	4.2	4.0	4.0	5.0

APPENDIX 8 - A COMPARISON OF REGULATOR PARAMETERS

The regulator consisted of two parts, an exponential basic dosage scheme and a PID regulator with fixed parameters. The input signals to the PID regulator was in g/L and the output was given in L/min. Sampling period, integrator time, derivator time etc were all in seconds.

In the exponential substrate dosage scheme four parameters were used: initial cell mass, growth rate, yield and concentration of fermentable sugar in the substrate (molasses). Actually, only the product of yield and concentration of fermentable sugar is needed. Analysis by Jästboalget AB, who delivered the molasses, indicate a sugar content of 50 % of the weight, i.e. 410 g/L. However, this analysis is difficult. See comments in appendix on cultivation conditions. In most of the cultivations the parameter sugar concentration in the dosage scheme was 480 g/L and the yield parameter was set to 50 %. Alternatively 410 g/L and 58.5 % could have been used, leaving the product invariant.

For the operators convenience, a simple algorithm was used in order to keep track of the volume of the broth during the fed-batch phase of the cultivation. This algorithm requires the initial value of the volume.

Standard parameters

The following parameters were used, if not otherwise stated. The sampling interval was 30 s for the regulator. The signals were prefiltered with a first order filter with a time constant of 10 s and then filtered in the computer with sampling interval 2 s and a time constant of 60 s. A second order Butterworth filter was used.

Parameters for the exponential basic dosage scheme were:

μ = 0.21 h^{-1}
G_{in} = 480 g(ferm. sugar)/L
Y = 0.50 g(yeast)/g(ferm. sugar)
VX_0 = 10 g(yeast)
V_0 = 4.00 L

Parameters for the PID regulator were:

K = 0.0020 (mL/min)/(g/L)
Ti = 2000 s
Td = 500 s

ro = 1 (bypass)
To = 30 s (anti-windup)
N = 3 (filter in the derivative part)

Experimental week: 8427-8450

The first cultivations were done using only PI control. Not until week 8450 the derivative part was used. Further, there were some changes of the parameters of the exponential dosage scheme.

Parameters for the exponential basic dosage scheme were:

μ = 0.23 h^{-1}
G_{in} = 420 and 480 g/L
Y = 0.50 and 0.52 g(yeast)/g(ferm. sugar)
VX_0 = 10 and 12 g
V_0 = 4.00 L

Parameters for the PID regulator were:

K = 0.0020-0.0030, Td = 400 s and N = 3.

Experimental week: 8506-8519

Parameters for the exponential basic dosage scheme were:

VX = 12 g at start of 8506 and 8509.

Parameters for the PID regulator were:

K = 0.0030 for cultivation 8506 and 8509.
K = 0.0030-0.0015, Ti = 2000-1200, Td = 500-300, N = 3, week 8509.

Experimental week: 8528-8547

During week 8532 the sampling period was 60 s.

The regulator was tuned during week 8547.

K = 0.0020-0.0025, Ti = 2000-1200, Td = 500-300, N = 3 - cultivation A.
K = 0.0020-0.0030, Ti = 2000-1200, Td = 500-300, N = 3 - cultivation B.

Experimental week 8606-8629

When a PRBS experiment was done early in the cultivation, it was found important to use a representative growth rate for the exponential dosage scheme over this period. The parameter μ = 0.24 h^{-1} in cultivation 8622 and 8628.

During the PRBS experiments the regulator were in some cases tuned to get a more exciting feed rate, i.e. the regulator was not so well damped. In two cultivations the control system oscillated and the regulator gain was decreased in a few steps. In week 8617 the ethanol sensor had double response time compared to the normal operation. In week 8628 the DO level was deliberately set low in the begining of the fed-batch phase and that seemed to have increased the process gain considerably and the nominal regulator parameters led to oscillating behaviour of the feed rate.

Parameters for the PID regulator were:

K = 0.0010-0.0042 Ti = 1300-2000 Td = 213-700 N = 3

APPENDIX 9 - A GUIDE TO EXPERIMENTAL RESULTS - WEEK BY WEEK

A one page summary of each cultivation is given below. These summaries give a short characteristic of difficulties that were met and observations done. A short summary of interesting parameters, like the average growth rate and the yield are given. A time table for special events and observations are also given. First a brief characterization of the different periods of our experimental work.

Experimental week: 8427-8450

Pioneer work with molasses were done during this period. The substrate molasses did not seem to disturb the ethanol sensor. The first fed-batch was run using manual control of the feed rate, based on the ethanol signal. Later, a PI control around an exponential basic dosage scheme was used. Good results were obtained during the first 15 h, week 8433. These data were presented at the conference in Munich, [Axelsson, 84]. During this period it was found that the growth rate at cell concentrations above 30 g/ℓ varied considerably from cultivation to cultivation. That meant that the maximal feed rate varied a factor 2. Further, attention was made to a strange sulphur odouer in the lab. Microscope studies did not show any apparent infection.

Experimental week: 8506-8519

This was a very good period. The three last cultivations, 8512 and the two during 8519, went through almost without any troubles. The stability of the ethanol measurement system was improved at the beginning of the period, when the gas flow regulator system was installed. The stirrer speed was now also logged by the computer. Further, the set-point of the ethanol concentration was increased to 0.4 g/L, after recomendation, (mr Wallström, personal communication). The characteristic sulphur smell was thought of as an indication of starving. The smell was not observed any more. At the start of this period, new molasses in plastic vessels were delivered. Before the molasses came in metal vessels.

Experimental week: 8528-8533

During this period lab assistant Fingal Morén was engaged in the practical work. The feed weight meassurement signal and the signal for cooling and heating were incorporated. The stirrer speed was also controlled from the computer and exponential increase in the stirrer speed were tried. A lot of practical problems, made the work during these weeks of less value. During the first three cultivations a lot of anti-foam came into the fementor by accident. Special effects were observed. The dynamic precision of the pump was documented for the first time during these experiments.

Experimental week: 8547

During this week two cultivations were done. Emphasis was on reproducing data from week 8519 and on making off-line analysis of the ethanol concentration in the broth. The two cultivations were succesful. The high growth rate from 8519 was reproduced. However, this time the yield was much higher. The off-line analysis showed a drift of the ethanol signal of 0.2 g/L, like preliminary results week 8519.

Experimental week: 8606-8629

During this period lab assistant Lena Nielsen was engaged in the practical work. The very first cultivation Lena did, 8606, went through after initial trouble with the ethanol sensor. This cultivation is almost comparable with 8519 and 8547. Otherwise this period was devoted to dynamical experiments in closed loop. A new improved version of the ethanol sensor, MGS-2, was used from week 8617. A PID regulator was used as usually and the reference value followed a PRBS sequence during 3-4 hours. Preliminary results were obtained week 8609. Various technical problems made cultivations week 8607, 8613 and 8617 of less value. Good results were obtained week 8621 and 8622. During week 8628 a cultivation with low DO level was made and a definite increase in the gain was found in the ethanol loop. Finally, in week 8629, a ±10% variation was done in the feed concentration in order to test the robustness of the regulator.

State of the art - cultivations week 8519 and week 8547

The representative cultivations were done during week 8519 and 8547. Two cultivations were done each of these weeks. Almost comparable are also cultivations 8512 and 8606. A good illustration of the control difficulties were obtained in cultivation 8433.

An idea of the parameter variation was obtained from these cultivations. It growths from 3.0±0.5 g/L to 66±1 g/L in 18 h and the volume of the broth increases from 4.0 L to 6.0 L. The average growth rate has been possible to reproduce with high accuracy while the yield varies more between cultivations. The average growth rate during 18 h has been 0.20±0.01 h^{-1} and the yield 0.27±0.03 g(yeast dry weight)/g(molasses).

Fed-batch cultivation - week 8427

After much trouble this cultivation went through. The pump was carefully checked on-line during the fed-batch phase and was found very stable and linear. The cooling was not sufficient during the later part, and the temperature rose to 30.6°. Anti-foam was given manually about evey 30 min. We had also problems with leakage from the fermentor where the samples were taken.

The ethanol set-point was 0.2 g/L and was kept well using manual control. The manual control made the cultivation change between ethanol production and consumption within half an hour.

1) Duration of the batch = 15 h.

2) Pause before the start of the fed-batch = 4.0 h.

3) Average growth rate μ = 0.206 h^{-1} during 16 h.
μ_1 = 0.211 h^{-1} 0-9 h and μ_2 = 0.202 h^{-1} 9-16 h.

4) Overall yield Y = 0.293 g(yeast)/g(molasses I).

5) Maximal feed rate = 4.1 mℓ/min.

6) Dosage of NaOH = 1.32 mℓ/g(yeast); pH_{Bstart} = 5.4; $pH_{FBstart}$ = 5.2;
Batch dosage = 0.000 ℓ.

7) Time 0.0 h.
A mini batch was given.

8) Time 1.5 h.
A new mini batch was given and manual control was started.

9) Time 7.0 h
An ethanol peak was detected. Anti-foam was given at this time.

10) Time 8.0 h.
A restart of the program was done.

11) Time 11.0 h.
A new ethanol peak was detected. The stirrer speed was changed at this time.

12) Time 17 h.
A large ethanol peak was detected.

[melass] = 0,628 g/l

Utbyte och tillväxt under fedbatch vecka: 8427

p(feed) = 1,2

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	$p\int$ Fdt	$\int$NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	15^{00}	0^{00}	3,15	12,62	12,62	12,62	0,0	0	0,006	—	4,006			
2	18^{25}	3^{25}	5,55	22,6	25,5	28,2	84,3		0,024	-0,030	4,064	0,171	0,189	
3	20^{00}	5^{00}	7,70	31,5	35,4	37,1	133		0,040	-0,060	4,091	0,210	0,291	
4	22^{00}	7^{00}	13,13	55,1	53,4	58,3	248		0,079	-0,090	4,196	0,280	0,327	
5	24^{00}	9^{00}	19,05	84,3	80,7	88,7	413		0,134	-0,120	4,427	0,213	0,282	
6	02^{00}	11^{00}	28,2	130,1	121,9	136,1	670		0,204	-0,150	4,612	0,217	0,284	
7	04^{00}	13^{00}	42,5	213,9	184,2	210,0	1071		0,319	-0,180	5,032	0,249	0,333	
8	07^{05}	16^{05}	60,5	347,8	347,8	347,8	1819		0,444	-0,210	5,750	0,158	0,285	
		16 h										0,206	0,293	

Fed-batch cultivation - week 8429

After some trouble this cultivation went through. The ethanol concentration was kept 0.1-0.2 g/ℓ using P control. The system was poorly damped. A couple of times the ethanol signal was seen to be affected by an increase of the stirrer speed. The growth rate decreased drastically after about 10 h fed-batch. The tubing for the substrate feeding went off its correct position at the pump and the feeding was stopped for about half an hour. This also happened in the middle of the cultivation. At the end a strange sulphur smell was noticed.

Glucose level was measured off-line and found to be 20-50 mg/ℓ. The ethanol analysis was not reliable. The pump was compared with data from v8427. There were some differences but the stability was good. Little drift was found for the ethanol sensor system.

1) Duration of the batch = 17 h.

2) Pause before the start of the fed-batch = 3.0 h.

3) Average growth rate $\mu = 0.155\ h^{-1}$ during 20 h.
$\mu_1 = 0.215\ h^{-1}$ 0-10 h and $\mu_2 = 0.095\ h^{-1}$ 10-20 h.

4) Overall yield Y = 0.229 g(yeast)/g(molasses I).

5) Maximal feed rate = 2.8 mℓ/min.

6) Dosage of NaOH = 1.75 mℓ/g(yeast); $pH_{Bstart} = 5.3$; $pH_{FBstart} = 5.3$;
Batch dosage = 0.022 ℓ.

7) Time 0.0 h.
A mini batch was given. Manual control was used for many hours.

8) Time 1-4 h.
A lot of ethanol was built up due to bad manual feed dosage.

9) Time 6.5 h
The stirrer speed was increased from 500 to 750 rpm and made the ethanol signal decrease.

10) Time 8.0 h.
The regulator was turned on.

11) Time 13 h.
The stirrer speed was increased from 900 to 1200 rpm and made the ethanol signal increase this time.

12) Time 15 h.
More tests were made in order to check the influence of the stirrer speed on the ethanol signal.

[melas] = 0,628 g/g ~~feed~~

Utbyte och tillväxt under fedbatch vecka: 8429

ρ(feed) = 1,205

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	$\rho \int$ Fdt	$\int$NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	11^{00}	0^{00}	3,50	14,15	14,15	14,15	25,6		0,022	0	4,043			
2	13^{00}	2^{00}	5,40	22,1	19,3	25,4	103,6		0,038	-0,030	4,094	0,223	0,162	
3	15^{00}	4^{00}	6,85	28,1	26,3	29,3	131		0,050	-0,060	4,099	0,120	0,349	
4	17^{00}	6^{00}	11,84	49,4	35,8	44,3	'235'		0,070	-0,090	4,175	0,282	0,326	
5	19^{00}	8^{00}	17,64	76,2	48,8	68,0	'400'		0,110	-0,120	4,322	0,217	0,259	
6	21^{00}	10^{00}	26,63	121,1	66,4	101,7	634		0,170	-0,150	4,546	0,232	0,306	
7	23^{00}	12^{00}	35,3	169,2	90,5	149,6	967		0,170	-0,180	4,792	0,167	0,230	
8	01^{00}	14^{00}	51,3?	257,7	123,3	195,0	1282		0,170	-0,210	5,024	0,210	0,447	
9	03^{00}	16^{00}	49,3	256,9	168,0	232,4	1542		0,170	-0,240	5,210	0,104	0,243	
10	05^{00}	18^{00}	–	–	–	–	–	–	0,170	–	–	–	–	
11	07^{00}	20^{00}	55,3	311,8	311,8	311,8	2094		0,170	-0,270	5,638	0,048	0,158	
12	10^{21}	23^{21}	60,1	352,4	523,4	356,3	2403		0,170	-0,300	5,864	0,037	0,269	
		20h										0,155	0,229	

Fed-batch cultivation - week 8432

This cultivation went through with some troubles. The batch was by accident grown without aeration. The fed-batch was manually controlled around 0.2 g/ℓ. During the fed-batch a period of high ethanol concentration was built up and later consumed. This was due to bad manual control strategy. The last few hours a P control was started and behaved properly. No data was logged at the disk.

The ethanol sensor calibration was about 4.1 times less sensitive. At the lower stirrer speeds a change of stirrer speed influenced the ethanol signal. At the higher speeds this effect was not noticed. Antifoam was automatically dropped at regular intervals.

The glucose level was measured off-line and found to be 20-60 mg/ℓ. The ethanol analysis was not reliable. No sulphur smell was noticed.

1) Duration of the batch = 16.5 h.

2) Pause before the start of the fed-batch = 0 h.

3) Average growth rate μ = 0.174 h^{-1} during 20.5 h.
μ_1 = 0.198 h^{-1}, 0-10 h and μ_2 = 0.153 h^{-1}, 10-20 h.

4) Overall yield Y = 0.276 g(yeast)/g(molasses I).

5) Maximal feed rate > 4.7 mℓ/min.

6) Dosage of NaOH = 1.03 mℓ/g(yeast); pH_{Bstart} = 6.0; $pH_{FBstart}$ = 5.9;
Batch dosage = 0.000 ℓ.

7) Time 6.5-11 h.
A lot of ethanol was produced and at the end of this period consumed.

8) Time 8.45 h.
The ethanol signal was abruptly increased when the stirrer speed was changed from 500 to 700 rpm.

9) Time 14-16 h.
A PID controller with different parameters were tried. There were problems with bumpless transfer and finally a P regulator was started.

10) Time 16-20 h.
A P regulator was tested. It was found that the ethanol signal slowly drifts away.

[melan] = 0,628 g/g (feed)

p(feed) = 1,21

Utbyte och tillväxt under fedbatch vecka: 8432

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	ρ∫ Fdt	∫NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	14^{15}	0	2,68	10,7	10,7	10,7	0?	0	0	0	4,000			
2	16^{00}	1^{45}	3,84	15,3	14,5	12,3	9?		0	-0,030	3,977	0,204	0,814?	
3	18^{00}	3^{45}	5,05	20,2	20,6	20,1	54,0		0,019	-0,060	4,004	0,139	0,173	
4	20^{00}	5^{45}	8,85	36,0	29,1	33,6	132		0,051	-0,090	4,070	0,289	0,323	
5	22^{18}	8^{03}	14,2	60,6	43,5	72,5	356		0,092	-0,120	4,266	0,289	0,175	
6	24^{00}	9^{45}	17,0	73,4	58,4	83,3	418		0,123	-0,150	4,318	0,113	0,329	
7	02^{00}	11^{45}	18,3	78,9	82,7	84,1	423		0,140	-0,180	4,310	0,036	1,752	??
8	04^{00}	13^{45}	26,2	116,9	117,2	116,1	607		0,170	-0,210	4,462	0,197	0,329	
9	06^{00}	15^{45}	36,0	171,6	166,0	169,7	916		0,250	-0,240	4,767	0,192	0,282	
10	08^{15}	18^{00}	48,3	251,8	245,6	255,2	1408		0,320	-0,270	5,214	0,219	0,260	
11	10^{00}	19^{45}	60,4	335,9	333,1	323,4	1801		0,373	-0,300	5,561	0,165	0,341	
12	10^{40}	20^{25}	64,8	374,1	374,1	374,1	2093		0,373	-0,330	5,773	0,162	0,208	
		20,5 h										0,174	0,276	

Fed-batch cultivation - week 8433

This cultivation was quite good and some of the data was later used at a conference in Munich. A PI controller with constant parameters was used for about 15 h and showed reasonable performance. During the first hours the system was poorly damped and later the feedback gain was not enough to compensate for the error in the basic dosage scheme. During the time 15-25 h the ethanol concentration was poorly controlled.

The inoculum had a fruit (plums) smell. Early during the cultivation a characteristic sulphur smell was noticed. Off-line glucose analysis gave values 10-30 mg/ℓ. After the cultivation the ethanol signal did not reach zero. The off-line ethanol analysis was not reliable.

Different kind of practical problems occured during this week. The computer halted during the batch phase and re-booted. During the fed-batch there were some leakage from the fermentor but this was stopped. There were also some problems with air in the substrate dosage tubing.

1) Duration of the batch = 15 h.

2) Pause before the start of the fed-batch = 0 h.

3) Average growth rate μ = 0.171 h^{-1} during 21 h.
μ_1 = 0.195 h^{-1}, 0-11 h and μ_2 = 0.144 h^{-1}, 11-21 h.

4) Overall yield Y = 0.289 g(yeast)/g(molasses I).

5) Maximal feed rate = 2.6 mℓ/min.

6) Dosage of NaOH = 1.05 mℓ/g(yeast); pH_{Bstart} = 6.3; $pH_{FBstart}$ = 6.2;
Batch dosage = 0.000 ℓ.

7) Time 2 h.
Manual control and initial fluctuations have passed.

8) Time 3 h.
The PI regulator was turned on. K=0.003 and Ti=2000s.

9) Time 4 h.
A short drop in feed rate due to bad operator commands.

10) Time 15 h.
Manual control.

11) Time 16.5 h.
The regulator was turned on again. Saw-teeth oscillations with a period of one hour during this period.

12) Time 20 h.
Manual control.

[melass] = 0,628 g/g(fed)

Utbyte och tillväxt under fedbatch vecka: 8433

p(feed) = 1,21

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	ρ∫ Fdt	∫NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	7^{45}	0	2,35	9,40	9,40	9,40	0		0	0	4,000			
2	9^{00}	1^{15}	2,74	10,9	11,6	11,6	12,1		0	-0,030	3,980	0,118	0,197	
3	11^{00}	3^{15}	3,83	15,2	16,4	16,6	39,8		0	-0,060	3,973	0,102	0,247	
4	13^{00}	5^{15}	6,23	24,8	23,1	25,6	89,0		0	-0,090	3,984	0,245	0,311	
5	15^{00}	7^{15}	9,68	38,8	32,5	38,5	"160"		0	-0,120	4,012	0,224	0,314	
6	17^{00}	9^{15}	13,85	56,8	45,7	56,3	258		0,035	-0,150	4,098	0,191	0,292	
7	19^{00}	11^{15}	19,83	84,3	64,4	86,1	422		0,081	-0,180	4,250	0,197	0,267	
8	21^{00}	13^{15}	27,70	123,8	90,6	127,5	650		0,143	-0,210	4,470	0,192	0,276	
9	23^{00}	15^{15}	37,10	175,5	127,5	176,5	920		0,211	-0,240	4,731	0,174	0,305	
10	01^{00}	17^{15}	44,80	226,7	179,5	239,7	1268		0,283	-0,270	5,061	0,128	0,234	
11	03^{00}	19^{15}	54,80	294,9	252,7	306,8	1637		0,329	-0,300	5,382	0,132	0,294	
12	05^{00}	21^{15}	63,8	355,8	355,8	355,8	1907		0,331	-0,330	5,577	0,094	0,359	
13	07^{00}	23^{15}	72,6	409,2	500,9	375,4	2015		0,331	-0,360	5,636	0,070	0,787	
14	09^{00}	25^{15}	76,2	438,8	705,1	408,8	2199		0,331	-0,390	5,758	0,035	0,256	
15	11^{15}	27^{30}	76,3	444,0	1036	428,8	2309		0,331	-0,420	5,819	0,005	0,075	
		21^{10} L										0,171	0,289	

Fed-batch cultivation - week 8444

After some minor problems this cultivation went through. A PI regulator was used with K=0.0030 and Ti=2000 s at the set-point 0.2 g/ℓ. The regulator performs reasonable for the first 9 hours but later the ethanol signal drifts away. At the end manual control was done in order to check the function of the ethanol sensor. It was ok.

The growth rate decrease rapidly after 9.5 h. This effect was companied with an increase in pH a couple of hours before. During this cultivation a lot of antifoam was automatically given. The DO signal made a dip at each dosage. The ethanol signal was disturbed several times when the stirrer speed was changed.

The pump was very good. No off-line analysis of ethanol and glucose. The ethanol signal did not go down to zero afterwards. A sulphur smell was felt.

1) Duration of the batch = 18.5 h. Batch media = 110 g molasses.

2) Pause before the start of the fed-batch = 0.

3) Average growth rate μ = 0.153 h^{-1} during 19.5 h.
μ_1 = 0.220 h^{-1} 0-9.5 h and μ_2 = 0.090 h^{-1} 9.5-19.5 h.

4) Overall yield Y = 0.276 g(yeast)/g(molasses I).

5) Maximal feed rate ≈ 2 mℓ/min.

6) Dosage of NaOH = 0.51 mℓ/g(yeast); pH_{Bstart} = 5.9; $pH_{FBstart}$ = 5.35;
Batch dosage = 0.000 ℓ.

7) Time 1.0 h.
Manual control and initial fluctuations have passed. A direct start of the feeding was done after the end of the batch phase. No mini-batch was given.

8) Time 8.5 h.
The pH goes up to about 5.3.

9) Time 9.5 h.
The feed rate levels off to a constant value off 2 mℓ/min. The growth rate goes down to about half within a couple of hours.

10) Time 14 h.
Manual control. The ethanol signal reacts properly.

11) Time 16 h.
The PI regulator was started without the exponential basic dosage term.

Utbyte och tillväxt under fedbatch vecka: 8444 ρ(feed) = 1,18

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	ρ∫ Fdt	∫NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	1400	-0 30	4,75	19,0	19,0	19,0	0		0	0	4,000			
2	1600	1 30	5,09	20,3	23,9	23,6	26,5		0,005	-0,030	3,997			
3	1800	3 30	8,40	34,2	32,5	37,6	107,2		0,035	-0,060	4,066	0,168	0,226	
4	2000	5 30	16,2	68,2	44,2	62,7	252		0,085	-0,090	4,209	0,345	0,374	
5	2200	7 30	22,6	99,0	60,1	93,0	427		0,140	-0,120	4,382	0,186	0,280	
6	2400	9 30	33,3	154,3	81,6	144,2	723		0,170	-0,150	4,633	0,222	0,297	
7	0200	11 30	41,0	198,8	111,0	194,3	1012		0,170	-0,180	4,848	0,127	0,245	
8	0400	13 30	50,25	254,3	150,8	244,0	1299		0,170	-0,210	5,061	0,123	0,308	
9	0600	15 30	52,6	276,2	205,0	288,9	1558		0,170	-0,240	5,250	0,041	0,135	
10	0800	17 30	62,4	341,9	278,7	338,8	1846		0,185	-0,270	5,479	0,107	0,363	
11	1000	19 30	67,1	378,8	378,8	378,8	2077		0,185	-0,300	5,645	0,051	0,254	
12	1200	21 30	67,7	394,5	514,9	422,1	2327		0,185	-0,330	5,827	0,020	0,100	
13	1300	22 30	slut	-	-	-	-	-	-	-	-	-	-	
14	1515	2345	71,3	415,7	727,2	427,8	2360		0,190	-0,360	5,830	0,026	1,023	0,208
		1930 h										0,153	0,276	

Fed-batch cultivation - week 8450

This cultivation went through despite some problems. A PID controller was used around a set-point 0.3 g/ℓ. At start, the system came into a steady oscillation. Then the D part was turned on and the system became well damped. Later the fermentor was diluted with one litre of water in order to see weather a hypothetical growth inhibiting factor could be less effective. The experiment was done twice. The obtained result was in slight favour of the hypothesis but the measurement errors were to large to say anything definitely. Another disturbing factor was frequent peaks in the ethanol signal. This peaks caused cut-offs in the feed rate. The feed dropped on the baffle due to a misstake when putting the fermentor together. This misstake has been done before but it does not seem to make any difference. The computer halted once during the batch period. The first four hours of the fed-batch were not logged.

During the fed-batch phase, several off-line analysis were done for HAc. The level of HAc was 25-55 mg/ℓ and a couple of times it was zero. It is not clear weather any sulphur smell was noticed.

1) Duration of the batch = 18 h. Batch media = 110 g molasses.

2) Pause before the start of the fed-batch = 1.0 h.

3) Average growth rate μ = 0.167 h^{-1} during 16 h.
μ_1 = 0.169 h^{-1} 0-10 h and μ_2 = 0.164 h^{-1} 10-16 h.

4) Overall yield Y = 0.282 g(yeast)/g(molasses I).

5) Maximal feed rate = 3.7 mℓ/min.

6) Dosage of NaOH = 1.15 mℓ/g(yeast); pH_{Bstart} = 5.8; $pH_{FBstart}$ = 5.0;
Batch dosage = 0.010 ℓ.

7) Time 0-4 h.
A direct start of the feeding was done after the end of the batch phase. No mini-batch was given. Manual control. A large ethanol peak was built up due to poor manual control strategy.

8) Time 4 h.
A PI controller was started. Parameters: K=0.0030, Ti=2000 s.

9) Time 6 h.
A PI controller was tuned. Parameters: K=0.0020, Ti=2000 s.

10) Time 7.5 h.
A PID controller was tuned. Parameters: K=0.0030, Ti=2000 s, Td=400, N=3. A well damped system was obtained.

11) Time 13 h.
One litre of water was poured into the fermentor.

12) Time 15.5 h.
One litre of water was poured into the fermentor.

0,628 g medan / g fed.

Utbyte och tillväxt under fedbatch vecka 8450

	Prov	Tid	Tid FB	X g/l	$\hat{V}X$ g	skatt VX	Feed g	V totalt	$\int$NaOH	Uttag	μ(VX)	Y(X/S)	$\hat{V}X_{Feed}$	
	1	15^{00}	0,00	5,30	21,2	21,2	00,0	4,000	0	0	–	–		
	2	17^{00}	2,00	–	–	29,6	–	–	–	-0,040	–	–		
	3	19^{00}	4,00	9,20	37,0	41,4	91,1	4,019	0,025	-0,080	0,139	0,276	37,3	
	4	21^{00}	6,00	12,8	52,1	57,9	176	4,071	0,047	-0,120	0,171	0,283	52,4	
	5	23^{00}	8,00	19,1	80,5	80,8	326	4,216	0,110	-0,160	0,218	0,301	78,9	
	6	01^{00}	10,00	26,2	115,2	113,0	540	4,395	0,155	-0,200	0,179	0,258	116,8	
	7	03^{00}	12,00	35,3	164,8	157,9	849	4,667	0,215	-0,240	0,179	0,256	171,6	
*	8	04^{10}	13,17	–	–	192,0	1042	4,850	0,240	–	–	–	205,7	
	9	05^{00}	14,00	37,9	226,6	220,6	1208	5,980	0,275	-0,280	0,159	0,274	235,1	
*	10	06^{20}	15,33	–	–	275,6	1495	6,269	0,330	–	–	–	286,0	
	11	07^{00}	16,00	41,9	368,3	308,3	1623	7,358	0,355	-0,320	0,154	0,313	308,6	
	12	09^{25}	18,42	–	–	462,2	2047	7,739	0,390	–	–	–	383,7	
										164 ⇒	0,167	0,282		

* / Utspädning med 1 l vatten

Fed-batch cultivation - week 8506

The cells were grown without NaOH dosage during the night and the pH was found at 3.7 in the morning. The feed control went almost unstable in the morning when the stirrer speed was increased. Maybe the low pH value increased the gain in the feed rate/ethanol loop?

In the middle, problems with the feed pump made the cells almost starve for about an hour and later a period of ethanol production started due to over feeding when the pump was corrected. Consumption of inhibitory substances during this incident?

In the end remarkably high feed and growth rate. High overall yield was also found. No sulphur smell was recogniced. Some problems with the DO electrode at start up. Therefore a very approximate calibration was done. It is likely the recorded value was higher than the true value.

1) Duration of the batch = 15 h.

2) Pause before start of fed-batch = 1.5 h.

3) Average growth rate μ = 0.205 h^{-1} for 18.5 h.
First 11.5 h μ = 0.22 h^{-1} and later 7 h μ = 0.18 h^{-1}.

4) Overall yield Y = 0.29 g(yeast)/g(molases II).

5) Dosage of NaOH = 1.15 mℓ/g(yeast); pH_{start} = 5.6.

6) Time 0 h.
Start of fed-batch with a 24 g feed batch. Initial fluctuation in the feed rate had died away after 40 min. A very good start up.

7) Time 7 h.
The stirrer speed was increased. The DO level was below a critical value and the change of stirrer speed induced an oscillation in the ethanol-feed rate loop.

8) Time 8 h.
The NaOH dosage was corrected. During the night no NaOH was given. The pH was 3.7 in the morning.

9) Time 9-10.5 h.
Trouble with the feed pump due to the tubing. To little feed and later to much.

10) Time 12 h.
The disturbance in the ethanol concentration was corrected. The later part of the cultivation went without trouble.

11) Time 17.5 h.
The NaOH dosage ceased abruptly.

12) Time 18.5 h.
At the end the feed rate was 4.1 mℓ/min and the stirrer speed was 1250 rpm. There were some trouble keeping high feed rate in the end due to air bubbles in the feed tubing. About 30 % less feed rate than nominal.

Volyms korrigerade räkningar

Utbyte och tillväxt under fedbatch vecka 8506

0,628 [illegible]/g [illegible] *g([illegible])/g ([illegible])*

Prov	Tid	Tid FB	X g/l	$\hat{V}X$ g	skatt VX	Feed g	V totalt	$\int$NaOH	Uttag	μ(VX)	Y(X/S)	$\hat{V}X_{Feed}$	
1	01^{30}	0^{00}	2,75	8,60	8,60	0	4,000	0					
2	03^{00}	1^{30}	4,4*	17,5	11,7	32,7	3,986	0	−0,040	–		14,5	
3	08^{15}	6^{45}	8,0	32,4	34,5	160,4	4,051	0	−0,040	0,193	0,234	37,6	
4	10^{10}	8^{40}	13,5	56,2	51,2	270,8	4,166	65	−0,040	0,287	0,343	57,6	
5	12^{00}	10^{30}	20,4	88,2	74,7	450,8	4,323	115	−0,040	0,246	0,283	90,1	
6	13^{00}	11^{30}	25,4	112,0	91,8	561	4,407	150	−0,040	0,239	0,344	110,1	
7	14^{00}	12^{30}	29,9	134,6	112,8	670,9	4,502	195	−0,040	0,184	0,327	129,9	
8	15^{00}	13^{30}	38,2	179,3	138,6	891	4,695	248	−0,040	0,287	0,323	169,7	
9	16^{00}	14^{30}	43,0	208,7	170,3	1082	4,854	292	−0,040	0,152	0,245	204,3	
10	17^{00}	15^{30}	49,9	251,2	209,3	1292	5,034	340	−0,040	0,185	0,322	242,3	
11	18^{00}	16^{30}	52,9	276,7	257,1	1533	5,230	380	−0,040	0,097	0,168	285,9	
12	19^{00}	17^{30}	61,3	336,1	315,9	1812	5,483	445	−0,040	0,194	0,339	336,3	
13	20^{00}	18^{30}	68,4	388,2	388,2	2097	5,675	445	−0,040	0,144	0,291	387,9	
										0,206	0,288		

*/Provet har [illegible]

Fed-batch cultivation - week 8509

The feed control went unstable during the night and actions were taken first in the morning. Due to some mistakes at restart of the program the feed rate was zero for a while after 15 hours of fed-batch. The oscillations in the beginning and this later misstake makes it difficult to exclude the possiblity of consumption of inhibitory substances. At the end the feed rate was very high.

The data is recalibrated for feed rate but the ethanol signal is left unchanged.

1) Duration of the batch = 13.5 h.

2) Pause before start of fed-batch = 2.5 h.

3) Average growth rate μ = 0.18 h^{-1} during 20 h.

4) Overall yield Y = 0.28 g(yeast)/g(molases II).

5) Dosage of NaOH = 1.71 mℓ/g(yeast); pH_{start} = 5.1; Batch-dosage = 10 mℓ.

6) Time 4 h.
The feed control became unstable. This is difficult to explain. It might be related to the long starvation period. An other possibility is that the low DO concentration made the cells more inclined to produce ethanol.
Two other effects that changed the loop gain were noticed, but these take out each other. The pump flow was later checked and was just 0.756 of the calibrated flow. On the other hand, the ethanol sensor showed an increase in gain by a factor 1.33 when calibrated after the fed-batch compared to the calibration before the batch phase. This effect was due to the new tubing. The tube gets more permeable to ethanol after some hours of use.

7) Time 10 h.
The oscillation was seen by the operator in charge. The stirrer speed was increased and this did not seem to help. After half an hour the regulator gain was halfed. This stopped the oscillations within an hour.

8) Time 15 h.
The program was restarted. New calibration of the pump and a good estimate of the cell mass was done in order to keep the basic dosage scheme on track. By accident a feed pulse entered the fermentor at program stop. The program was stopped for about 15 min and so was the feed rate. During this time the program was started once for a few minutes and the feed pump was set to a medium value. See the printer paper.

9) Time 20.5 h.
An increase in DO was observed when the NaOH dosage ceased.

10) Time 21-22.5 h
At the end the feed rate was high. A maximal feed rate fo 5.3 mℓ/min was obtained. About 20 % less feed rate than nominal value. Further, the stirrer speed was higher then normal, now 1500 rpm. More evaporation of the ethanol out from the fermentor?
The last hour the feed rate was met manually in order to check where the critical feed rate was. A drastic response was recorded.

Utbyte och bildning under fedbatch nr 6509

g(cell)/g(substrat)

Prov nr	Tid	Tid FB	X g/l	$\hat{V}X$ g	$\hat{V}X$ g	Feed g	$\hat{V}_{tot}$	$\sum$ NaOH	Provvikt g	μ_{VX}	$Y_{X/S}$	$\hat{V}X_{Feed}$
0	23.45	0	2,37	9,48	9,48	0,0	4,000	0,00	-0,035			
1	01.30	1.45	3,04	12,13	13,0	29,5	3,990	0,008	-0,070	0,14	0,143	14,7
2	9.15	9.30	11,43	48,06	52,9	230	4,205	0,085	-0,105	0,178	0,285	49,9
3	11.00	11.15	16,23	69,92	72,7	354	4,308	0,120	-0,140	0,214	0,281	71,7
4	12.00	12.15	20,14	88,17	87,1	441	4,378	0,153	-0,170	0,232	0,334	87,0
5	13.00	13.15	25,17	113,2	104,3	562	4,494	0,200	-0,120	0,250	0,329	108,3
6	14.00	14.15	29,13	137,0	125,1	694	4,704	0,280	-0,150	0,19	0,287	131,5
7	15.00	15.15	33,10	159,5	149,9	820	4,818	0,320	-0,180	0,15	0,324	153,7
8	16.00	16.15	38,05	190,4	179,6	1010	5,005	0,380	-0,210	0,18	0,259	187,1
9	17.00	17.15	45,10	236,3	215,2	1245	5,239	0,450	-0,240	0,215	0,311	228,4
10	19.00	19.15	56,95	329,8	309,2	1773	5,791	0,595	-0,270	0,167	0,282	321,2
11	20.00	20.15	61,5	370,5	370,5	2049	6,029	0,635	-0,300	0,12	0,235	369,8
12	21.18		63 ?	398,9		2451	6,332	0,635	-0,330	0,06		440,5
13			↑									
			67,5 effektivt av feed							0,181	"0,280" ↑	
											Skattning	log!

Fed-batch cultivation - week 8512

The batch was inoculated three days after the sterilization of the fermentor and batch media. A white precipitation on the fermentor walls was seen. The feed control was started before the batch phase ended. The ethanol concentration never came below 0.4 g/ℓ. A PID regulator with fixed parameters was used as usually. The cultivation went through without much problem.

The noise level of the ethanol sensor increased abruptly after about 6 hours of fed-batch and the noise level remained high for 6-7 hours. Further, the increases of RPM caused a drop in the ethanol concentration several times. One reason is certainly the very low DO concentration when the RPM was changed.

The off-line analysis was done with greater care this time. The ethanol analysis was done immediately. The ethanol values was around 400 mg/ℓ and in agreement with the measurement signal. Although the method gave 100% different values between two measurements on the same probe. The glucose and sucrose concentration was analysed after freezing. Carbon-dioxide ice was used for cooling the sampling equipment. The glucose value was around 12-20 mg/ℓ and the sucrose level 20-50 mg/ℓ.

The ethanol concentration in the outlet air was measured approximately. A definite increase in the signal was shown between 15 and 18 h FB.

The TS values were very low a couple of times?

No drops in the feed rate occured. So in this cultivation no hypothetical growth inhibiting factors could have been consumed. The growth rate is 0.23 h^{-1} for the first 12 hours and goes down within an hour to 0.15 h^{-1} the last 6 hours. Is this an effect of the old batch media or is it a true effect of a growth inhibiting factor? Remarkably is that the lower growth rate was not followed by a sulphur smell.

1) Duration of the batch = 14.5 h.

2) No pause before start of the fed-batch.

3) Average growth rate μ = 0,20 h^{-1} during 18 h.
First 12 hours μ = 0.23 h^{-1} and later 6 hours μ = 0.15 h^{-1} .

4) Overall yield Y = 0.25 g(yeast)/g(molases II).

5) Dosage of NaOH = 1.37 mℓ/g(yeast); pH_{start} = 5.9; Batch-dosage = 0.

6) Time 5.75 h.
The noise level of the ethanol sensor increases abruptly and remains at the higher level throughout.
The NaOH dosage starts at the same time.

7) Time 12 h.
The growth rate decreases from μ=0.23^{-1} to μ=0.15^{-1} . This change occurs within 2 hours.

8) Time ≈ 21 h.
The feed tube was broken. Maximal feed rate was 3.4 mL/min. The feed rate was about 20 % less than nominal value.

Utbyte och tillväxt under fedbatch vecka 85/2

g(just)/g(analys)

Prov	Tid	Tid FB	X g/l	$\hat{V}X$ g	skatt VX	Feed g	V totalt	$\int$NaOH	Uttag	$\mu(VX)$	Y(X/S)		$\hat{V}X_{Feed}$
1	00^{15}		2,44 *	9,76 *	6,77	0	4,000	0	0				
2	01^{00}		2,08	8,28	8,28	10	3,980	0	-0,030				
3	8^{15}		10,78	44,5	35,3	193	4,130	0,030	-0,060	0,232	0,315		37,1
4	10^{15}		16,52	69,1	52,6	263	4,180	0,060	-0,090	0,213	0,559		48,2
5	12^{15}		24,01	106,8	78,5	523	4,450	0,140	-0,120	0,187	0,231		89,1
6	13^{15} *		24,7	112,4	95,9	651	4,550	0,180	-0,150	0,051	0,670		109,3
7	14^{15}		29,7	139,3	117,2	794	4,690	0,220	-0,180	0,215	0,299		131,9
8	15^{15} *		33,2	160,8	143,1	951	4,843	0,265	-0,210	0,144	0,218		156,6
9	16^{15}		37,4	186,7	174,8	1122	4,991	0,301	-0,240	0,149	0,245	0,151	183,6
10	17^{15} *		41,0	212,9	213,5	1337	5,193	0,355	-0,270	0,131	0,194	0,172	217,4
11	18^{15}		48,5	262,5	260,8	1570	5,412	0,410	-0,300	0,209	0,339		254,2
12	19^{15} *		47,7	269,4	318,5	1836	5,647	0,455	-0,330	0,026	0,041		296,1
13	23^{35} *		57,9	338,9		2109	5,853	0,465	-0,360	0,20	0,251	0,160	
										01^{00} ↑ 18^{15}			
	*/ Dessa	TS prover	lagrum	av DPA	; övriga	av CFI							

Fed-batch cultivation - week 8519a

This cultivation went through without much trouble. A lot of multiple TS measurements were done in order to check the accuracy of the method. Further, the ethanol concentration was measured off-line with improved techniques and a reproducible measurement in rough agreement with the measurement signal was obtained. The value obtained off-line was about 0.2 g/ℓ below the measured value. A couple of off-line measurements were done the last hours of the fed-batch with a stirrer speed of 1500 rpm. No anomalities were found. The membrane gas sensor system was calibrated after the fed-batch and little drift was found.

The condensor was found not to have sufficient capacity. The outlet tubing had droplets of water near the condensor. This finding was done early during the fed-batch.

During the first 10 hours the stirrer speed was set to a higher value than usually in order to have abundance of DO in the morning. The growth rate during this period was a little bit higher than usually.

At the end of the fed-batch a very high feed rate was obtained. Approximately a feed rate of 4.7 mL/min. The pump gain decreased gradually and at the end the actual feed rate was about 15 % lower than nominal. Measured manually during the cultivation.

1) Duration of the batch = 14 h.

2) Pause before the start of the fed-batch = 2 h.

3) Average growth rate μ = 0.20 h^{-1} during 18 h. First 11 hours μ = 0.23 h^{-1} and later 7 hours μ = 0.16 h^{-1}.

4) Overall yield Y = 0.25 g(yeast)/g(molases II).

5) Dosage of NaOH = 1.68 mℓ/g(yeast); pH_{start} = 5.3. Batch-dosage = 0.

6) Time 1.8 h.
Ethanol and feed rate have passed initial fluctuations.

6) Time 10 h.
The growth rate decreased from μ = 0.25 h^{-1} to 0.19 h^{-1}.

7) Time 11.5 h.
A peek in the ethanol signal. The regulator compensated the disturbance within 20 minutes.

9) Time 18 h.
The fed-batch stopped at a feed rate $\approx$ 5.0 mℓ/min and RPM = 1500.

[melass] = 0,628 g/g(feed)

ρ(feed) = 1,22

Utbyte och tillväxt under fedbatch vecka: 8519 A

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	ρ∫ Fdt	∫NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	6^{00}	0	2,60	10,4	10,4	10,4	0	0	0,000	0	4,000			
2	1^{15}	1^{15}	3,10	12,35	13,4	13,6	≈ 20	21,3	0,005	-0,040	3,981	0,137	0,155	
3	8^{00}		15,0	64,2	52,5	60,7	314	323	0,120	-0,100	4,278	0,244	0,281	
4	10^{00}		24,1	108,1	78,6	96,6	538	547	0,205	-0,160	4,487	0,261	0,312	
5	11^{00}		28,4	131,4	96,3	120,7	689	696	0,260	-0,200	4,626	0,195	0,246	
6	12^{00}		33,7	160,1	117,8	147,3	855	868	0,310	-0,260	4,752	0,191	0,275	
7	13^{00}		38,8	190,6	144,2	177,6	1044	1067	0,365	-0,310	4,912	0,174	0,257	
8	14^{00}		44,1	225,9	171,6	213,8	1270	1297	0,430	-0,350	5,123	0,170	0,249	
9	15^{00}		50,0	266,3	216,2	253,5	1518	1556	0,490	-0,410	5,327	0,165	0,259	
10	16^{00}		55,0	306,0	264,6	297,7	1794	1852	0,560	-0,470	5,583	0,139	0,229	
11	17^{00}		60,4	351,7	324,6	343,5	2080	2194	0,625	-0,510	5,823	0,139	0,254	
12	18^{00}		65,1	396,6	396,6	395,9	2407	2582	0,665	-0,550	6,092	0,120	0,219	
		18 h										0,202	0,255	

Fed-batch cultivation - week 8519b

This cultivation went through without much trouble, though DO concentration came accidentally below a critical value for a couple of hours in the middle. A lot of multiple TS measurements were done in order to check the accuracy of the method. Further the ethanol concentration was measured off-line with improved techniques and a reproducible measurement was obtained 0.2 g/ℓ below the signal from the membrane gas sensor system.

The condensor was found not to have sufficient capacity, cf. week 8519a.

There were indications that the oxygen supply was not sufficient during time 9-11.5 hours. This shortage resulted in a drift to a higher ethanol signal and a corresponding lower feed rate. When the stirrer speed was increased the feed rate began to increase at a higher rate within a half an hour.

There was also another indication of cross coupling between ethanol and DO dynamics. This was found at a change of stirrer speed at 4h of fed-batch.

At the end the feed rate was stable for a couple of hours and decreased from 3.7 to 3.4 mL/min. Pump gain close to 1 and actually increased a few percent near the end. The NaOH dosage also ceased the last few hours.

1) Duration of the batch = 13 h.

2) No pause before the start of the fed-batch.

3) Average growth rate μ = 0.20 h^{-1} during 18 h. First 11.5 hours μ = 0.24 h^{-1} and later 7 hours μ = 0.13 h^{-1}.

4) Overall yield Y = 0.25 g(yeast)/g(molases II).

5) Dosage of NaOH = 1.25 mℓ/g(yeast); pH_{start} = 5.3; Batch dosage = 0.

6) Time 2.5 h.
Manual control and initial fluctuations have passed.

7) Time 4 h.
A ringing in the ethanol signal occured and was induced by an increase in the stirrer speed.

8) Time 9 - 11.5 h.
The culture seemed to be growing under both oxygen and substrate limitation. An increase in the stirrer speed increased the rate of increase in the feed rate.

9) Time 15 - 18.5 h.
The NaOH dosage ceased. The feed rate came to a platau and was 3.4 mℓ/min only. The stirrer speed was 1300 rpm.

[medium] = 0,628 g / g(feed)

Utbyte och tillväxt under fedbatch vecka: 8549 B

ρ(feed) =

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	ρ∫ Fdt	∫NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	2030	0	2,52	10,1	10,1	10,1	0	0	0	0	4,000			
2	2200	1,5	3,56	14,2	13,6	14,6	29,0	27,3	0,006	-0,040	3,990	0,227	0,225	
3	2400	3,5	5,84	23,3	20,2	22,4	78,3	75,8	0,023	-0,100	3,987	0,247	0,294	
4	0805	11,58	34,5	165,9	100,0	141,1	834,4	671	0,285	-0,160	4,810	0,243	0,300	
5	0900	12,5	38,2	187,8	120,6	165,0	986,4	796	0,325	-0,220	4,915	0,135	0,229	
6	1000	13,5	42,3	215,1	146,3	195,5	1181	948	0,375	-0,260	5,085	0,136	0,223	
7	1100	14,5	48,0	253,6	178,3	229,6	1398	1122	0,435	-0,300	5,283	0,165	0,283	
8	1200	15,5	50,7	278,3	217,3	268,1	1643	1312	0,480	-0,340	5,489	0,093	0,161	
9	1300	16,5	58,0	329,1	264,9	310,9	1916	1523	0,480	-0,380	5,673	0,168	0,296	
10	1400	17,5	60,2	352,7	322,9	354,1	2191	1752	0,480	-0,420	5,859	0,069	0,137	
11	1500	18,5	65,3	393,6	393,6	393,6	2445	1964	0,480	-0,460	6,028	0,109	0,256	
		18,54										0,198	0,250	

Fed-batch cultivation - week 8528

After much trouble this cultivation went through. During this cultivation a lab assistant, Finngal Morén, was brought up, but only the spread in TS values can be accounted on him. The long time for the batch was due to the high melass content of the media. Due to a misstake, only E, DO, NaOHdos, rpm and mark signals was logged. The control variable can be reconstructued from the ethanol signal if wanted for further analysis. The start-up of the fed-batch was good but after a few hours the ethanolsingal went high and the regulator stopped the feeding. A few feedpulses during the next 5 hours indicates instability of the regulator. The later part was well regulated.

1) Duration of the batch = 17.5 h. Batchmedia contained 110 g melass.

2) Pause before the start of the fed-batch = 2h.

3) Average growth rate μ = 0.176 h^{-1} during 18 h.

4) Overall yield Y = 0.268 g(yeast)/g(melass II).

5) Dosage of NaOH = 1.64 mℓ/g(yeast); pH_{start} = 5.70; $pH_{FBstart}$ = 5.12;
Batch dosage = 0.006 ℓ.

6) Maximal feedrate = 5.1 mℓ/min. A gradual decrease of the pump gain to 80 % of the nominal gain, according to manual measurement during the cultivation.

7) Time 1.0 h.
Manual control and initial fluctuations have passed.

8) Time 3.5 h.
The ethanol signal went up without reason.

9) Time 8.5 h.
Manual control.

10) Time 10.0 h.
The regulator was started.

11) Time 15 h.
The regulator parameter was changed:
from K = 0.0020; Ti = 2000; Td = 500; N = 3;
to K = 0.0025; Ti = 1500; Td = 400; N = 3;

12) Time 17 h.
The feed rate reached a value of 5.1 mℓ/min. The stirrer speed was 1500 rpm.

$VX(Y)$ $\rho \int F\,dt$

$\rho \approx 1{,}23$ $1/\rho = 0{,}81$

0,628 g [illegible] /g feed

Utbyte och tillväxt under fedbatch vecka 8528

VX(TJ) VX(μ)

Prov	Tid	Tid FB	X g/l	$\hat{V}X$ g	skatt VX	Feed g	V totalt	∫NaOH	Uttag	μ(VX)	Y(X/S)	VX(Y)	
1	03[50] 05[00]	0	4,14	16,43	16,43	0	3,970	0	-0,030*			16,43	
2	13[05]	8[05]	17,8	77,4	68,2	371	4,345	105	-0,060	0,192	0,262	78,9	
3	14[05]	9[05]	19,9	88,8	81,3	407	4,360	120	-0,090	0,137	0,504	84,9	
4	15[05]	10[05]			96,9	≈485	4,423	150	-0,120			98,1	
5	16[00]	11[05]			115,6	605	4,505	185	-0,150			118,3	
6	17[00]	12[00]	29,6	138,0	135,8	746	4,661	237	-0,180			142,0	
7	18[05]	13[05]	33,9	163,9	164,4	922	4,834	297	-0,210	0,159	0,234	171,6	
8	19[00]	14[00]	41,6	208,2	193,1	1098	5,004	355	-0,240	0,261	0,401	201,2	
9	20[00]	15[00]	46,8	243,6	230,3	1325	5,208	405	-0,270	0,157	0,248	239,4	
10	21[00]	16[00]	51,5	281,9	274,6	1585	5,474	490	-0,300	0,146	0,235	283,2	
11	22[00]	17[00]	56,0	322,8	327,5	1882	5,765	570	-0,330	0,136	0,219	333,2	0,2
12	23[00]	18[00]	64,2	390,5	390,5	2226	6,083	640	-0,360	0,190	0,313	390,5	
13	23[30]	18[30]	59,5?	370,8		2398	6,232	680	-0,390				
										0,176	0,268		

*/ [illegible] 23[45].

Fed-batch cultivation - week 8529

After much trouble this cultivation went through. Finngal Morén was at charge. The long batch time was due to the high molass content of the media during the batch. During the batch a lot of antifoam dropped by accident into the fermentor. This was the effect of a leakage of the antifoam tubing. A dark grey precipitation was found on the fermentor walls. The growth rate was very slow and the DO was high. It is likely that the antifoam formed to a monolayer around the cells and decreased the masstransfer rate. After about 14 h fed-batch a strong ethanol smell was felt when one entered the lab.

1) Duration of the batch = 21 h. Batch media contained 110 g molasses.

2) Pause before the start of the fed-batch = 2.5 h.

3) Average growth rate μ = 0.070 h^{-1}.

4) Overall yield Y = 0.253 g(yeast)/g(molasses II).

5) Maximal feed rate = 0.8 mℓ/min.

6) Dosage of NaOH = 1.58 mℓ/g(yeast); pH_{Bstart} = ?; $pH_{FBstart}$ = 5.14; Batch dosage = 0.

7) Time 1.5 h.
Manual control and initial fluctuations have passed.

8) Time 12 h.
The DO electrode went bad. It remained on a low level through out.

9) Time 15.5 h.
The ethanol signal jumped up. Half an hour later, the set-point was changed to 0.8 g/ℓ.

Utbyte och tillväxt under fedbatch vecka: 8529 ρ(feed) = 1,23

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	$\rho\int$ Fdt	$\int$NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	?	?	5,85	23,4		—	0		0,005	0	4,005			
2	10^{10}	0^{40}	6,31	25,2		25,2	21,8		0,010	-0,030	3,998			
3	12^{00}	2^{30}	6,20	24,7		28,4	42,0		0,017	-0,060	3,991			
4	13^{00}	3^{30}	6,80	27,0		30,1	52,9		0,020	-0,090	3,973			
5	14^{00}	4^{30}	7,27	28,7		32,0	64,4		0,022	-0,120	3,954			
6	15^{00}	5^{30}	8,00	31,5		33,8	76,2		0,025	-0,150	3,937			
7	16^{00}	6^{30}	9,57	37,4		34,6	81,2		0,025	-0,180	3,911			
8	17^{00}	7^{30}	9,47	36,9		37,4	98,4		0,030	-0,210	3,900			
9	18^{00}	8^{30}	10,0	39,0		39,2	109,8		0,030	-0,240	3,900			
10	20^{00}	10^{30}	11,7	45,5		44,6	143,9		0,040	-0,270	3,887			
11	01^{00}	15^{30}	17,6	71,4		71,4	313		0,100	-0,300	4,054			
12	05^{10}	19^{40}	—			107,7	541		0,170	-0,330	4,280			
												0,070	0,253	154

Fed-batch cultivation - week 8532

After much trouble this cultivation went through. Finngal Morén was at charge. The long batch time was due to the high molass content of the media during the batch. During the batch a lot of antifoam dropped by accident into the fermentor. This was the effect of a leakage of the antifoam tubing. A dark grey precipitation was found on the fermentor walls. The computer halted twice due to external disturbances. The 12-chanal printer went out of order and a 2-chanal printer was borrowed for a few hours. Most of the data was only recorded by the computer. The ethanol signal gave a couple of peaks at the start and at the end. The sample time was chosen to 60 s, by rule of thumb, in order to improve possibilities for good identification. The RPM was continuously controlled from the computer according to a basic scheme.

1) Duration of the batch = 18 h. Batch media contained 110 g molasses.

2) Pause before the start of the fed-batch = 2.0 h.

3) Average growth rate μ = 0.161 h^{-1} during 18 h.

4) Overall yield Y = 0.281 g(yeast)/g(molasses II).

5) Maximal feed rate = 4.2 mL/min. Abrupt decrease in the pump gain, and at the end the pump gain was only 40 % of the nominal value.

6) Dosage of NaOH = 1.71 mℓ/g(yeast); pH_{Bstart} = 5.4; $pH_{FBstart}$ = 5.1;
Batch dosage = 0.008 ℓ.

7) Time 1.5 h.
Manual control and initial fluctuations have passed.

8) Time 2.0 h.
Ethanol peak.

9) Time 4.0 h.
Computer halted due to external disturbances. The computer was restarted within 15 min.

10) Time 4.5 - 13 h.
Good control of the ethanol signal. Small oscillation around the set-point.

11) Time 14 h.
Ethanol peak.

12) Time 15 h.
Ethanol peak. The feed pump did not manage to pump in feed at sufficiently high rate. Therefore the ethanol level went down.

Utbyte och tillväxt under fedbatch vecka: 8532 ρ(feed) = 421

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	$\rho\int$ Fdt	$\int$NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	7^{00}	0	5,50	22,0	22,0	22,0	0	0	0	0	4,000			
2	8^{00}	1^{00}	5,52	22,1			30,2	30	0,005	-0,030	4,000			
3	13^{10}	6^{10}	12,7	52,9			189,9	183	0,070	-0,060	4,167	0,142	0,259	
4	14^{00}	7^{00}	13,75	57,4			221,9	209	0,080	-0,090	4,173	0,098	0,224	
5	15^{00}	8^{00}	16,2	68,1			270,6	253	0,100	-0,120	4,204	0,171	0,350	
6	16^{00}	9^{00}	—	—			344,0	320	0,133	-0,150	4,267	—	—	
7	17^{05}	10^{05}	—	—			447,7	416	0,170 ?	-0,150	4,390	—	—	
8	18^{00}	11^{00}	27,3	126,8			573,4	541	0,220	-0,150	4,643	0,207	0,309	
9	19^{00}	12^{00}	31,5	147,4			708,9	685	0,275	-0,180	4,681	0,151	0,242	
10	20^{00}	13^{00}	37,0	180,8			890,0	880	0,360	-0,210	4,886	0,204	0,293	
11	21^{00}	14^{00}	41,7	211,6			1088	1131	0,415	-0,240	5,074	0,157	0,248	
12	22^{00}	15^{00}	49,4	261,8			1312	1471	0,485	-0,270	5,299	0,213	0,357	
13	23^{00}	16^{00}	53,7	297,0			1561	1920	0,540	-0,300	5,530	0,126	0,225	
14	24^{00}	17^{00}	59,1	343,4			1841	2508	0,620	-0,330	5,811	0,145	0,264	
15	01^{00}	18^{00}	65,7	400,2			2143	3265	0,685	-0,360	6,096	0,153	0,299	
		18 h										0,161	0,281	

Fed-batch cultivation - week 8533

After much trouble this cultivation went through. Finngal Morén was at charge during the preparations. The batch was redone due to an accident with the shaking bulb. The computer halted three times due to external disturbances. The first time was during the growth phase during the night and the feed was halter for 5 h. The other two stops were alomost immidiately detected by the operator. The RPM was continuously controlled from the computer according to a basic scheme.

During work with recovering the data files after all this computer stops a mistake was done by JanPeter and most of the logged data on the files from this cultivation was lost.

1) Duration of the batch = 15 h. Batch media contained 40 g molasses.

2) Pause before the start of the fed-batch = 1.0 h.

3) Average growth rate μ = 0.127 h^{-1} during 20 h.

4) Overall yield Y = 0.283 g(yeast)/g(molasses II).

5) Maximal feed rate = ? mℓ/min.

6) Dosage of NaOH = 2.07 mℓ/g(yeast); pH_{Bstart} = 5.2; $pH_{FBstart}$ = 5.1;
Batch dosage = 0.015 ℓ.

7) Time 1.15 h.
Automatic control was turned on, but it did not perform very well. The ethanol concentration oscillates for a couple of hours and then the computer halted.

8) Time 4.5 h.
The computer halted due to external disturbances.

9) Time 10.0 h.
The computer was found aborted and a restart was done within 10 minutes.

10) Time 20.0 h.
The computer was found aborted and a restart was done within 10 minutes.

11) Time 20.45 h.
The computer was found aborted and a restart was done within 10 minutes.

12) Time 23.20 h.
The pump tubing was corrected.

0,628

Utbyte och tillväxt under fedbatch vecka: 8533 p(feed) = 1,22

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	ρ∫ Fdt	∫NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	00:00	0	2,10	8,40		8,40	0		0	0	4,000			
2	16:00		6,57	26,7		25,2	94,5		0,025	-0,045	4,057	0,117	0,308	
3	15:00		11,0	46,0		49,1	229		0,070	-0,075	4,183	0,109	0,229	
4	18:00		18,0	79,6		85,0	431		0,170	-0,105	4,418	0,183	0,265	
5	20:00		23,6	106,8		108,3	562		0,210	-0,145	4,526	0,147	0,331	
6	22:00		29,6	138,7		138,7	733		0,270	-0,185	4,686	0,131	0,297	
										-0,225				
											Under 12h	0,137	0,279	
											Σ	0,127	0,283	

Fed-batch cultivation - week 8547a

This cultivation went through without much problem. The emphasis of this experiment was to reproduce the cultivation from week 8519 and do careful off-line analysis of the ethanol concentration. These results indicated a drift of the sensor system as the cultivation proceeds towards higher ethanol signal than the off-line measurements gave. The stirrer speed was controlled using a basic scheme, but during the later half, the stirrer was controlled manually to compensate for bad tuning of the scheme.

After about ten hours there were a decrease in the ethanol signal and the DO level was low. After an increase in the stirrer speed, the DO level and the ethanol signal increased. The feed rate was increased considerably. The regulator showed good stability and the bandwidth could have been increased. The regulator parameters are actually changed later. During the last hours the ethanol signal fluctuates considerably.

Remarkably was the high growth rate during the later part of the cultivation. The overall yield was also high, compared to week 8519. Note also the low NaOH dosage per cell. This effect was due to the fact that the NaOH dosage ceased early, i.e. after 15 h.

1) Duration of the batch = 12.5 h. Batch media contained 40 g molasses.

2) Pause before the start of the fed-batch = 2.0 h.

3) Average growth rate μ = 0.190 h^{-1} during 17.5 h.

4) Overall yield Y = 0.293 g(yeast)/g(molasses II).

5) Maximal feed rate = 4.0 mL/min. A 15 % decrease in the pump gain.

6) Dosage of NaOH = 0.985 mℓ/g(yeast); (1.24 after 16 h).
pH_{Bstart} = 5.0; $pH_{FBstart}$ = 5.2; Batch dosage = 0.015 ℓ.

7) Time 2.0 h.
Initial fluctuations have ceased and the regulator was turned on.

8) Time 10 h.
The DO level was found low in the morning. The approximate basic dosage scheme of stirrer speed was to low and stirrer speed was set in manual mode. Note changes in DO and E levels. The regulator behaved well. The feed rate increased considerably without stability problems in the ethanol loop.

9) Time 13.5 h.
The PID parameters were changed:
K=0.00025 (0.0020); Ti=1200 (2000); Td=300 (500); N=3.

10) Time 15.5 h.
A large disturbance in th ethanol signal. It lasted for half an hour. The feed rate also reached a plateau here. The NaOH dosage ceased also.

11) Time 19 h.
The cultivation was stopped at a stirrer speed of 1300 rpm and the feed rate had reached a plateau of 3.9 mℓ/h.

0,628

Utbyte och tillväxt under fedbatch vecka: 8547A p(feed) = 1,16

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	p∫Fdt	∫NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	0^{00}		3,50	14,0	14,0	14,0	0	0	0	0	4,000			
2	11^{05}		24,5	114,1	112,8	120,4	578,4		0,198	-0,040	4,657	0,191	0,276	
3	13^{00}		34,5	170,2	164,8	178,2	892,4		0,242	-0,080	4,932	0,200	0,284	
4	16^{00}		54,2	305,6	296,1	311,9	1619		0,361	-0,120	5,639	0,195	0,297	
5	17^{30}		64,8	386,9	386,9	386,9	2027		0,381	-0,160	5,971	0,157	0,317	
6	19^{05}		69,6	437,9	514,2	463,7	2444		0,381	-0,200	6,291	0,083	0,195	
											17,5h	0,190	0,293	
											19h	0,181	0,276	

Fed-batch cultivation - week 8547b

This cultivation went through without much trouble. The emphasis on this experiment was to reproduce results overall results from week 8519 and also from this week. Many off-lina analysis were done of the ethanol concentration. A drift of 0.2 g/L at the end of the cultivation, was confirmed for the fourth time. The stirrer speed was well controlled using a basic scheme. The DO level was comparatively high. Most of the time above 30 %.

At about 8 am, the ethanol signal decreased slightly and the feed rate increased considerably. There was a drop in the DO level that was not understood. There might have come a person in the room. No comments in the notebook.

Remarkably high growth rate and yield throughout, compared to 8519. Note that the NaOH dosage ceased early, i.e. after 16 h.

1) Duration of the batch = 13 h. Batch media contained 40 g molasses.

2) Pause before the start of the fed-batch = 2.0 h.

3) Average growth rate μ = 0.205 h^{-1} during 18 h.

4) Overall yield Y = 0.301 g(yeast)/g(molasses II).

5) Maximal feed rate = 3.9 mL/min. During the last hours a decrease to about 3.4 mL/min. Not much drift.

6) Dosage of NaOH = 1.17 mℓ/g(yeast); (1.71 after 16 h). pH_{Bstart} = 5.0; $pH_{FBstart}$ = 5.1;
Batch dosage = 0.020 ℓ.

7) Time 1 h.
The PID regulator was turned on and the ethanol level reached set-point with some over-shoot.

8) Time 8 h.
A sudden drop in the DO level from 30 to 10 %. A slow slight decrease in the ethanol signal accompanied with a considerable increase in the feed rate.

9) Time 13.5 h.
The PID parameter were changed:
K=0.0030 (0.0020); Ti=1200s (2000s); Td=300s (500s); N=3.

10) Time 16 h.
A platau in the feed rate was reached. The NaOH dosage ceased. The regulator showed poorly damped actions.

11) Time 19 h.
The cultivation was finished at a stirrer speed of 1500 rpm and a feed rate of 3.3 mℓ/min.

0,628

Utbyte och tillväxt under fedbatch vecka: 8547B p(feed) = 1,24

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	ρ∫Fdt	∫NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	0^{10}	0	2,58	10,3	10,3	10,3	0	0	0	0	4,000			
2	10^{10}	10^{00}	21.4	96,0	80,3	95,7	452		0,165	-0,040	4,488	0,223	0,302	
3	13^{00}	12^{50}	35,0	172,8	143,6	174,2	867*		0,320	-0,080	4,937	0,207	0,295	
4	16^{00}	15^{50}	51,6	287,5	265,9	295,2	1507*		0,480*	-0,120	5,571	0,170	0,285	
5	18^{00}	17^{50}	66,8	401,0	401,0	401,0	2070		0,500	-0,160	6,003	0,166	0,321	
6	19^{00}	18^{50}	68,8	424,6	492,4	450,5	2329		0,500	-0,200	6,171	0,057	0,145	
											17^{50} h	0,205	0,301	

* Framtaget från datafilen -

Fed-batch cultivation - week 8606

This cultivation went through after some trouble. Lena Nielsen was at charge. A hole in the membrane of the ethanol probe caused some damage in the gas detector unit. Two units were therefore coupled together and the batch was started after a 24 h delay. The batch media was under this period chilled to about 15 C. A grey precipitation was found on the fermentor walls this day. The calibration of the pH sensor had drifted over 2 pH units during autoclaving.

The fedbatch phase went through without any problems. A high overall growth rate was obtained combined with a low yield. This cultivation is almost comparable with v8519 and v8547.

1) Duration of the batch = 13 h. Batch media contained 40 g molasses.

2) Pause before the start of the fed-batch = 2.0 h.

3) Average growth rate μ = 0.217 h^{-1} during 17 h.

4) Overall yield Y = 0.249 g(yeast)/g(molasses II).

5) Maximal feed rate = 4.7 mL/min.
Pump was monitored the first few hours and a couple of times near the end. No drift was found.

6) Dosage of NaOH = 1.69 mL/g(yeast). pH(Batch start) = 5.5; pH(FB start) = 5.4; Batch dosage = 0.015 L.

7) Time 1.1 h.
Initial fluctuations ceased and the PID regulator was turned on.

8) Time 9 h.
The DO level was low due to bad tuning of the rpm scheme. DO was below 10%. The rpm began to increase here and soon the DO level rose. A corresponding increase in feed rate was also observed. Minor fluctuations in the ethanol signal.

9) Time 17.5 h.
A considerable increase in the ethanol signal; E = 0.78 g/l.

10) Time 21 h.
A calibration of the ethanol sensor with 0.30 g ethanol/l gave the signal E = 0.27 g/l.

0,628

Utbyte och tillväxt under fedbatch vecka: 8606 p(feed) = 1,22

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	$\rho\int$ Fdt	$\int$NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	23^{00}	0	2,29	9,16	9,16	9,16	0		0	0	4,000	–	–	
2	8^{20}	9^{20}	18,3	82,8	69,4	79,1	447		0,180	-0,020	4,526	0,236	0,262	
3	10^{05}	11^{05}	26,5	127,4	101,5	119,1	703		0,280	-0,050	4,806	0,246	0,277	
4	12^{00}	13^{00}	36,4	190,4	153,8	179,9	1092		0,415	-0,080	5,230	0,210	0,258	
5	13^{00}	14^{00}	43,1	236,8	191,1	221,0	1355		0,493	-0,110	5,494	0,218	0,281	
6	14^{00}	15^{00}	47,6	273,3	237,4	260,6	1608		0,563	-0,140	5,741	0,143	0,230	
7	15^{00}	16^{00}	51,7	311,3	295,6	309,9	1923		0,615	-0,170	6,021	0,130	0,192	
8	16^{00}	17^{00}	58,1	364,5	366,4	363,8	2268		0,615	-0,200	6,274	0,158	0,246	
9	16^{45}	17^{45}	61,2?	392,6	431,2	395,4	2470		0,620	-0,230	6,414	0,074	0,221	
											7L	0,217	0,249	

Fed-batch cultivation - week 8607

This cultivation was never completed. Tubing for the heating system of the fermentor broke up due to bad "plumbing" and over pressure in the tubing. This resulted in a water leakage and water came into the computer and it stopped. All anti-foam was found pumped into the fermentor. All this happened during the early hours, only a few hours after the fed-batch was started. Lena Nielsen at charge.

It is also worth noticing that at the start of the fed-batch phase the air filter was found a little bit wet. This resulted in an over pressure in the fermentor. This might be an explanantion for the lower feed rate than the calibrated feed rate.

1) Duration of the batch $\approx$ 12.5 h (not clear from the note book).

2) Pause before the start of the fed-batch = 4.0 h.

3) Time 1.3 h.
The regulator was turned on.

4) Time 2.67 h. Warm water leakage was found and the computer was found halted.

Fed-batch cultivation - week 8609

This cultivation were prepared for educational purpose (laboration in the course: control of biotechnological processes). Ulla Dissing made the preparations together with Lena Nielsen. Between student groups a very good PRBS experiment was performed. The ethanol sensor MGS-1 was used. A substrate pulse of 20 g started the fed-batch as usual and the ethanol conc was kept at 0.40-0.50 g/L until start of the PRBS signal. No dry weight measurement was taken. No weight measurement signal from the feed vessel.

Later, various troubles made us stop the cultivation. For instance: the nitrogen gas tube was emptied long before what was calculated and this happened twice. The computer went down due to problems with the disc dx0.

1) Duration of the batch = 14 h.

2) Pause before the start of the fed-batch = 4.0 h.

3) The pH probe was calibrated 4.7 was actually 5.7.
No NaOH dosage during the batch phase.

4) Time ≈ 1 h.
The regulator was turned on (note book is not clear).

5) Time 2-2.5 h.
Manual control during 20 min here.

6) Time 4 h.
K=0.0042, Ti=1500, Td=213, N=3.

7) Time 6.5 h.
The computer halted because ref voltage equipment was installed at the same current source (!). About 20 min later the regulator was on.

8) Time 8.5 h.
Start of PRBS sequence 0.45±0.15 g/L and min period 4 min (8 samples).
K=0.0030, Ti=1300, Td=200, N=3.

9) Time 10.5 h.
During the PRBS experiment the tubing for nitrogen gas to the MGS-1 system jumped off! However, Jan Peter happened to be around and fixed it within 3 min. During this time the regulator was set in manual mode and after this 3 min the regulator was turned on (sample number 254 in the PRBS sequence).

10) Time 12.25 h.
The feed pump was tested: signal 2.4 mL/min was 2.11 when measured.

11) Time 12.5 h.
The feed rate was set at a constant value during the night in order to "save" the cultivation for students next day.

12) Time ≈ 21 h.
The nitrogen gas tube was found empty and changed.

13) Time ≈ 28 h.
The feed tubing had ruptured. Later troubles with the disc dx0 in the computer.

Utbyte och tillväxt under fedbatch vecka: 8609 ρ(feed) =

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	ρ∫ Fdt	∫NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	2350	-1¹⁰	2,09				0		0,000	0,000	4,000			
2	0100	0⁰	—						0,000	-0,030				
3	0200	0100					28,5		0,020	-0,030				
										-0,060				

Fed-batch cultivation - week 8613

Lena Nielsen was at charge. This cultivation was not completed according to the schedule. The batch took 4 hours longer time than normally. To much anti-foam was probably added before the media was inocculated and this could be a reason for the slow growth in the batch. A dark brown precipitation could be seen on the fermentor walls. Another reason for the slow growth during the batch phase could be that the inocculum was overaged. The media was muddy, however microscope study did not reveal any foreign organisms in the culture. The cell conc at start was only 2.0 g/l and to much sugar in the media was not the cause (which it was cultivations: 8528, 8529 and 8532).

1) Duration of the batch = 17 h. Batch media contained 40 g molasses.

2) Pause before the start of the fed-batch = 9 h.

Fed-batch cultivation - week 8617

This cultivation was terminated before the fed-batch was completed. The main reason being that the feed tubing ruptured. During these 14 h of the fed-batch the growth rate was very high and the yield was reasonable. The ethanol probe had an extremly long response time. A later calibration showed at least 12-13 min response time. There was minor fluctuations in the rate of feed around the critical point. This because the regulator was oscillating and because of the PRBS. Lena Nielsen at charge.

1) Duration of the batch = 14 h.

2) Pause before the start of the fed-batch = 2.0 h.

3) Average growth rate μ = 0.243 h^{-1} during 14 h.

4) Overall yield Y = 0.261 g(yeast)/g(molasses II).

5) Maximal feed rate (in steady state) = 4.5 mL/min. Transient 6.0 mL/min. Good stability of the pump up to 14 h of cultivation. Drift at the higher feed rates that was set manually.

6) Dosage of NaOH = 2.75 mL/g(yeast). pH(Batch start) = 5.5;
pH(FB start) = 5.1; No NaOH dosage during batch.
The ethanol signal drifted 1.0 V during the batch.

7) Time 1 h.
The regulator was turned on even though it oscillated. Regulator parameters were: K=0.0020, Ti=2000, Td=500, N=3.

8) Time 2.8 h.
K=0.0015, Ti=2000, Td=700, N=3.

9) Time 3 h.
K=0.0015, Ti=2500, Td=900, N=3.

10) Time 3.2 h.
K=0.0010, Ti=2500, Td=900, N=3.

11) Time 4 h.
Start PRBS.

12) Time 9 h.
Start PRBS.

13) Time 11.3 h.
The pH-value decreased to 4.40 due to absence of NaOH.

14) Time 13.2 h.
Drift of the feed pump because the tubing worn out. Pump signal 3.2 mL/min while measured value 2.6 mL/min. Regulator parameters were changed: K=0.0020, Ti=1500, Td=500, N=3. The pump for NaOH was changed.

15) Time 14.2 h.
The tube for the feed ruptured and was mended in 10 min. The feed rate was controlled manually.

16) Time 15.5 h.
The tube ruptured again and was mended in 10 min. During this period the feed rate was zero. The ethanol signal went down. A drift in the signal could be the reason for measured value 0.2 g/L. The regulator was turned on.

17) Time 16.8 h.
K=0.0020, Ti=1500, Td=400, N=3.
Feed rate zero for 5 min.

18) Time 17.5 h.
The tube ruptured and the the experiment was terminated.

Utbyte och tillväxt under fedbatch vecka: 8617

ρ(feed) = 1,32

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	ρ∫ Fdt	∫NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	[illegible]	0	[illegible]	[illegible]	7,84	7,84	0	0	0	0	[illegible]	-	-	
2	[illegible]	[illegible]	[illegible]	[illegible]			[illegible]	[illegible]	[illegible]	[illegible]	4,391	0,288	0,279	
3	[illegible]	9,5	[illegible]	106,8			560	0,459	0,218	[illegible]	4,617	0,224	0,285	
4	15^{30}	14	42,82	236,1			1392	1,141	0,493	[illegible]	5,514	0,176	0,247	
										14 u	$\bar{\mu}$ = 0,243	$\bar{Y}$ = 0,261		

Fed-batch cultivation - week 8621

This cultivation went through without much trouble. Lena Nielsen was at charge of the lab. The goal of this experiment was to make 3 hours PRBS-identification at start, in the middle and near the end of the cultivation. The first was very good the second was also good but the last PRBS period was a failure. Drift in the ethanol sensor signal (accumulation of acetate?) made the PRBS signal around 0.45±0.15 actually touch the zero level of ethanol as far as one can judge from the dynamic response. (When there is no ethanol, small changes in the feed rate does not change the ethanol concentration). Also interesting DO responses during the second PRBS test.

A remarkable high growth rate combined with a relatively high yield was the outcome of this cultivation. Is this an effect of the on/off switching of the feed rate and a hypothetical consummation of inhibitory substances? Although there still was a drift in the ethanol signal during the last hours of fedbatch. A test afterwards indicated almost no drift!

During the preparation a couple of things ought to be mentioned here. The ethanol sensor was improved since last time. Time delay 90 s and time constant xxx s. After sterilization of the fermentor some (about one third) of the KCl (2M) in the pH electrode was found to have disapeard. It might have vapoured during sterilization, but then the Al-folie at the top of the electrode used to have a grey precipitation. It is more likely that the KCl had come in to the fermentor. The pH electrode was later changed. The last two cultivations we have had trouble with the feed pump. The tubing had broken a couple of times. Before start of this cultivation the wheels of the pump was thoroughly cleaned and some moisture was given that they should rotate easily.

It is also worth noticing that this was the last cultivation with molasses II. The dry weight measurement was found very light?

1) Duration of the batch = 13 h.

2) Pause before start of the fed-batch = 2.0 h.

3) Average growth rate μ = 0.221 h^{-1} during 17 hours.

4) Overall yield Y = 0.281 g(yeast)/g(molasses II).

5) Maximal feed rate = 4.2 mL/min. Pump gain decreased gradually during the latter half of the cultivation. Drift about 40 %.

6) Dosage of NaOH = 1.15 mL/g(yeast); pH_{Bstart} = 5.65; $pH_{FBstart}$ = 5.19. No dosage of NaOH during the batch phase.

7) Time 1.15 h.
The regulator was turned on. K=0.0020, Ti=2000, Td=500, N=3.

8) Time 3.00 h.
Start of PRBS sequence. E=0.45±0.05 g/L. It lasts for exactly 3 hours. Constant RPM.

9) Time 7.30 h.
Stop and restart of the computer.

10) Time 7.40 h.
The computer is running again.

11) Time 11.00 h.
Start of PRBS sequence. E=0.45±0.15 g/L. Regulator: K=0.0040, Ti=1300, Td=180, N=3. To much damping was found and the derivative part was tuned a little the hours before. Constant RPM. The VX and V was set manually in order to compensate for the high growth rate.

12) Time 14.30 h.
Something happens with the pump?

13) Time 15.00 h.
Start of PRBS sequence. E=0.45±0.15 g/L. The system is not in equilibria. Judging from the little response of the changes in the substrate feeding, there is no ethanol in the fermentor at this point!

14) Time 16.50 h. The fed-batch is finished.

0,628

Utbyte och tillväxt under fedbatch vecka: 8621 ρ(feed) = 1,22

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	$\rho\int$ Fdt	$\int$NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	00 15	0 00	2,51	10,04	10,04	10,04	0,0		0,000	0	4,000	–	–	
2	02 15	2 00	3,63	14,58	15,6	18,0	45,2		0,010	-0,030	4,017	0,187	0,160	
3	03 15	3 00	4,61	18,51	19,5	21,8	66,5		0,020	-0,060	4,014	0,239	0,294	
4	06 15	6 00	9,31	38,39	37,8	40,9	175		0,070	-0,090	4,123	0,243	0,292	
5	11 15	11 00	29,7	141,5	114,1	141,9	748		0,270	-0,120	4,763	0,261	0,287	
6	14 15	14 00	48,0	259,7	221,5	261,5	1426		0,392	-0,150	5,411	0,202	0,278	
7	17 05	16 50	67,2	414,2	414,2	414,2	2292		0,465	-0,180	6,164	0,165	0,284	
										=17L	μ=	0,221	0,281	
										2-17L				

Fed-batch cultivation - week 8622

Lena Nielssen was at charge during the cultivation. This cultivation went through without much trouble. However, during calibration of the ethanol sensor the response time was found 12 min instead of about 6 min. Carl Fredrik made some adjustments and previous response time was obtained. The work with the sensor was done during the batch phase of the cultivation and the probe was autoclaved another time and put into the fermentor.

The goal of this experimental week was to do dynamic experiments at different cell concentrations in order ot get data for test of model of the ethanol dynamics. The data from week 8609 and 8621 is also useful, however during this experiment it was decided to have a shortest period 6 min instead of 4 min. The DO level was high during the different test periods here. These data seems to be very useful. It is interesting to see that the average growth rate during the dynamical test periods were a little higher than if the ethanol signal had been kept constant. Loss in yield was not apparent.

A few different remarks ought to be done here. During the autoclaving of the fermentor there was over pressure due to a closed tubing. Could have been dangerous! Molasse from a new plastic container was used, nr III. There were also the standard problem with the wet filter for air out flow and this results in a slight over-pressure in the fermentor. Leakage in the anti-foam tubing was also found during the last hours of the cultivation.

1) Duration of the batch = 13 h.

2) Pause before start of the fed-batch = 2.0 h.

3) Average growth rate μ = 0.222 h^{-1} during 17.5 hours.

4) Overall yield Y = 0.270 g(yeast)/g(molasses III).

5) Maximal feed rate = 4.0 mL/min. The pump was very stable. Neglible drift.

6) Dosage of NaOH = 1.63 mL/g(yeast); pH_{Bstart} = 5.60; $pH_{FBstart}$ = 5.12. Dosage of NaOH during the batch phase = 7 mL.

7) Time 0.50 h
The regulator was turned on with parameters:
K=0.0020 Ti=2000 Td=500 N=3

8) Time 2.40 h.
The ethanol reaches steady state at 0.45 g/L.

9) Time 3.00 h.
Start of PRBS 0.45±0.05 g/L. Shortest period is 12 sample, i.e. 6 min.
K=0.0030 Ti=1300 Td=500 N=3.

10) Time 8.0 h.
Stop and restart of the computer because a PRBS sequence with another amplitude will be used soon. The procedure took about 10 min. The feed rate was controlled by a ref voltage.

11) Time 9.0 h.
Start of PRBS 0.45±0.15 g/L. Shortest period is 12 sample, i.e. 6 min.
K=0.0030 Ti=1300 Td=200 N=3.

12) Time 13.4 h.
Stop and restart of the computer. The procedure took 15 min.

13) Time 14.5 h.
Start of PRBS 0.65±0.15 g/L. Shortest period is 12 sample, i.e. 6 min.
K=0.0030 Ti=1300 Td=200 N=3.

14) Time 16 h.
Leakage in the antifoam tubing. Antifoam was further on given manually.
The air filter was also found wet and was changed.

15) Time 17.5 h.
The pump was turned off.
The ethanol sensor was later calibrated.

Utbyte och tillväxt under fedbatch vecka: 8622 ρ(feed) = 1.22

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	ρ∫ Fdt	∫NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	0000	0	2.04	8.09			00		0.007	-0.040	3967			
2	0300	3	4.00	16.1			66	0.054	0.032	-0.070	4.016	0.229	0.193	
3	0800	8	13.4	57.3			301	0.247	0.127	-0.100	4.274	0.254	0.279	
4	0925	9.4	18.7	82.6			446	0.366	0.179	-0.130	4.415	0.261	0.278	
5	1100	11	25.5	118.2			656	0.538	0.256	-0.160	4.634	0.224	0.270	
6	1400	14	40.2	210.8			1203	0.986	0.447	-0.190	5.243	0.192	0.270	
7	1600	16	50.5	294.0			1746	1.431	0.611	-0.220	5.822	0.166	0.244	
8	1730	17.5	62.5	389.5			2252	1.846	0.636	-0.250	6.232	0.188	0.300	
											17,5 L	0,222	0,270	

Fed-batch cultivation - week 8628

Lena Nielsen was at charge during this cultivation, however Jan Peter was present during start-up and most of the fed-batch part of the cultivation. The last few hours and shut down was done by Lena. The inocula was new made. This time the batch took only 12 h. Before start there was some trouble with the MGS, having to long a response time. The system was adjusted and recalibrated. The response time was now 6-7 min, corresponding to a time delay of 120-140 s and a time constant of 120-130 s. There was some difficulties when the probe was mounted in the fermentor before start of the batch.

The fed-batch was done at low DO levels from the start. The goal was this week to do PRBS test at low DO concentrations and see if there was any change in the ethanol dynamics. However, the dynamics seemed to have changed so much that it was difficult to have a well damped closed loop. The ethanol signal kept oscillating although the regulator gain was decreased from 0.0030 to 0.0010. The PRBS sequence was never started. Acetaldehyd was measured using an enzyme-kit. None was found. Acetaldehyd has boiling point at about 20° C. Not much is likely in the fermentor!?

1) Duration of the batch = 12 h (new inocula?).

2) Pause before start of the fed-batch = 3.0 h.

3) Average growth rate μ = 0.203 h^{-1} during 16 hours.

4) Overall yield Y = 0.282 g(yeast)/g(molasses III).

5) Maximal feed rate = 4.0 mL/min. Good stability up to 14 h. At the higher feed rates the obtained feed rate was about 15 % lower.

6) Dosage of NaOH = 1.88 mL/g(yeast); pH(B start) = 5.30;
pH(FB start) = 4.86. No dosage of NaOH during the batch phase.

7) Time 1.0 h.
Start of the PID regulator. K=0.0020 Ti=2000 Td=500 N=3

8) Time 3.25 h.
K=0.015 Ti=2000 Td=600 N=3

9) Time 4.25 h.
The stirrer speed was increased in order to obtain a higher DO level and in this way stabilize the ethanol loop. Not much improvement!

10) Time 7.00 h.
K=0.0010 Ti=2000 Td=750 N=3

11) Time 8.25 h.
Increase in stirrer speed in order to get a higher DO level and in this way stabilize the ethanol loop!? A clear improvement was obtained!

12) Time 13 h.
Restart of the computer in order to load appropriate PRBS sequence. However, the ethanol loop was now very sensitive and stability was lost here. The restart operation took about 20 min.

13) Time 14.5 h.
Manual control.

Metanhalt: 0,628

Utbyte och tillväxt under fedbatch vecka: 8628 p(feed) = 1,22

Nr	Tid	Tid FB	X g/l	VX(TS) g	VX(μ) g	VX(Y) g	Feed g	ρ∫ Fdt	∫NaOH	Uttag	V totalt	μ(TS)	Y(X/S)	
1	8^{00}	0^{00}	2,41	9,64	9,64	9,64	0,0		0,0	- 0	4,000			
2	10^{05}	2^{05}	3,45	13,8	14,7	18,1	47,5		0,015	-0,040	4,014	0,172	0,139	
3	14^{00}	6^{00}	8,02	32,7	32,6	35,5	146		0,055	-0,100	4,075	0,220	0,306	
4	16^{30}	8^{30}	12,5	52,1	54,1	54,7	254		0,100	-0,140	4,168	0,186	0,286	
5	19^{00}	11^{00}	21,0	92,9	89,8	96,7	491		0,200	-0,180	4,422	0,231	0,274	
6	21^{30}	13^{30}	33,3	159,6	149,2	160,4	≈850		0,315	-0,220	4,792	0,216	0,296	
7	24^{00}	16^{00}	46,7	247,8	247,8	247,8	1343		0,465	-0,260	5,306	0,176	0,285	
											164	0,203	0,282	

Fed-batch cultivation - week 8629

This cultivation went through without much trouble. Lena Nielsen was at charge, Jan Peter made the start-up and Carl Fredrik was the operator during the later half of the cultivation. The cell culture was new since cultivation 8628. During the first hours after start of the fed-batch phase, an electrical disturbance was found in the ethanol signal. The disturbance was not found before filtration, but after filtration! The disturbance was found to be correlated with the on-off level control of a process on the other bench. The disturbance vanished when the electrical current to the on-off control was taken from another source. During the start-up of the fed-batch phase glucose analysis was done every 15 min during 1.5 hours.

The goal for this week was to test influence of abrupt variations in the incoming substrate concentration. The concentration was manually changed between 0.90 and 1.10 of the nominal concentration. In the middle of the cultivation the substrate tubing moved out of position in the pump and the feed rate was zero for 10-15 minutes. The regulator managed to control the situation throughout. The regulator was not turned off during the few minutes work with the pump. Typical for this cultivation was also that the DO concentration was unnecessarily low due to a low stirrer speed during the later half of the cultivation. During the last few hours the stirrer speed was increased and the culture responded in an increase of substrate demand.

1) Duration of the batch = 15 h.

2) Pause before start of the fed-batch = 2.0 h.

3) Average growth rate μ = 0.185 h^{-1} during 18 hours.

4) Overall yield Y $\approx$ 0.25 g(yeast)/g(molasses III).
Variations in the substrate conc were neglected.

5) Maximal feed rate = 5.0 mL/min. A gradual decrease in the pump gain with about 15 % of the nominal value. A high increase in the feed rate the last few hours due to increased stirrer speed.

6) Dosage of NaOH = 1.92 mL/g(yeast); pH_{Bstart} = 5.23;
$pH_{FBstart}$ = 5.11. No dosage of NaOH during the batch phase.

7) Time 0.50 h.
A second substrate pulse was given.

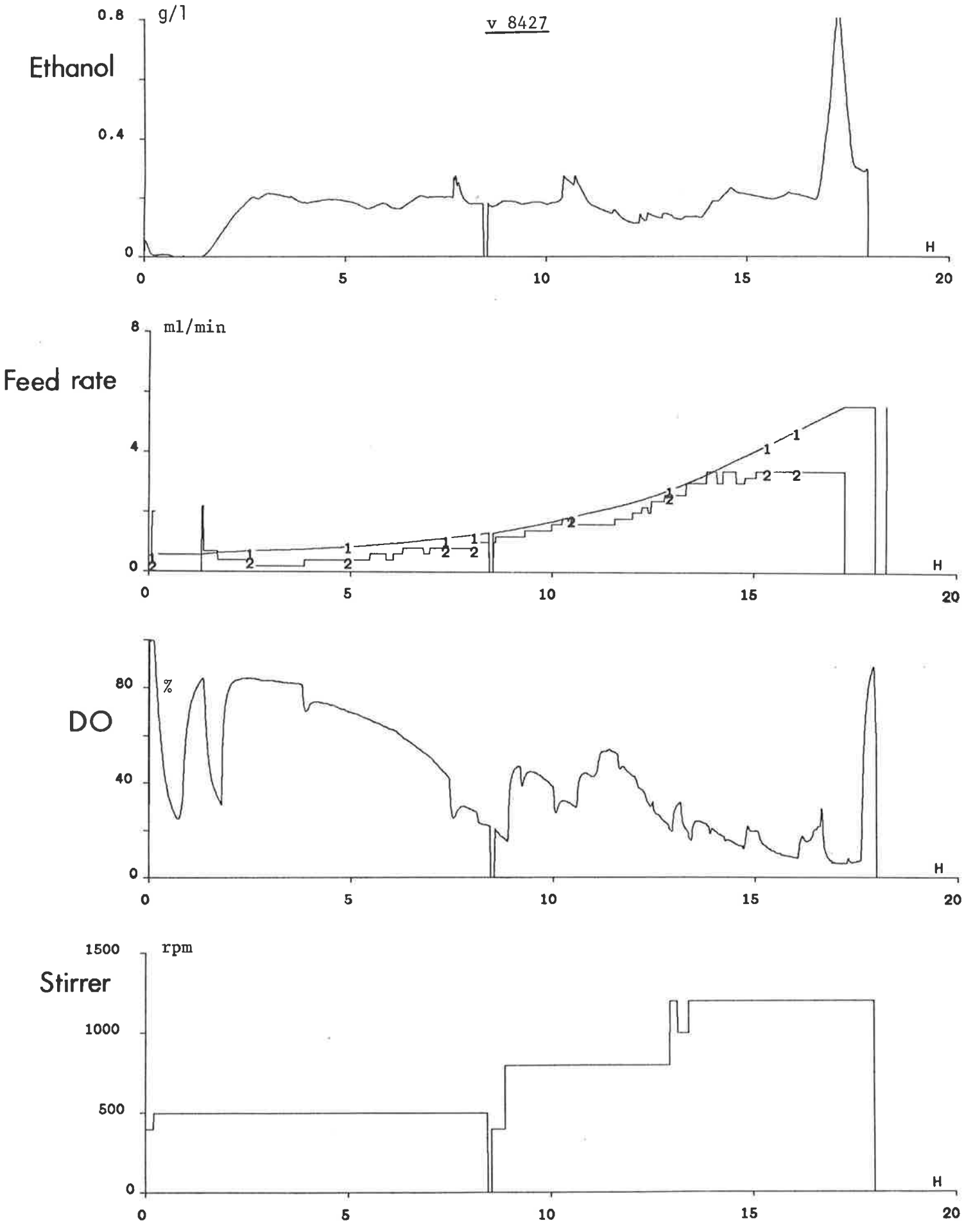
8) Time 1.50 h.
Samples for glucos analysis were done every 15 min. Immediate filtering (15-30 s) and deep freezing were done. The samples were later analysed using an enzym-kit.

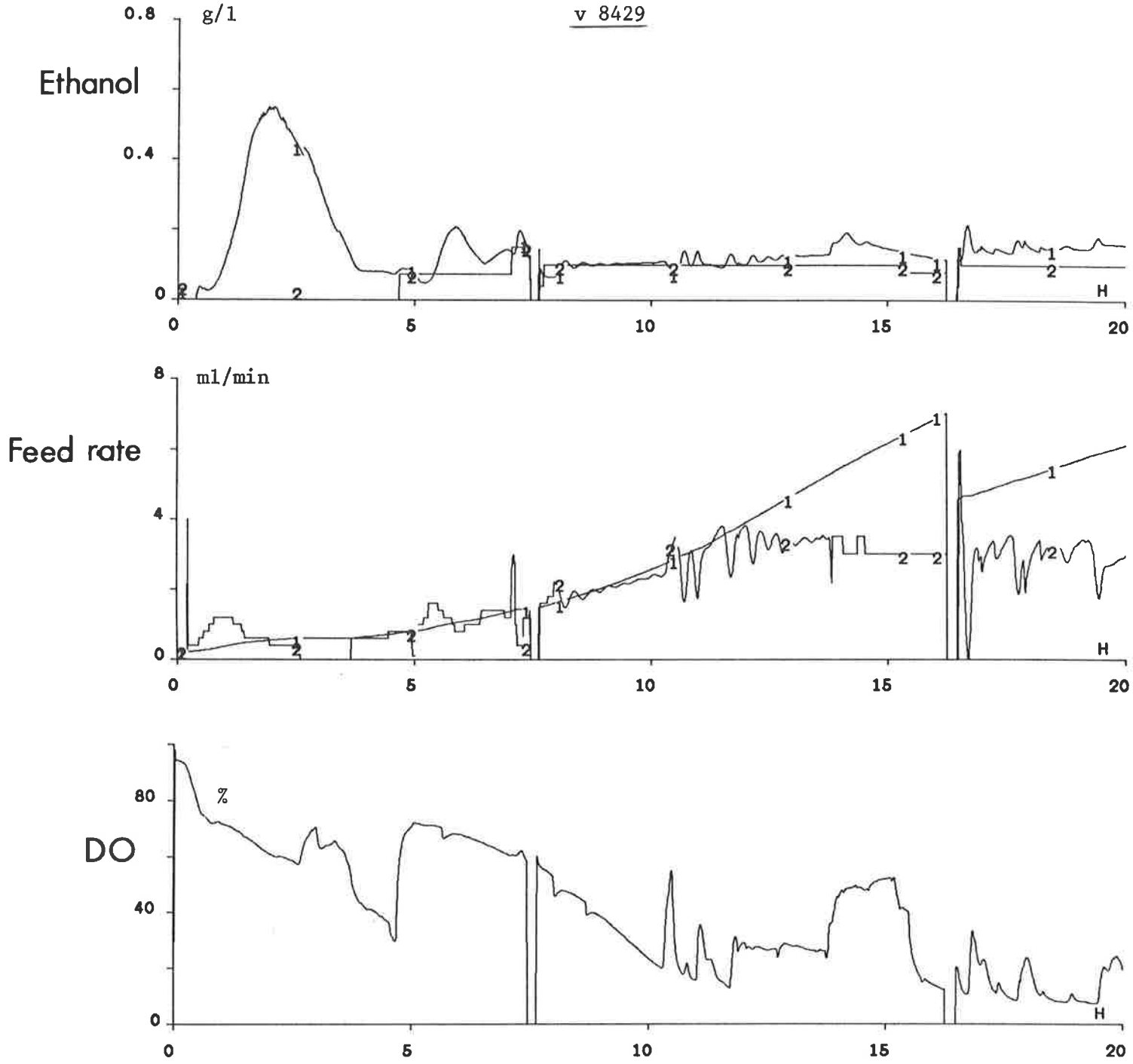
9) Time 2.25 h.
Electrical disturbance on the ethanol signal was eliminated and transients in the ethanol control had vanished.

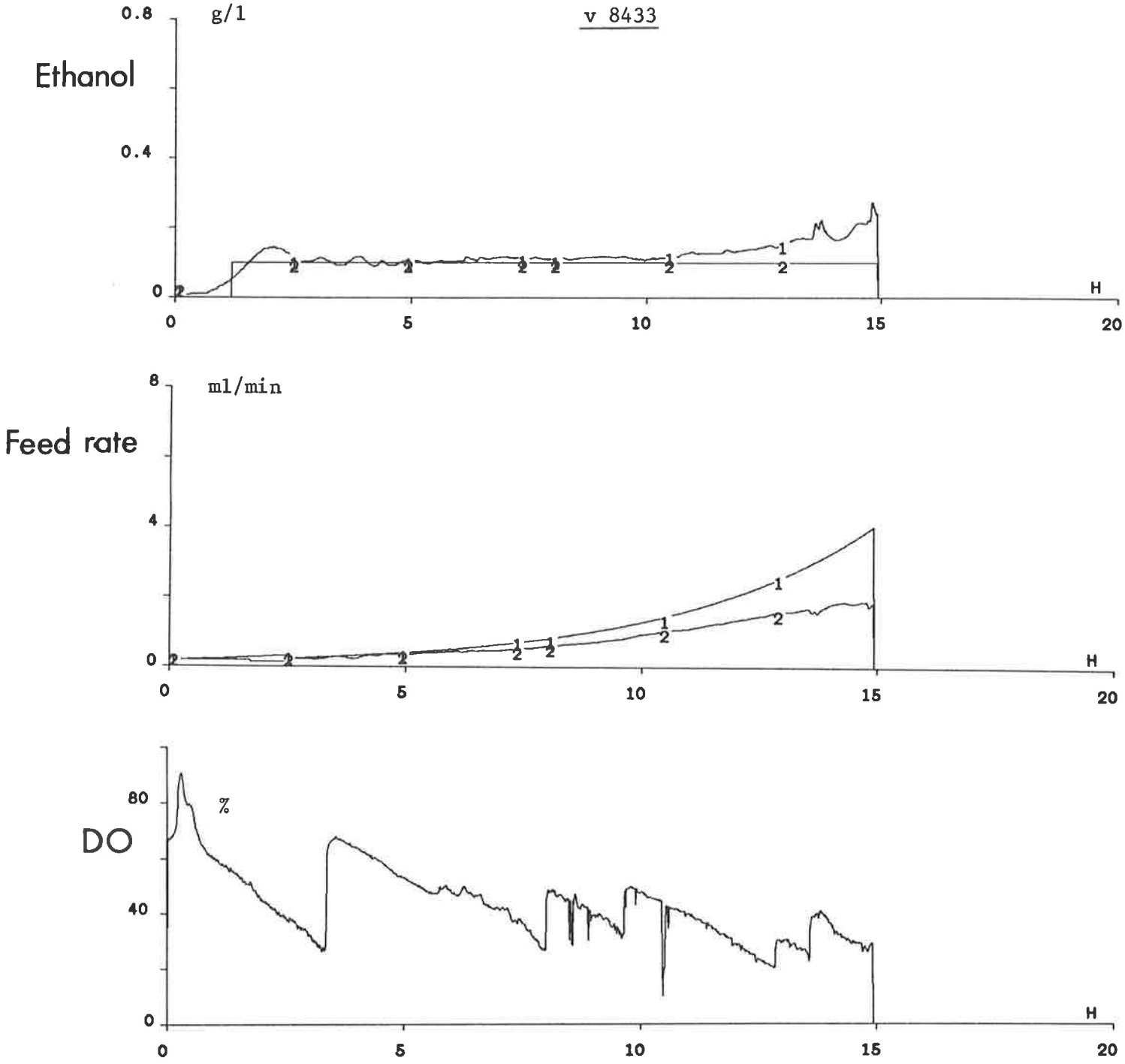
10) Time 9 h.
Trouble with the substrate tubing.

11) Time 16 h.
Unknown disturbance.

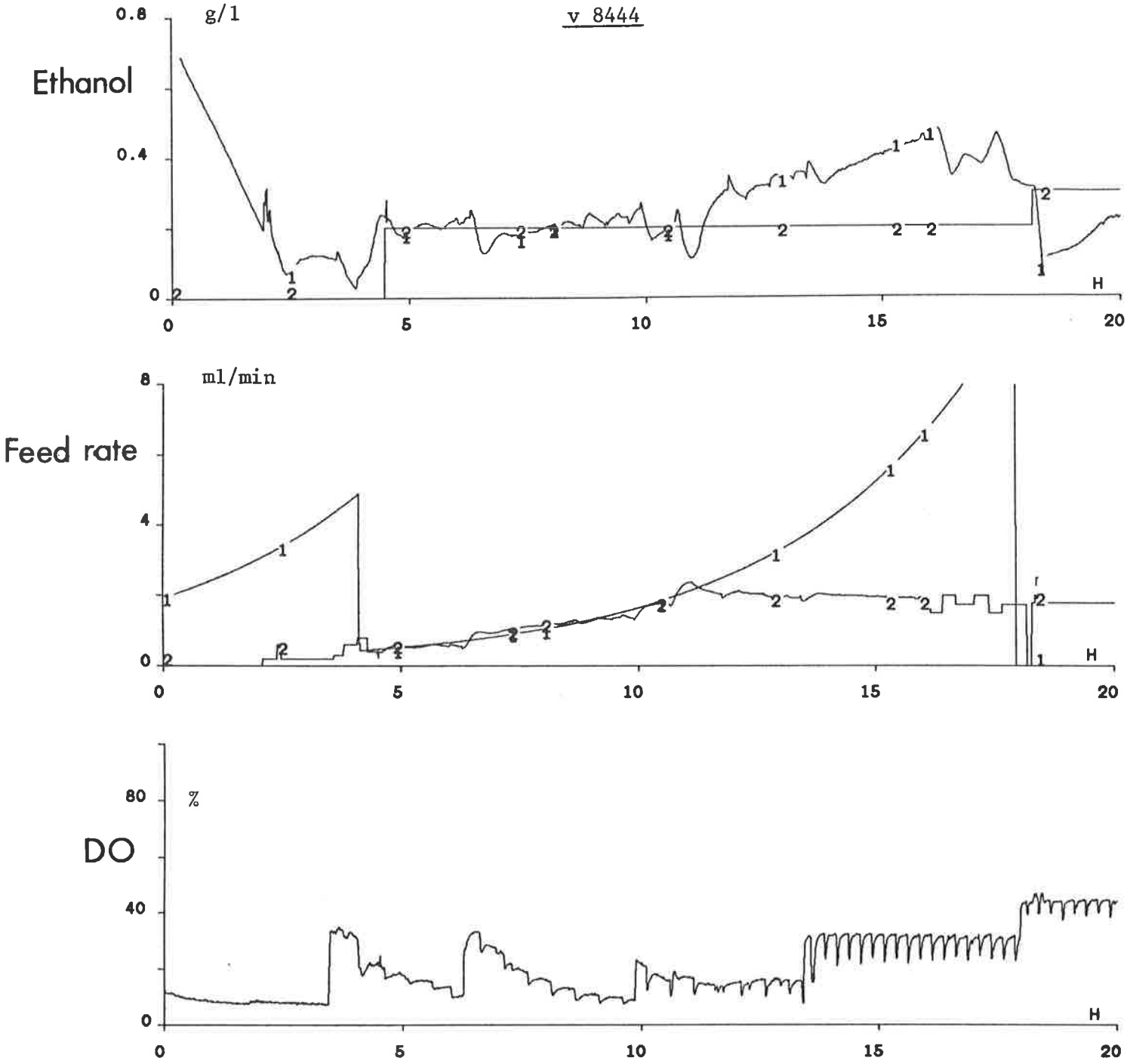
12) Time 18.5-19 h.
Increase in the stirrer speed and suddenly an increase in the DO level.

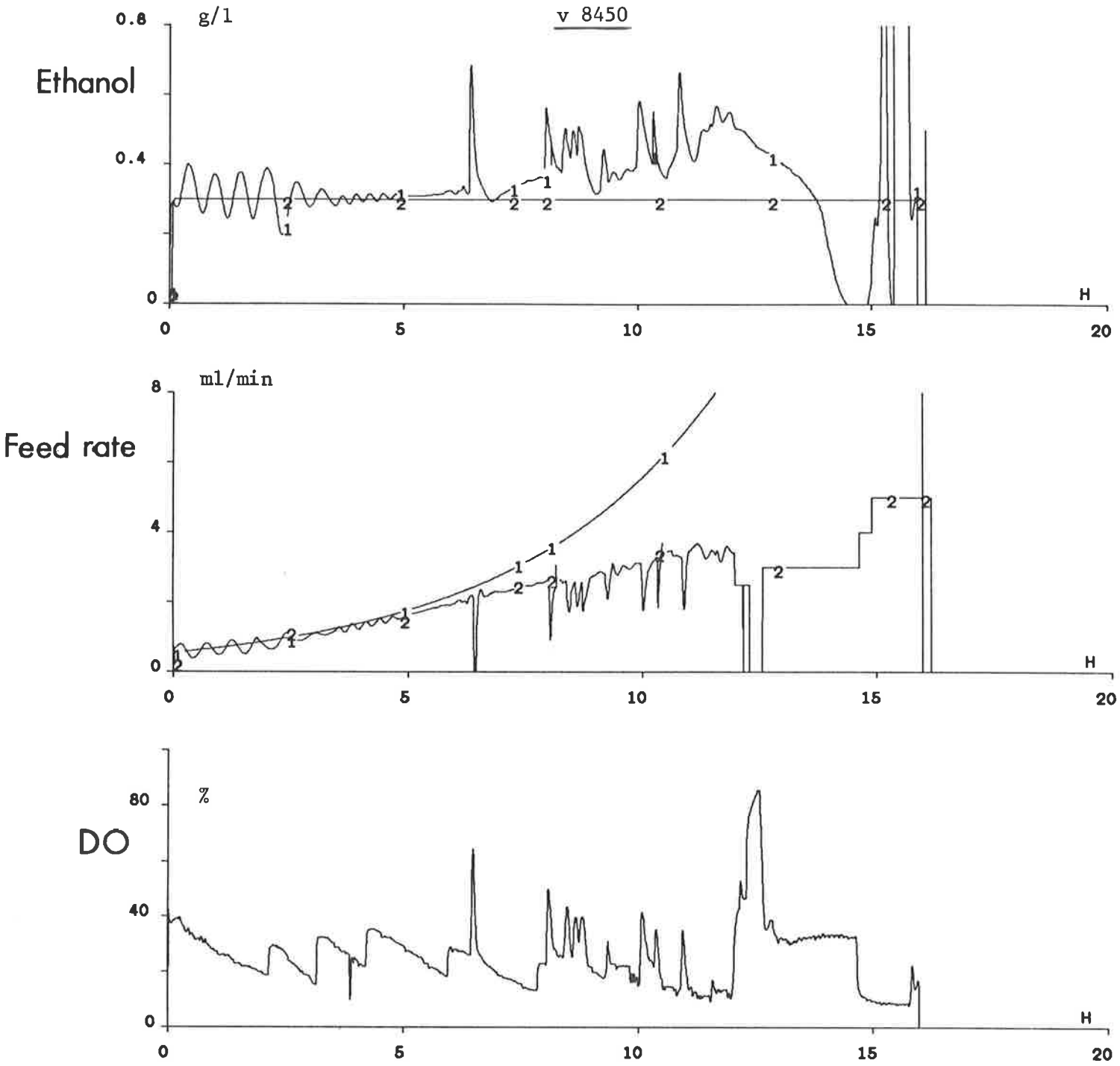

v 8427
Ethanol
g/l
0.8
0.4
0
H
0
5
10
15
20
Feed rate
ml/min
8
4
0
1
2
DO
%
80
40
0
Stirrer
rpm
1500
1000
500

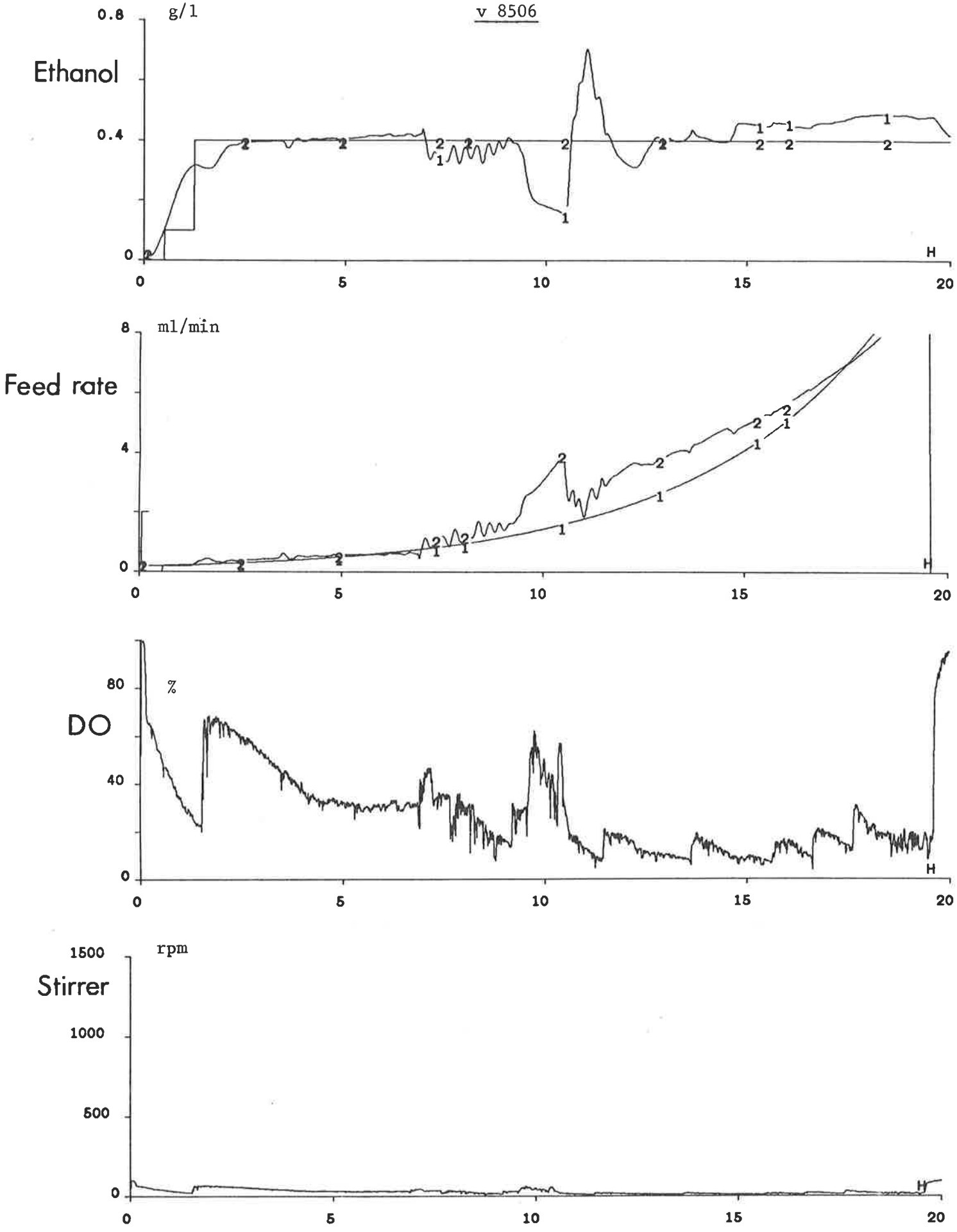

v 8429
Ethanol
g/l
0.8
0.4
0
0
5
10
15
20
H
Feed rate
ml/min
8
4
0
DO
%
80
40
0

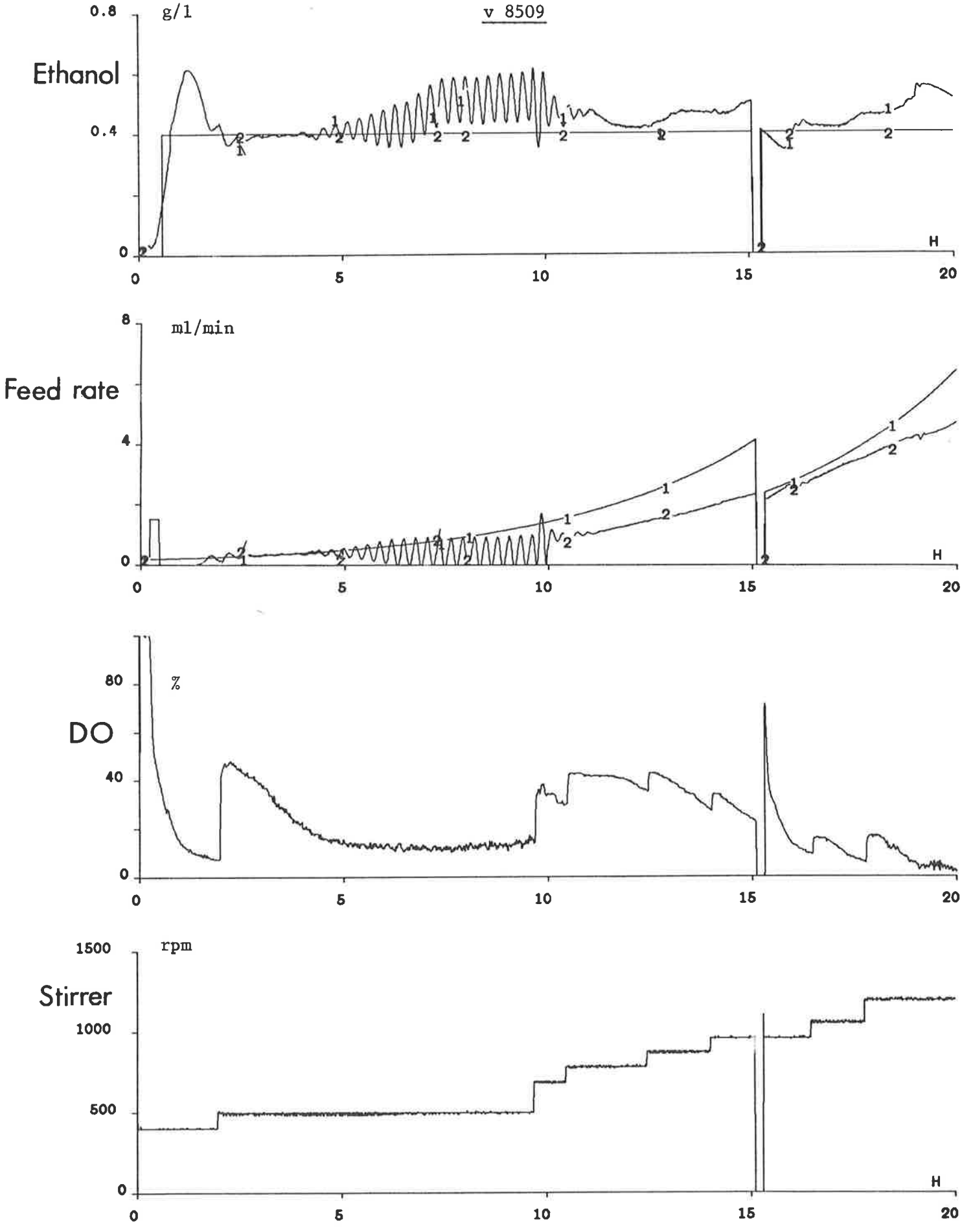

v 8433
Ethanol
g/l
0.8
0.4
0
H
0
5
10
15
20
Feed rate
ml/min
8
4
0
H
0
5
10
15
20
DO
%
80
40
0
H
0
5
10
15
20

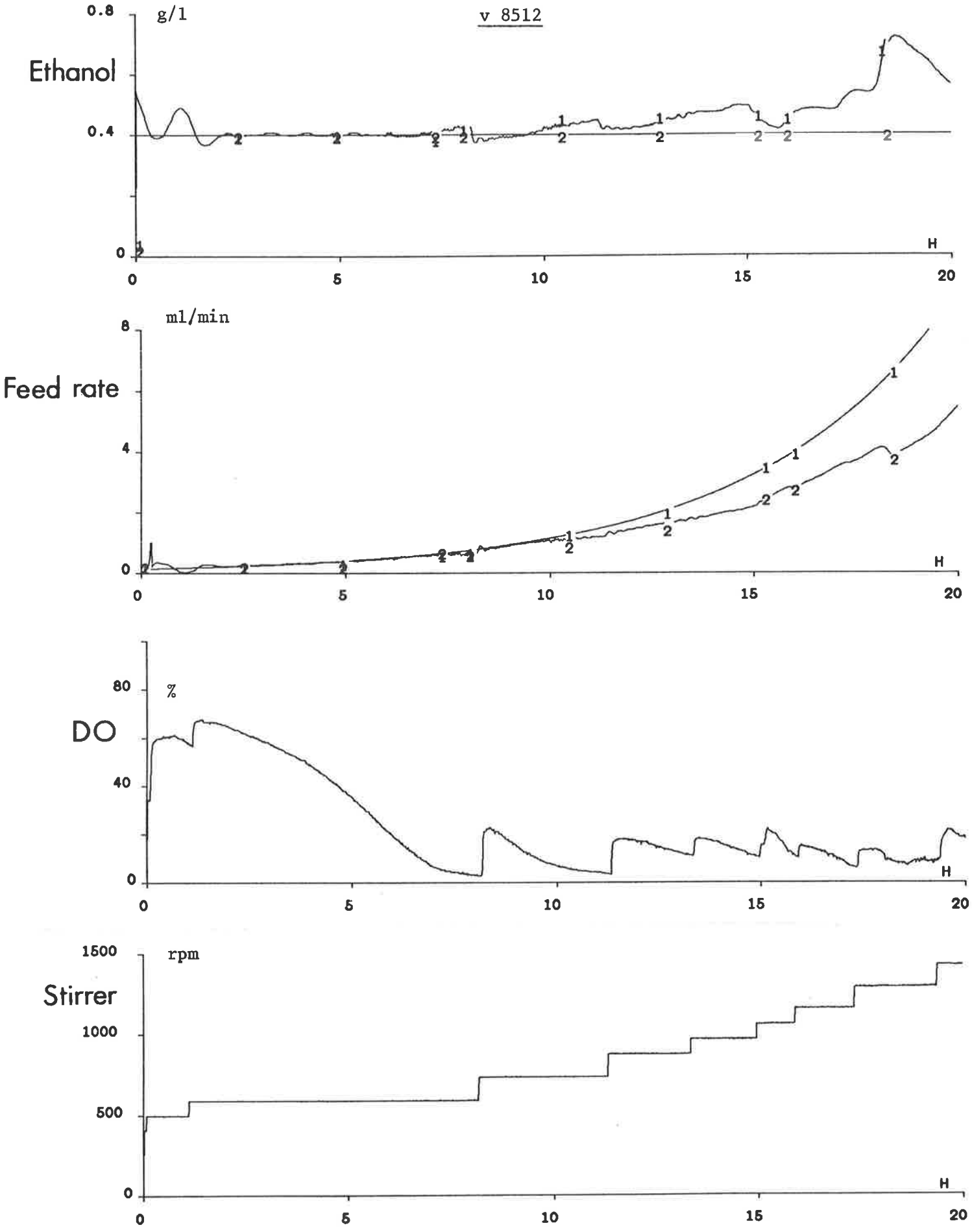
v 8444

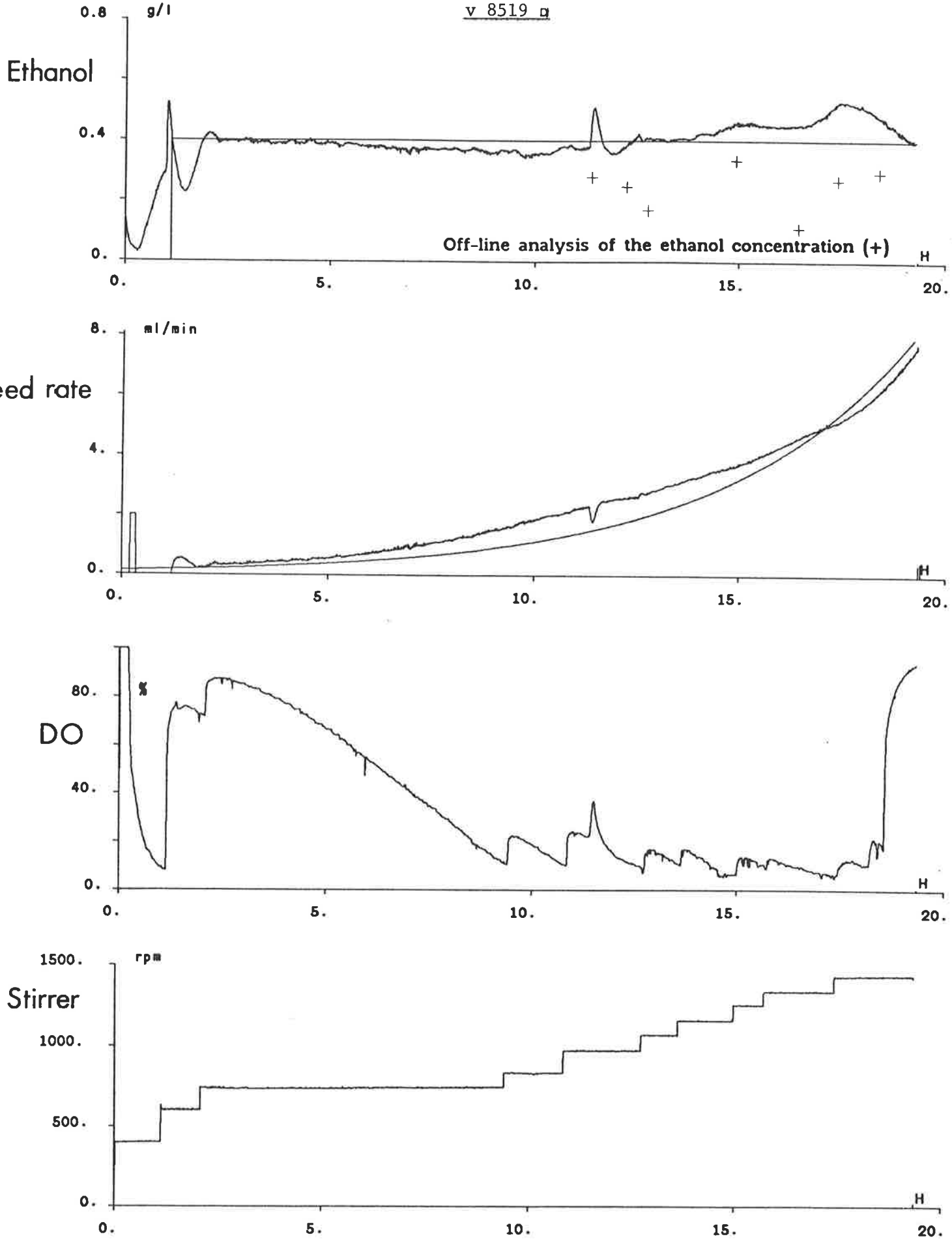

Ethanol
g/l
0.8
0.4
0
H
0
5
10
15
20
Feed rate
ml/min
8
4
0
H
0
5
10
15
20
DO
%
80
40
0
H
0
5
10
15
20

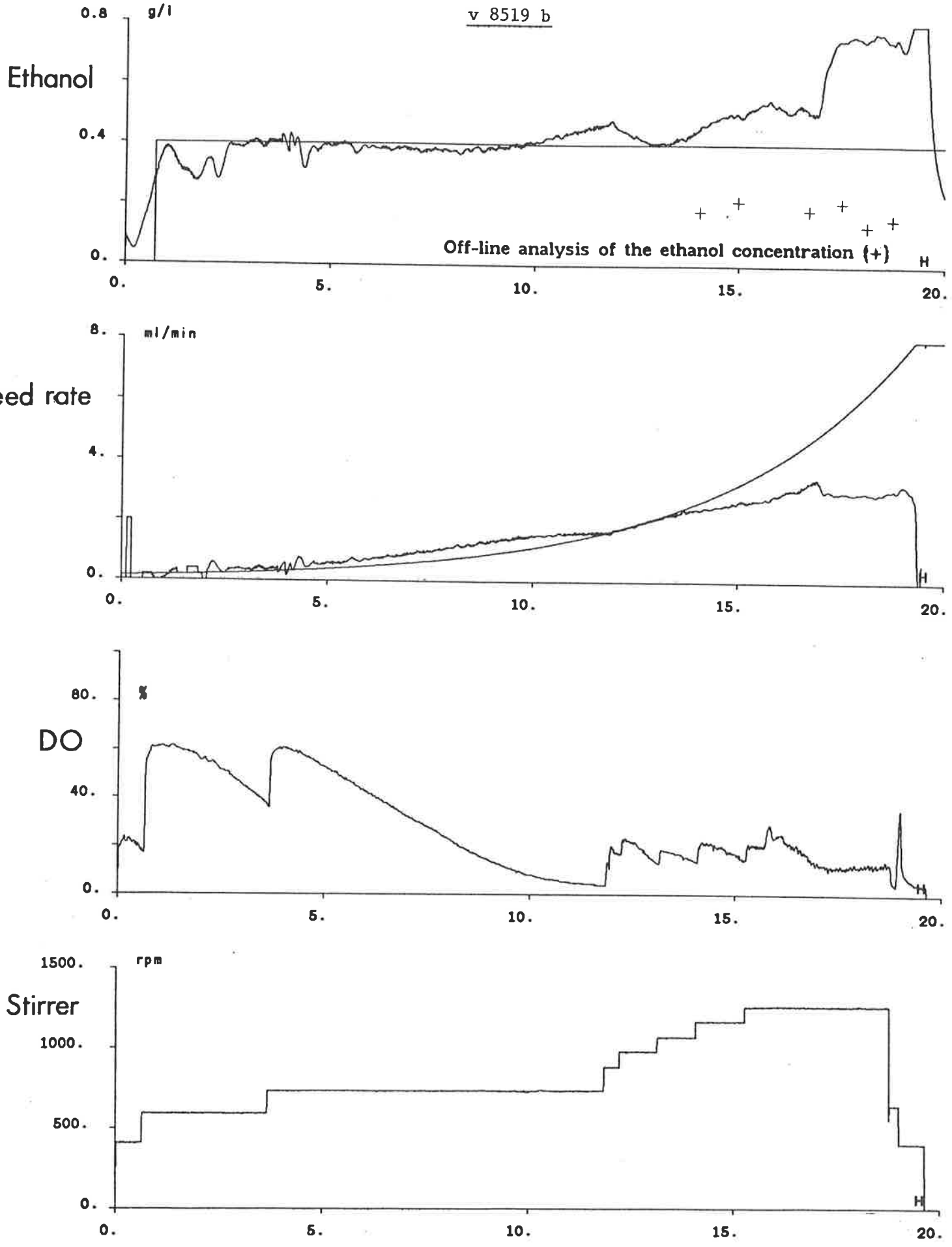

v 8450
Ethanol
g/l
0.8
0.4
0
H
0
5
10
15
20
Feed rate
ml/min
8
4
0
H
0
5
10
15
20
DO
%
80
40
0
H
0
5
10
15
20

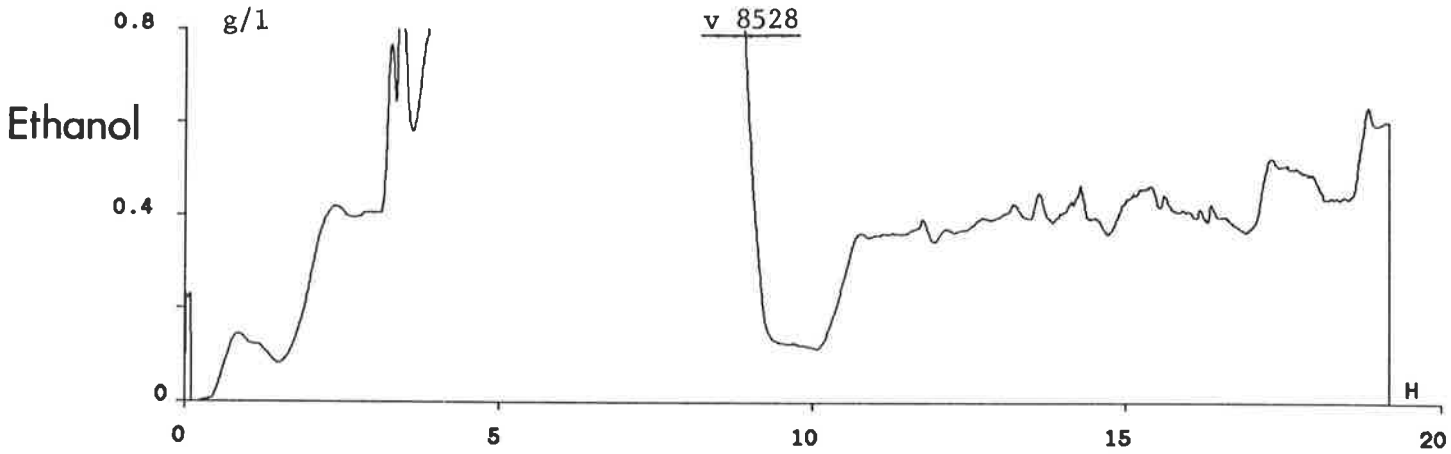

v 8506
Ethanol
g/l
0.8
0.4
0
H
0
5
10
15
20
Feed rate
ml/min
8
4
0
DO
%
80
40
Stirrer
rpm
1500
1000
500


v 8509
Ethanol
g/l
0.8
0.4
0
Feed rate
ml/min
8
4
0
DO
%
80
40
0
Stirrer
rpm
1500
1000
500
0
0
5
10
15
20
H

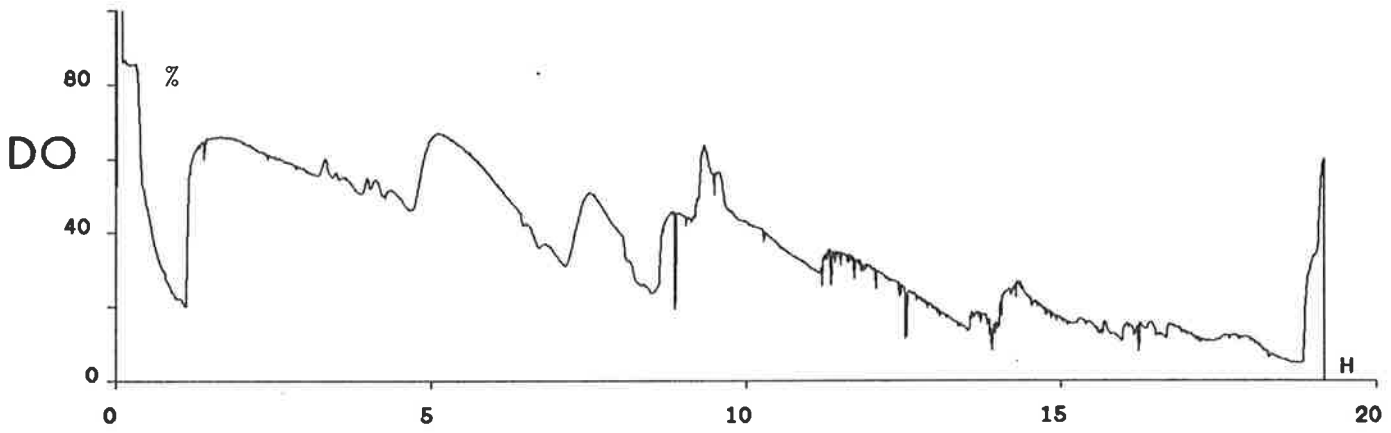

v 8512
Ethanol
g/l
0.8
0.4
0
H
0
5
10
15
20
Feed rate
ml/min
8
4
0
DO
%
80
40
Stirrer
rpm
1500
1000
500

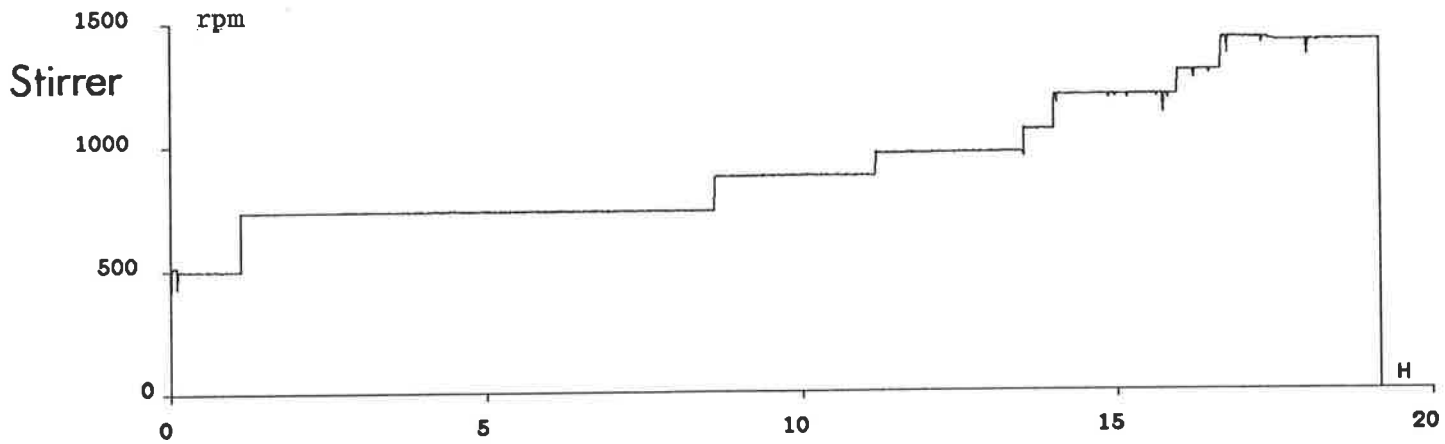

v 8519 a
Ethanol
0.8
g/l
0.4
0.
Off-line analysis of the ethanol concentration (+)
H
0.
5.
10.
15.
20.
Feed rate
8.
ml/min
4.
0.
H
0.
5.
10.
15.
20.
DO
80.
%
40.
0.
H
0.
5.
10.
15.
20.
Stirrer
1500.
rpm
1000.
500.
0.
H
0.
5.
10.
15.
20.

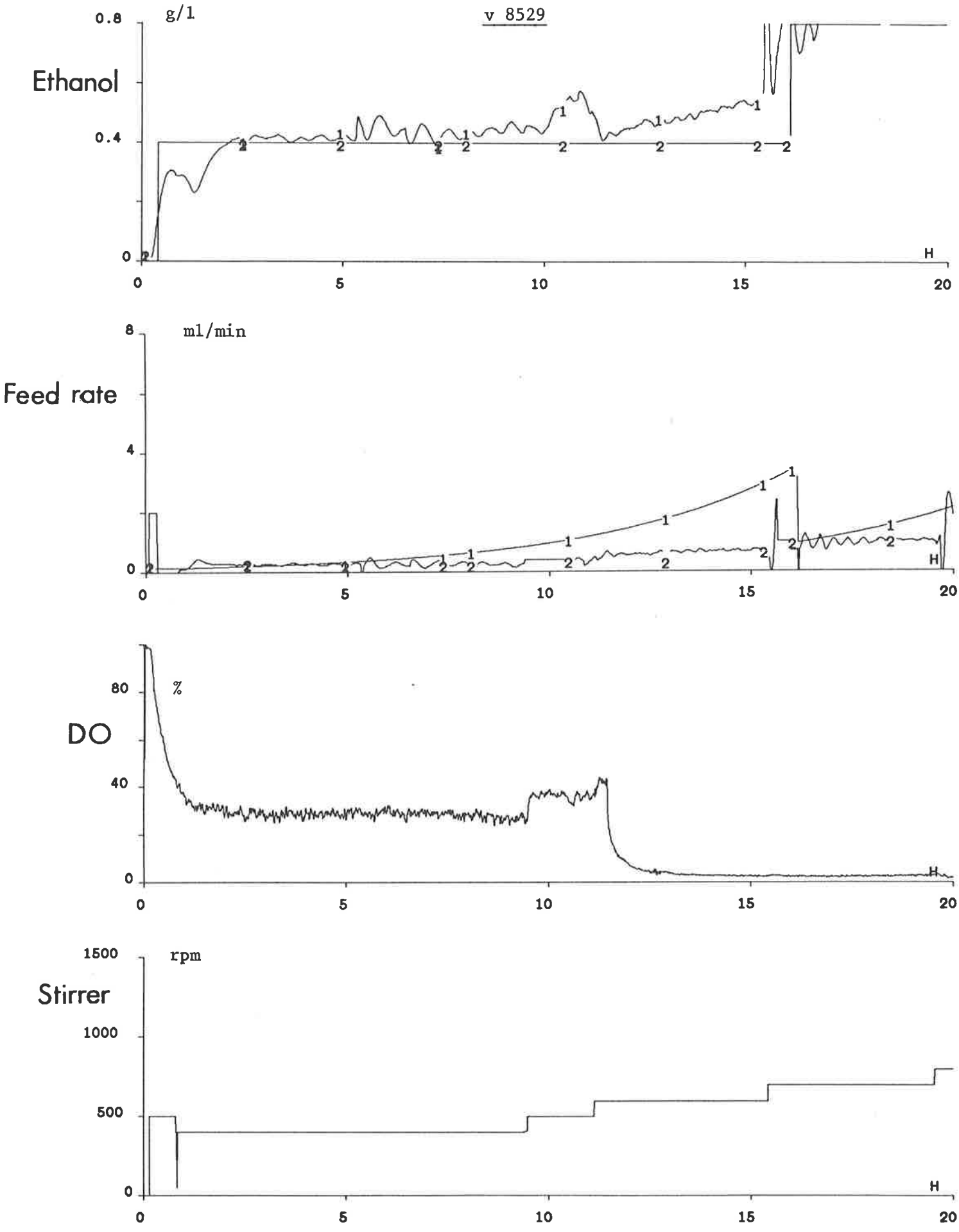

v 8519 b
Ethanol
g/l
0.8
0.4
0.
Off-line analysis of the ethanol concentration (+)
H
0.
5.
10.
15.
20.
Feed rate
ml/min
8.
4.
0.
DO
%
80.
40.
0.
Stirrer
rpm
1500.
1000.
500.
0.

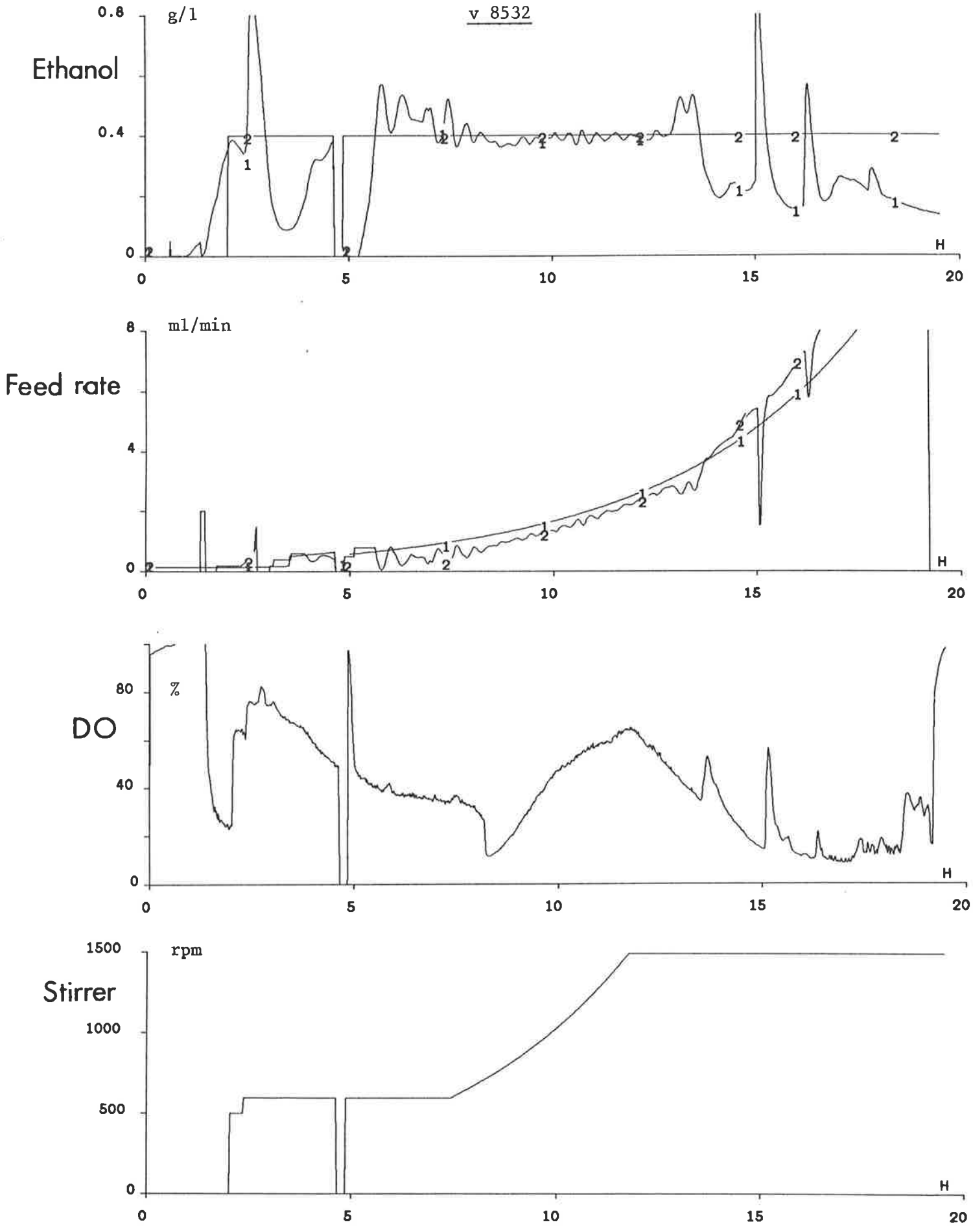

Ethanol
g/l
0.8
0.4
0
v 8528
H
0
5
10
15
20

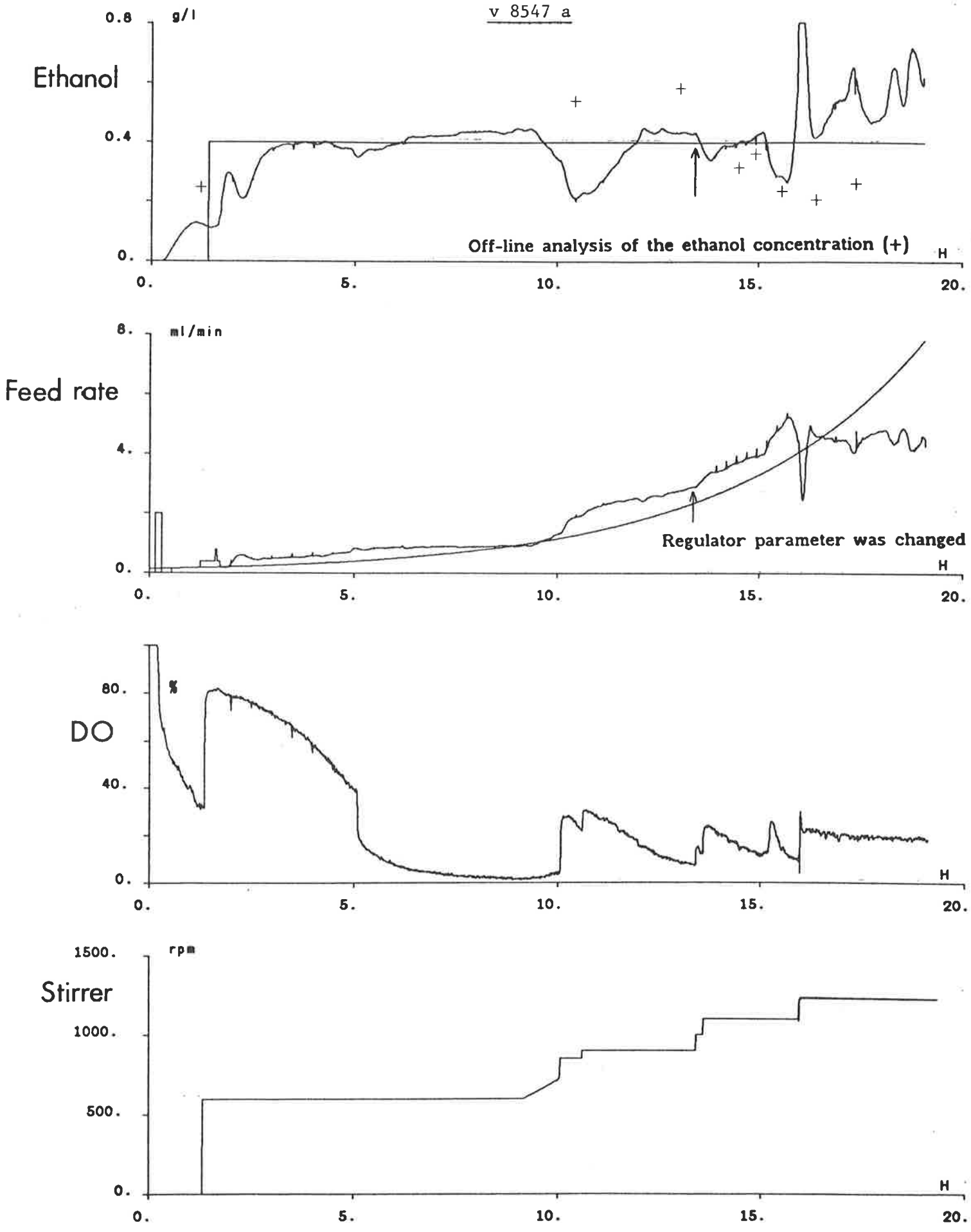
Feed rate


DO
%
80
40
0
H
0
5
10
15
20

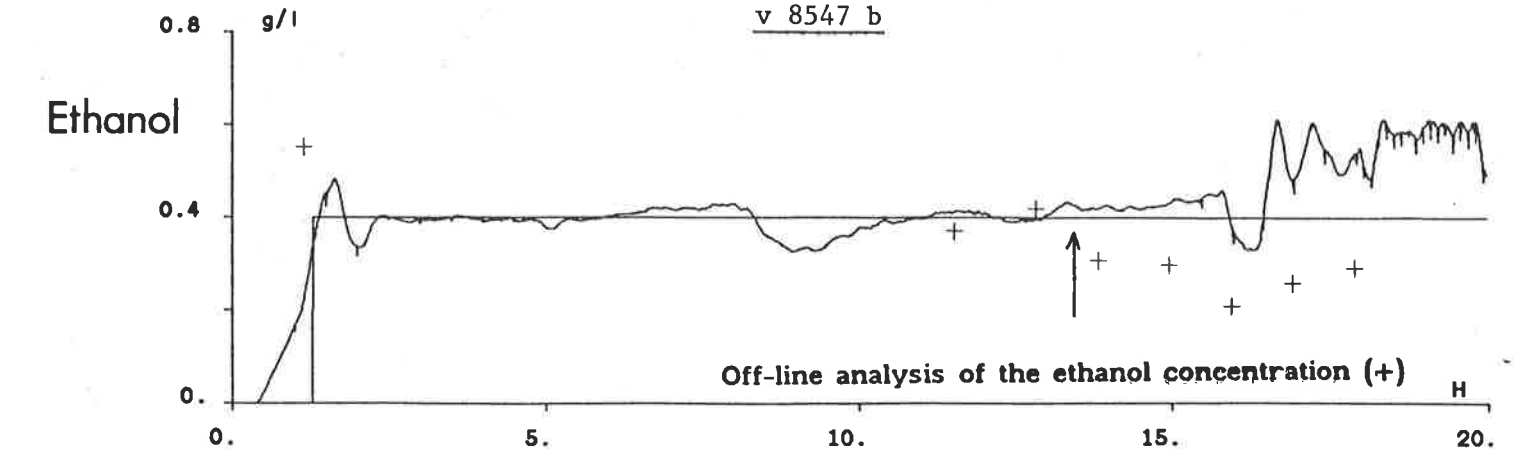

Stirrer
rpm
1500
1000
500
0
H
0
5
10
15
20

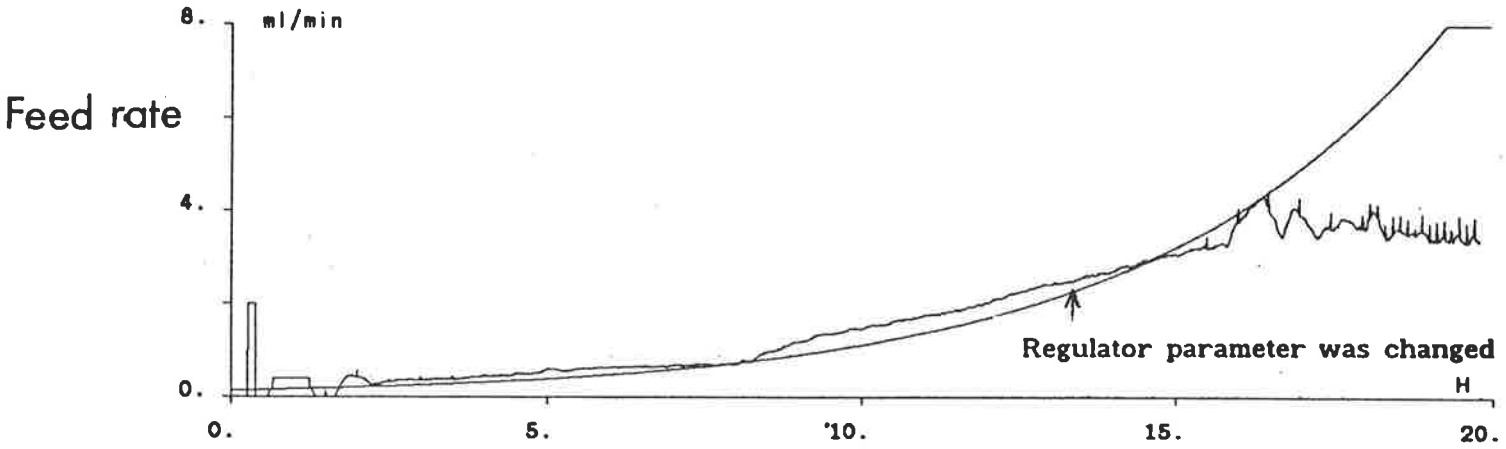

v 8529
Ethanol
g/l
0.8
0.4
0
H
0
5
10
15
20
Feed rate
ml/min
8
4
0
DO
%
80
40
Stirrer
rpm
1500
1000
500

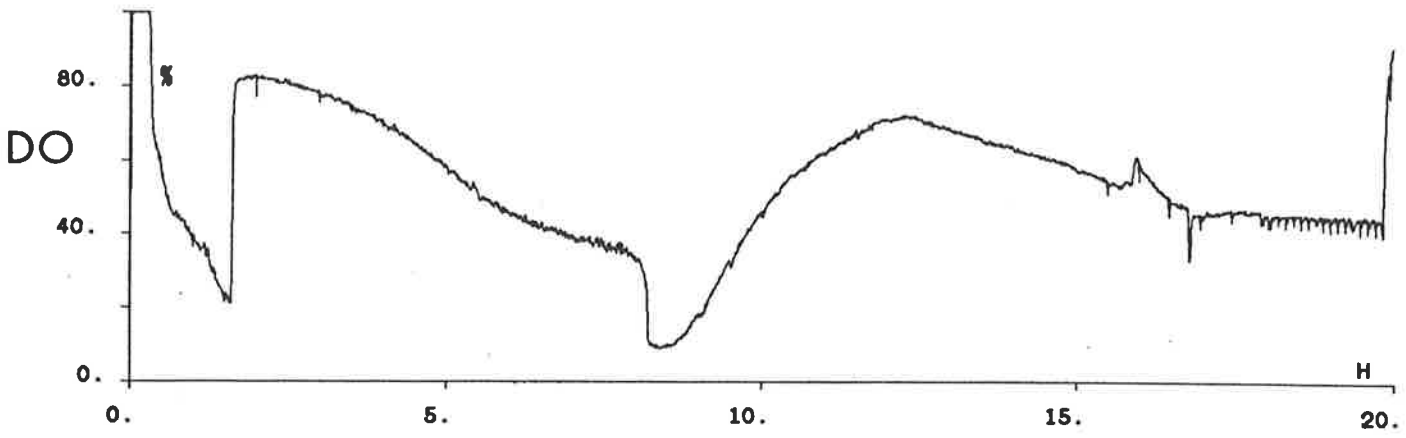

v 8532
Ethanol
g/l
0.8
0.4
0
H
0
5
10
15
20
Feed rate
ml/min
8
4
0
DO
%
80
40
Stirrer
rpm
1500
1000
500

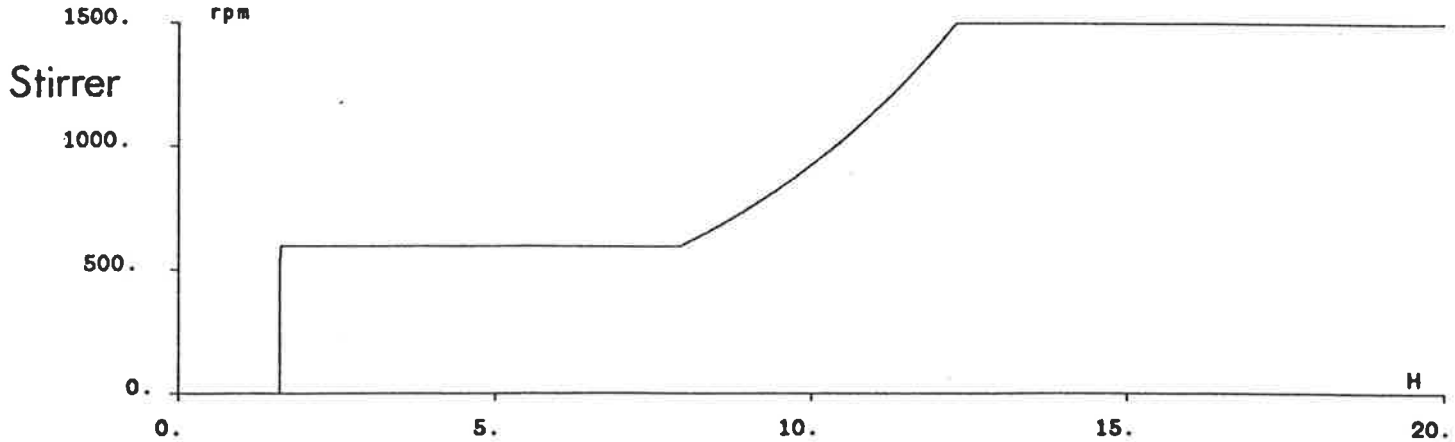

v 8547 a
Ethanol
g/l
0.8
0.4
0.
Off-line analysis of the ethanol concentration (+)
H
0.
5.
10.
15.
20.
Feed rate
ml/min
8.
4.
0.
Regulator parameter was changed
H
0.
5.
10.
15.
20.
DO
%
80.
40.
0.
H
0.
5.
10.
15.
20.
Stirrer
rpm
1500.
1000.
500.
0.
H
0.
5.
10.
15.
20.

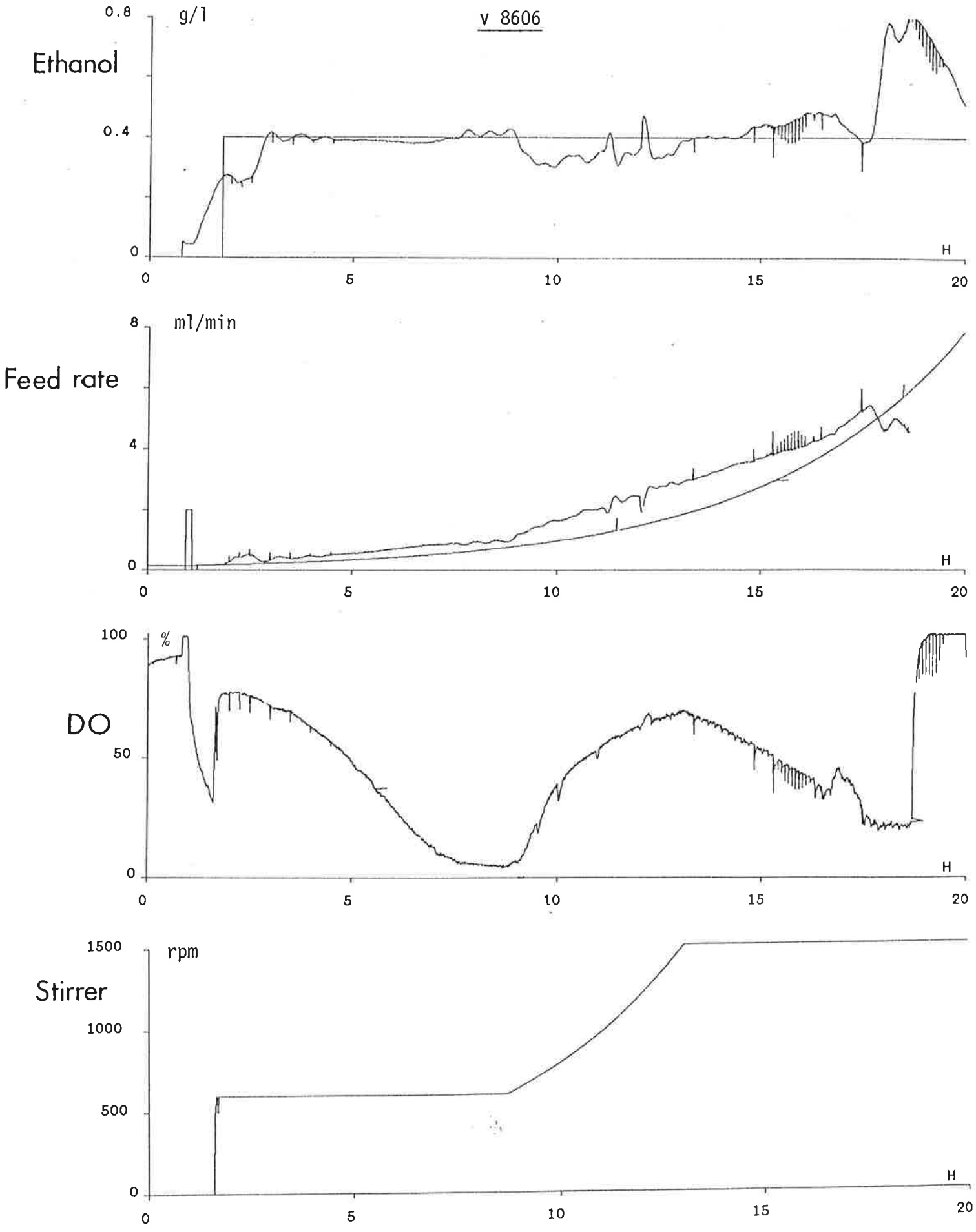
v 8547 b

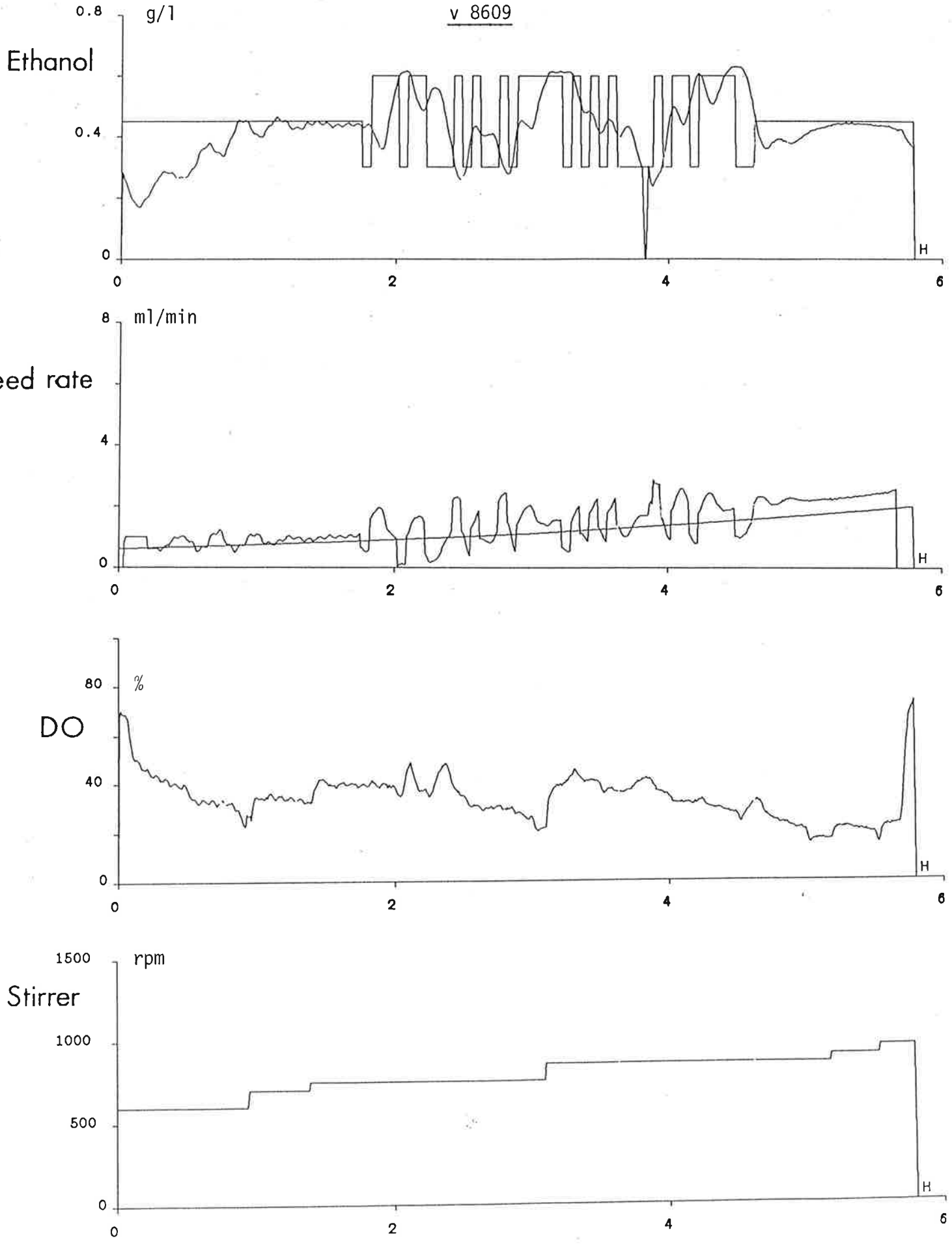

Ethanol
g/l
0.8
0.4
0.
Off-line analysis of the ethanol concentration (+)
H
0.
5.
10.
15.
20.

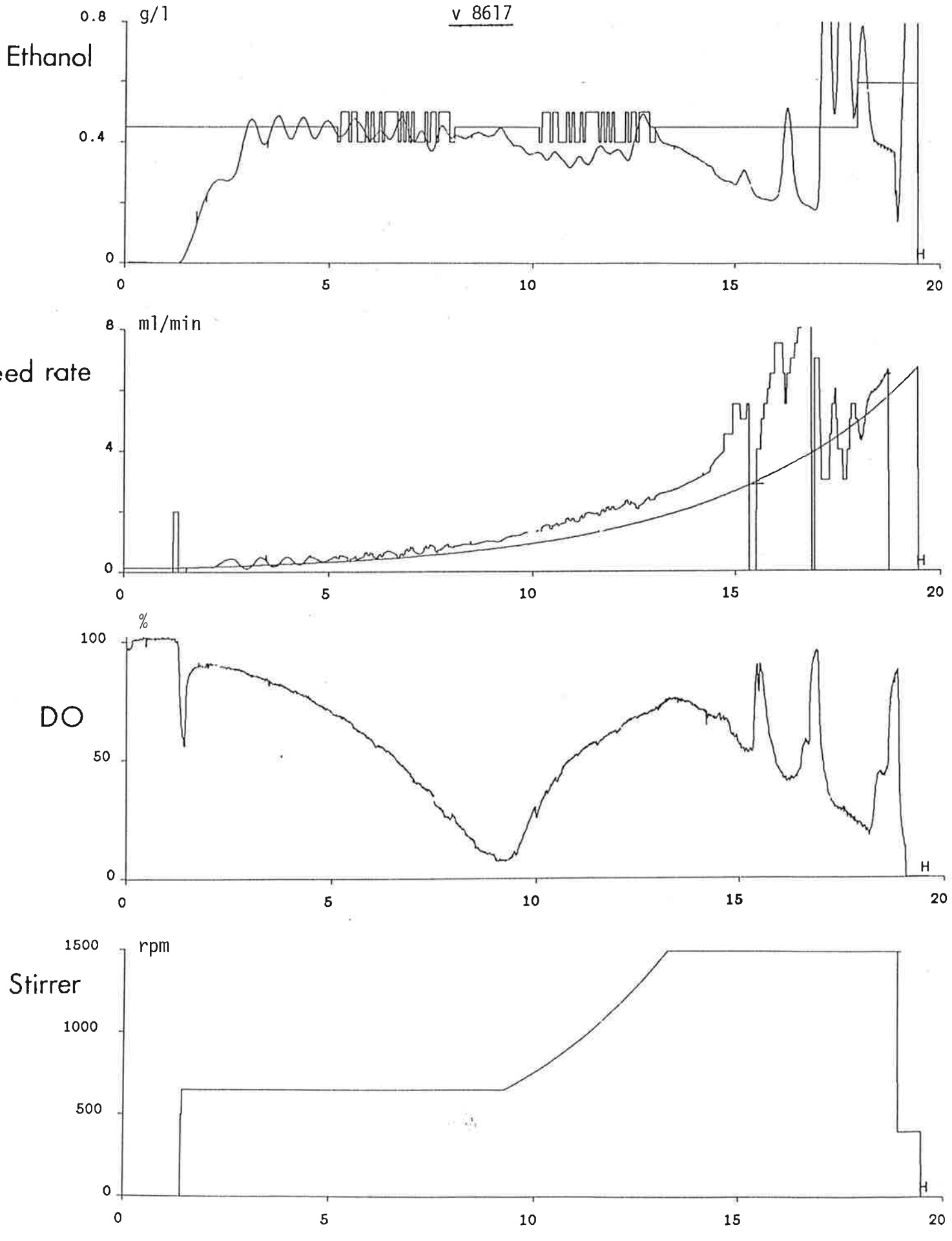

Feed rate
ml/min
8.
4.
0.
Regulator parameter was changed
H
0.
5.
10.
15.
20.

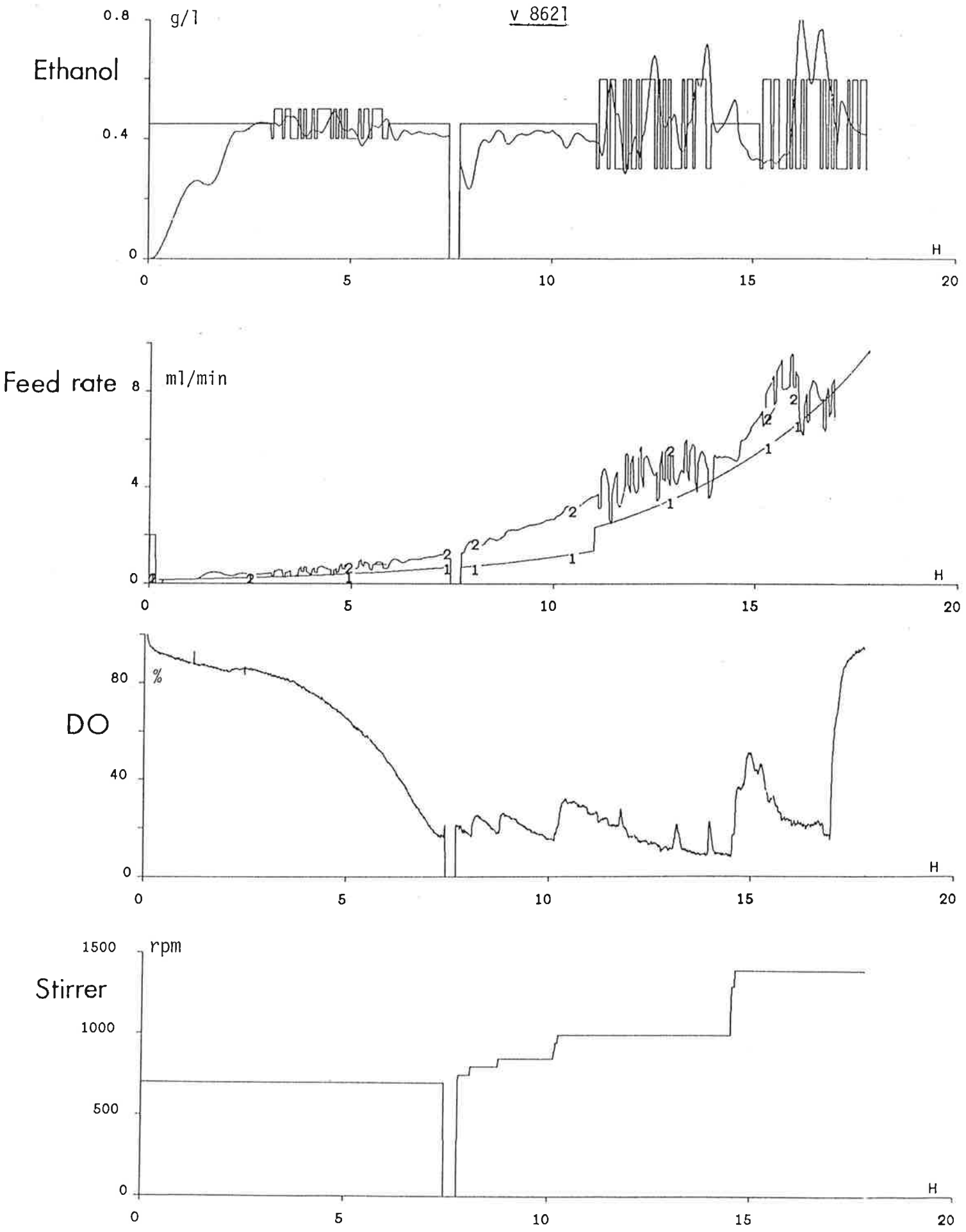

DO
%
80.
40.
0.
H
0.
5.
10.
15.
20.

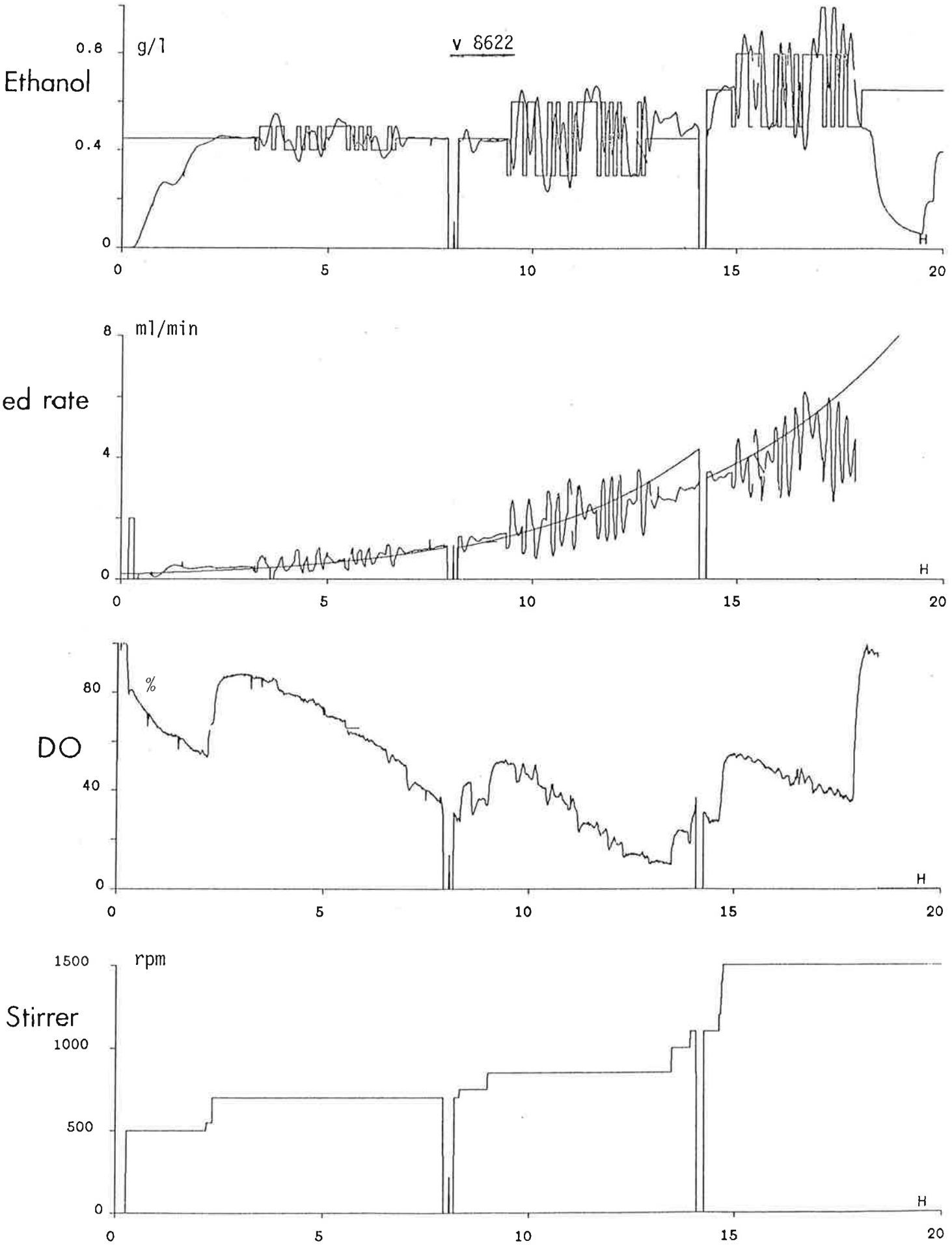

Stirrer
rpm
1500.
1000.
500.
0.
H
0.
5.
10.
15.
20.

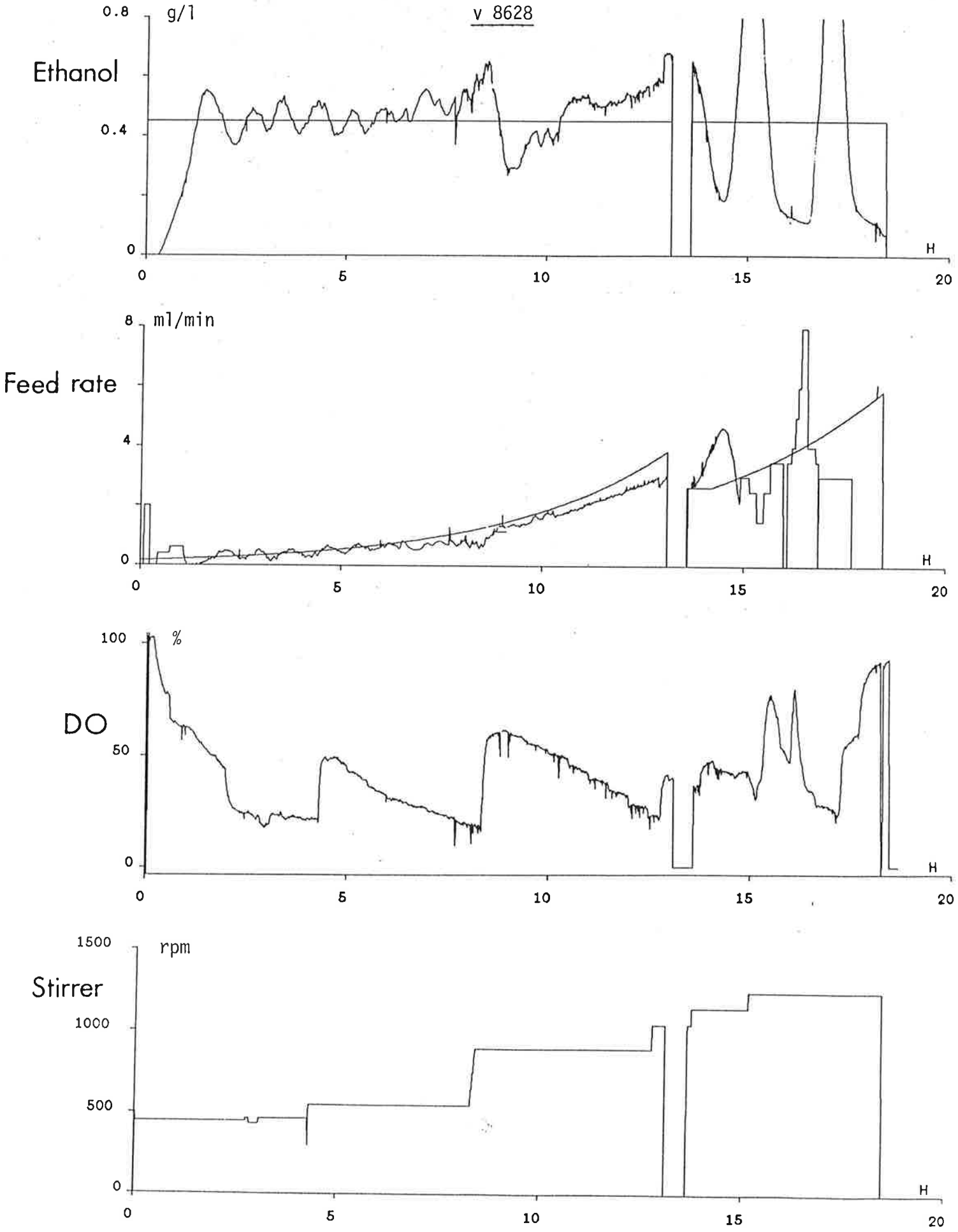

Ethanol
g/l
v 8606
0.8
0.4
0
H
0
5
10
15
20
Feed rate
ml/min
8
4
0
H
0
5
10
15
20
DO
%
100
50
0
H
0
5
10
15
20
Stirrer
rpm
1500
1000
500
0
H
0
5
10
15
20

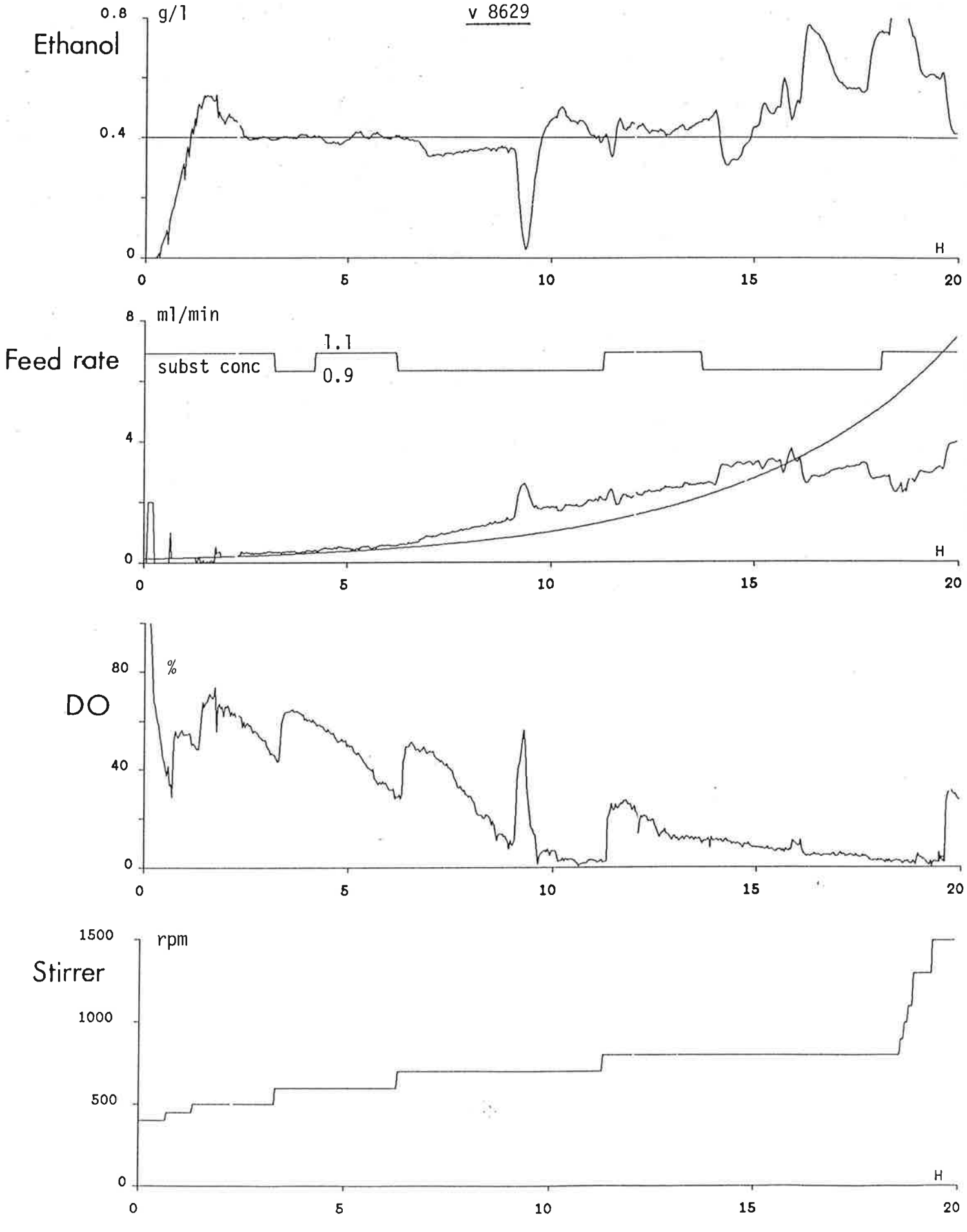

v 8609
Ethanol
g/l
0.8
0.4
0
Feed rate
ml/min
8
4
0
DO
%
80
40
0
Stirrer
rpm
1500
1000
500
0
0
2
4
6
H


v 8617
Ethanol
g/l
0.8
0.4
0
Feed rate
ml/min
8
4
0
DO
%
100
50
0
Stirrer
rpm
1500
1000
500
0
0
5
10
15
20
H


v 8621
Ethanol
g/l
0.8
0.4
0
H
0
5
10
15
20
Feed rate
ml/min
8
4
0
DO
%
80
40
Stirrer
rpm
1500
1000
500


Ethanol
g/l
V 8622
0.8
0.4
0
0
5
10
15
20
H
Feed rate
ml/min
8
4
0
0
5
10
15
20
H
DO
%
80
40
0
0
5
10
15
20
H
Stirrer
rpm
1500
1000
500
0
0
5
10
15
20
H


v 8628
Ethanol
g/l
0.8
0.4
0
H
0
5
10
15
20
Feed rate
ml/min
8
4
0
H
0
5
10
15
20
DO
%
100
50
0
H
0
5
10
15
20
Stirrer
rpm
1500
1000
500
0
H
0
5
10
15
20


v 8629
Ethanol
g/l
0.8
0.4
0
H
0
5
10
15
20
Feed rate
ml/min
8
4
0
subst conc
1.1
0.9
DO
%
80
40
Stirrer
rpm
1500
1000
500