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Axelsson, Jan Peter

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Jan Peter Axelsson

DEPARTMENT OF AUTOMATIC CONTROL
LUND INSTITUTE OF TECHNOLOGY

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

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Author(s)
Jan Peter Axelsson

Supervisor

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Abstract

In this report is summarized experimental experience from our preliminary work on fed-batch cultivation of baker's yeast. This work was carried out at the end of 83 and in the first months of 84 with brand new equipment. Problems of various kinds were tackled. However, some interesting data was recorded. The emphasis of this report is to present data rather than to interpret them.

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FED-BATCH CULTIVATION OF BAKER'S YEAST ON GLUCOSE MEDIA

In this report is summarized experimental experience from our preliminary work on fed-batch cultivation of baker's yeast. This work was carried out at the end of 83 and in the first months of 84 with brand new equipment. Problems of various kinds were tackled. However, some interesting data was recorded. The emphasis of this report is to present data rather than to interpret them.

Simple dynamical experiments were done in order to reveal the dynamics from feed-rate to ethanol concentration in the broth at different cell concentrations. These results were presented in Axelsson (84).

During the work, autonomous oscillations in the ethanol and dissolved oxygen concentrations was frequently recorded. The period time of these oscillations were between 7 and 25 min. These oscillations are here called fast because they cannot be related to the cell cycle. Oscillations in synchronously growing cultures have a period of 1.5-3 h. Due to problems with the equipment, some of the early measurement data is poorly calibrated and only logged at printer-paper. Although this data might be interesting if further investigations will be carried out.

The preliminary work done, using glucose semi-synthetic media, was characterized by these fast oscillations and also of slow growth rate at cell concentrations above 10 g/ℓ. In later work molasses is used and these problems have not occurred. The relatively high maximal feed rates during week 8413 and 8414 is difficult to explain. A common characteristic of these two cultivations were that the feed rate was changed stepwise manually.

Simple dynamical experiments

In the literature, simple dynamics from feed rate to ethanol concentration in the broth is proposed, Woehrer (81) and Dairaku (82). In a fed-batch reactor the dynamics could be described by a first order system followed by an integrator. These results are here confirmed, cf. fig.1. and fig.2.

The linearized equations around a quasi steady state gives:

$$T \cdot \frac{d}{dt}\Delta G = -\Delta G + b_1 u \qquad (1)$$

$$\frac{d}{dt}\Delta E = b_2 \Delta G \qquad (2)$$

where

$$\Delta G = G - G_c \qquad \Delta E = E - E^\circ \qquad u = F - F^\circ$$

T	is the time constant for the glucose dynamics	1-10 min.
G	is the glucose concentration	250 g/ℓ.
G_c	is the critical glucose concentration.	100-200 mg/ℓ.
E	is the ethanol concentration	0.1-0.3 g/ℓ.
F°	is the exponential basic dosage scheme	0.2-4.0 mℓ/min.

The first experiment, fig.1., shows the dominating dynamics due to integral action. Dynamics due to metabolic activity of the cells and to the sensor system are noticeable. In fig.2. the response of the ethanol signal to an oscillating feed rate obtained by high gain proportional control is shown. The data was fitted to a first order linear model by the maximum likelihood method and it was found that T=4.0 min. The pure integrator of the ethanol dynamics, was not revealed in this experiment. The reason is, that the input signal does not contain much energy in the low frequency domain. However, from a control point of view it is important to have a good model of the process near the bandwidth of the desired closed loop.

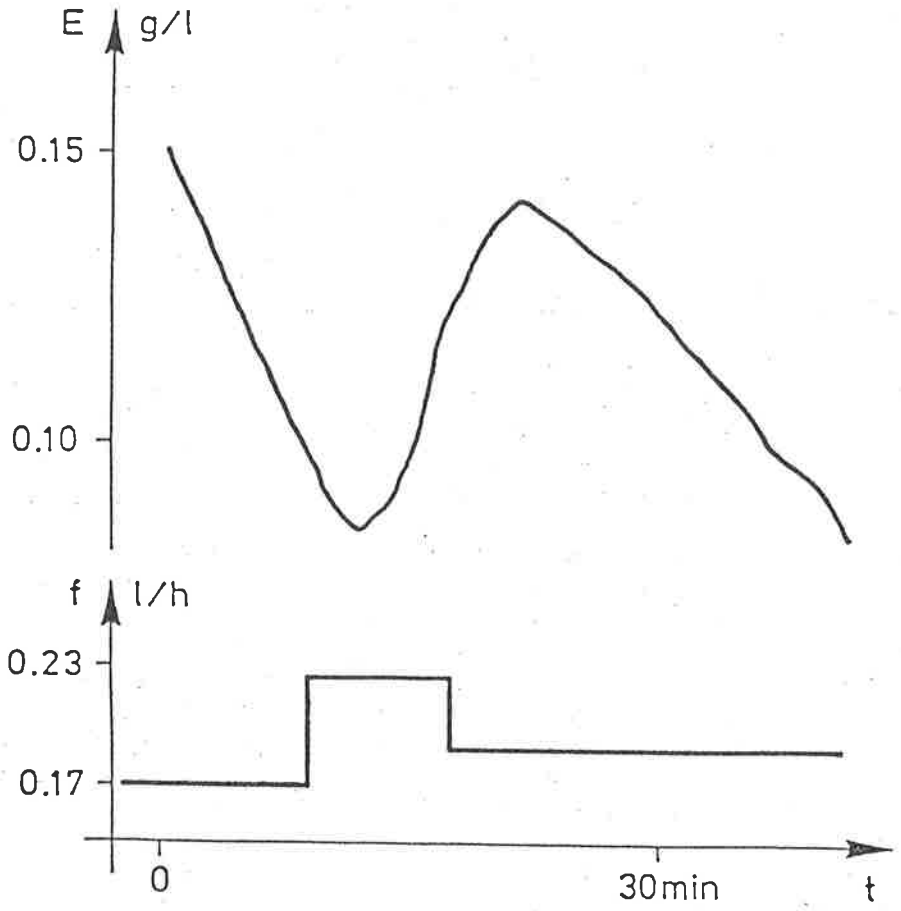

Fig. 1. Response of the ethanol signal to step changes in the feed rate at a cell concentration X≈45 g/l. The dominant behaviour is due to integral action. Dynamics with time constants less than 7 min are due to the cells and the sensor system, and smoothens the corners. The delay in the response to the changes in the feed rate was due to a 3 min time delay in the ethanol sensor system. Data is from experimental week 8413.

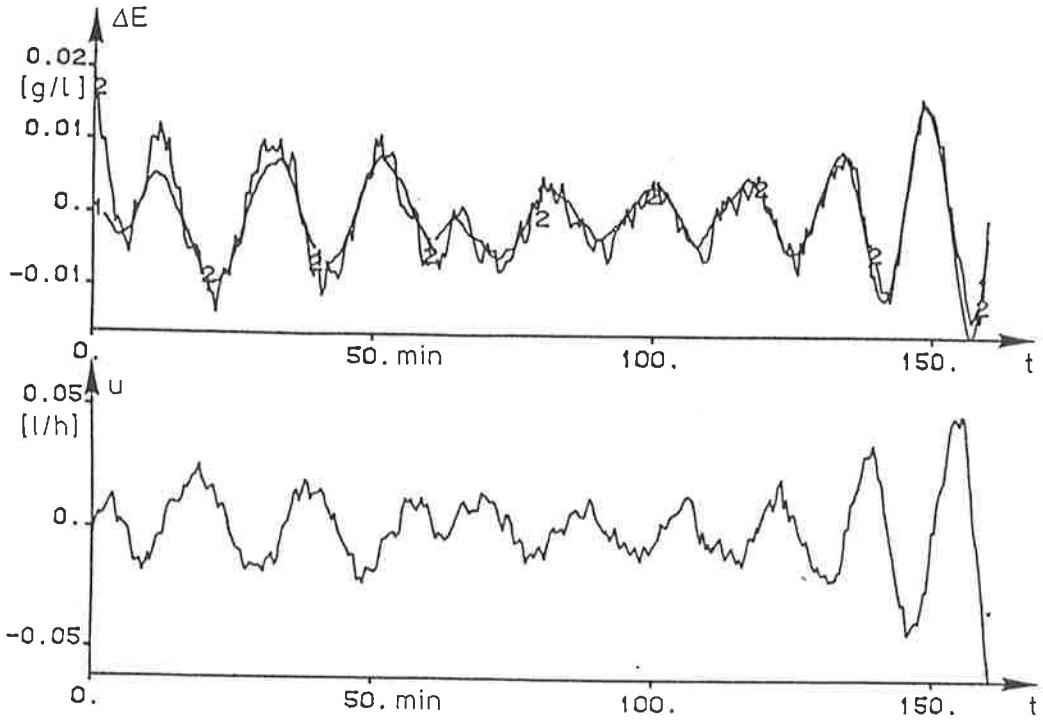

Fig. 2. The response of the ethanol signal to an oscillating feed rate. Cell concentration X≈10g/ℓ. The data is fitted to a first order linear model with T=4.0 min. The upper diagram shows ethanol concentration, where 1) is simulation and 2) is experimental. The lower diagram shows feed rate. Experimental week 8414.

Fast autonomous oscillations in ethanol and dissolved oxygen concentrations

Two different kinds of fast oscillations (period 7-25 min) were observed. The DO signal could start oscillating independently of the ethanol signal but the more frequent observation was the onset and continuation for many hours of a correlated oscillation in these two signals. This phenomena made it impossible to control the feed rate based on the ethanol measurement.

Autonomous oscillations are sparsely reported in the literature. Some work deal with effects related to synchronously growing cultures, Meyenburg (73). The individual yeast cell produces and excret ethanol for about half an hour to an hour at the initiation of the budding phase. Usually, the cells in the fermentor are more or less uniformly distributed over the cell cycle, and the effect averages out. However, certain changes in the environment enhance synchrony. Further, synchrony of cell growth is more likely in a well defined synthetic media, Rieger (83) and Meyer (84). There is also some work analysing oscillations that are not correlated with the cell cycle. A recent study by by Heinzle (83) discuss autonomous oscillations at growth rates less than 0.15 h^{-1}. These oscillations are explained as an effect of interaction between the metabolic flow and reserve material in the cell like glycogen and trehalose. The DO concentration has also an influence of the oscillations. However, the oscillations discussed by Heinzle are slower than ours.

In conclusion, these oscillations seems to be poorly understood. One reason might be lack of on-line sensors for important biological variables.

Our records of autonomous oscillations are difficult to relate to the literature. Here a resumé:

o The start of the 'independent' oscillation in the DO-signal took place when the yeast began to produce ethanol. See fig. 3.

o The more frequent phenomena was that ethanol- and DO-signal started to oscillate with good correlation. Within a couple of periods a stable oscillation was built up. It could continue for several hours. Different ways to stop the oscillation were tried. At the start of the oscillations the feed rate was usually controlled using the ethanol signal. Therefore the feed rate also came into oscillation. When the regulator was turned off and the feed rate was set to a constant value, the oscillations continued. Only when the feed rate was set to zero the oscillations did stop. See fig. 4. and fig. 5.

Weather these oscillations in the ethanol- and DO-signal reflected the physical reality in the reactor was not analysed in detail. The purpose of our work was not to study these oscillations. They were treated as a hindrance of progress of our work. Although some observations were done and most of them are in favour of the reliability of the signals:

- The ethanol sensor dynamics has a time constant of about 2 min. These fast recorded changes in the ethanol signal therefore indicate an approximately double magnitude of the amplitude in the true oscillation in the ethanol concentration in the reactor.

- A number of times the oscillations were observed to be in synchrony with the NaOH dosage. However manual NaOH dosage could not change the rhythm of the oscillations.

+ The DO- signal responded properly to an increase in both aeration rate and in stirrer speed, cf fig. 5.

+ The oscillations stopped when the feed rate was set to zero.

+ Addition of yeast extract also stopped the oscillations, cf. week 8414.

+ Later, molasses has been used as substrate, and no oscillations of this kind has been recorded.

Additions of various substances during the cultivation

During a few experiments different complements to the media were added. It was found that Zn and Fe had a pronounced effect of the rate of ethanol production/consumption rate. Further, yeast extract, rich of essential substances, always stopped oscillations rather abruptly. During the week 8412 the oscillations stopped after addition of a mixture of well-defined substances. The added substances were: $FeCl_3$, inositol, trace elements, biotin and niacin. A few hours later, the yeast cells were able to grow for several hours without oscillations.

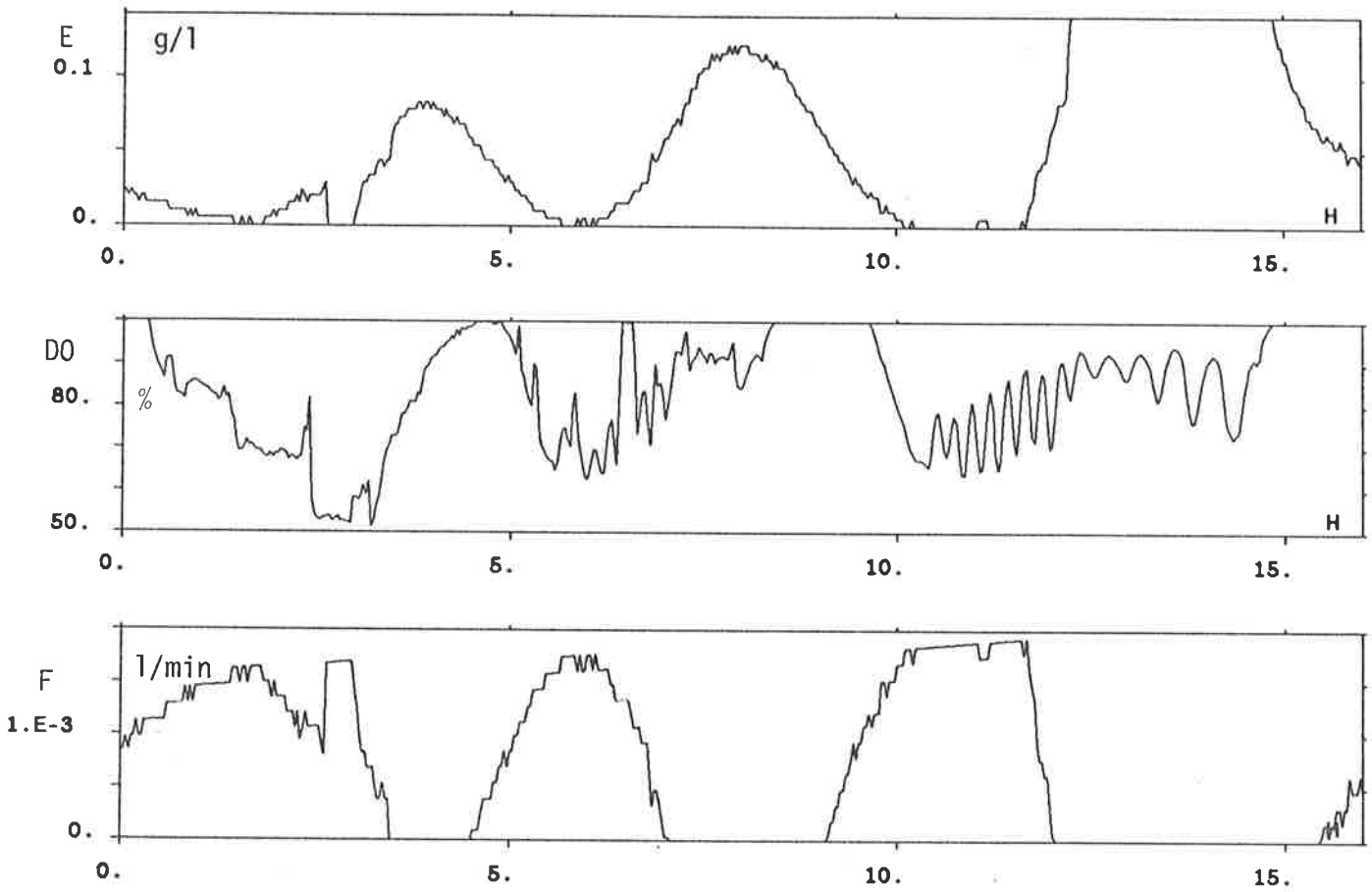

Fig. 3. After about 4 h of fed-batch. Cell concentration X ≈ 10 g/ℓ. Slow oscillation in the ethanol signal induced by the regulator. Proportional control was used. Note the onset of fast oscillations in the DO-signal during the ethanol consummation. The second train of oscillations in the DO-signal was more regular. Measurement of the period time gave 13 min for the first 8 periods and then the period time increased from 22 to 29 min and finally ceased. A careful exam of the unfiltered ethanol signal (which was only logged at the printer paper) revealed small oscillations correlated with the oscillations in the DO-signal. A high DO-signal was correlated with a slightly higher ethanol-signal. At the end the ethanol signal raised abruptly due to sensor malfunction. Data is from experimental week 8406.

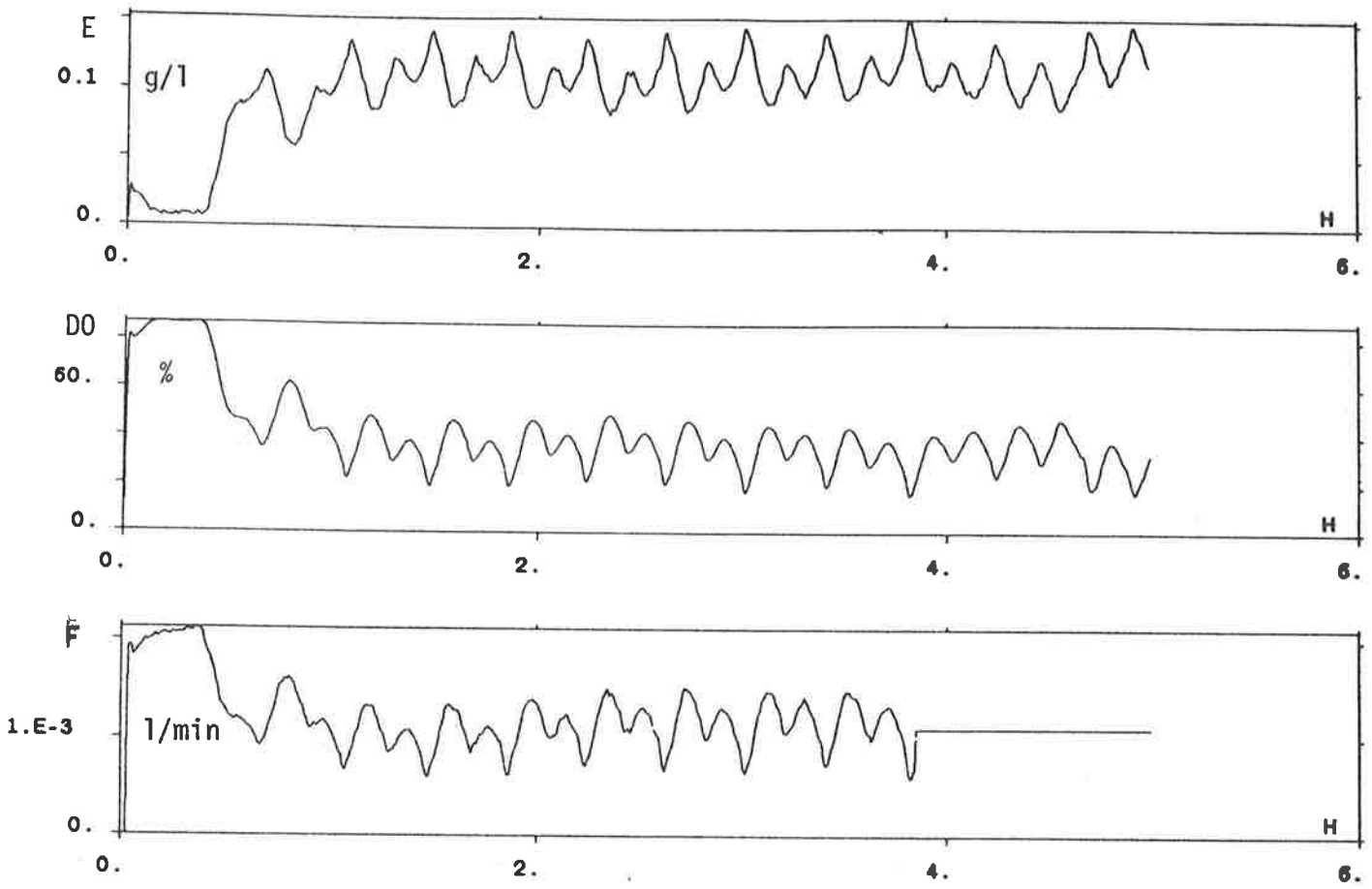

Fig. 4. After about 5 h of fed-batch. X ≈ 15 g/ℓ. Rapid autonomous oscillations started. When the feed rate was set to a constant value the oscillation rhythm changed slightly. The double peak pattern breaks into a single peak pattern. Experimental week 8414.

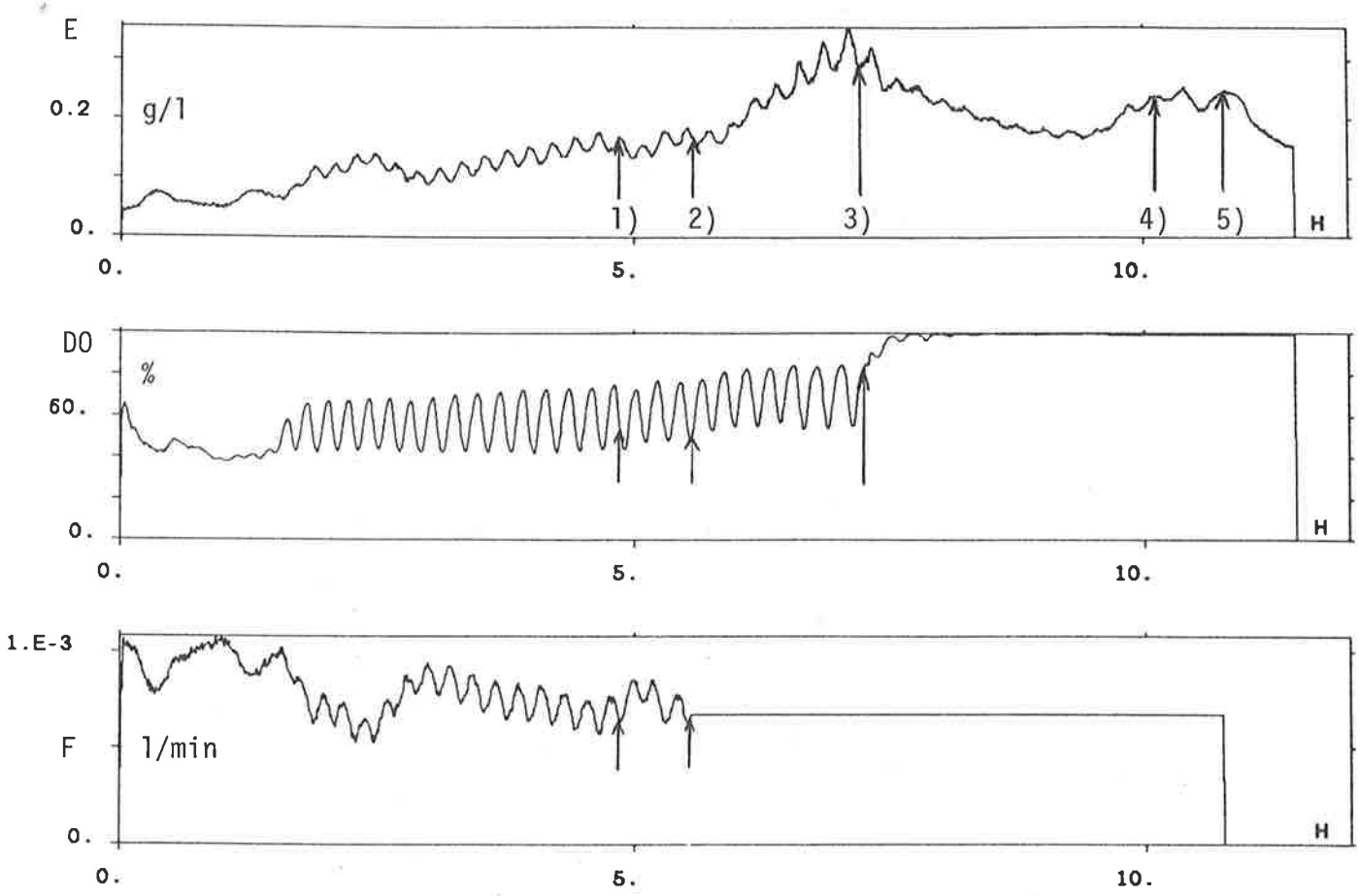

Fig. 5. Autonomous oscillations ocurred 10 h after the start of the mini-batch. Cell concentration X $\approx$ 18 g/ℓ Different ways to stop the oscillations were tried. Actions taken were: 1) The air-flow was doubled; 2) The feed rate was set to the constant value 1.0 mℓ/min; 3) The stirrer speed was increased from 500 to 750 RPM; 4) Addition of NH_4Cl; 5) The feed rate was finally set to zero; Measurements off the cell concentration indicate a low growth rate, $\mu \approx 0.09\ h^{-1}$. One of the measurements, just after the onset of oscillations have a very high value. If this measurement is regarded as OK, then the period before had a high normal growth rate, $\mu \approx 0.18\ h^{-1}$, and reserved material has to be metabolized in order to explain the high 'yields'. The period of oscillations then is a time of no growth and just a waste of substrate. The data is from experimental week 8412.

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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 between 400-1200 rpm.

After the batch culture was completed the feed was started. The feed pump was a peristaltic voltage controlled pump (Ismatec). The concentrated feed entered the fermentor as droplets of about 20 $\mu\ell$. The droplets were assumed to be immediately mixed in the fermentor.

The inoculum preparation

The batch culture (4ℓ) was inoculated with 200mℓ (5% v/v) inoculum. This was cultivated in shaker flasks for 24h at 30°C on YM broth (Difco) supplemented with $Na_2HPO_4 \cdot 2H_2O$ (0.6 g/ℓ), KH_2PO_4 (0.4 g/ℓ) and $MgSO_4 \cdot 7H_2O$ (0.2 g/ℓ).

The batch media

The medium in the batch was composed as follows: glucose, 10g/ℓ; yeast extract, 3.8g/ℓ; peptone, 5g/ℓ; malt extract, 3.3g/ℓ; $MgSO_4 \cdot 7H_2O$, 3.8g/ℓ; KH_2PO_4, 0.5g/ℓ; $Na_2HPO_4 \cdot 2H_2O$, 3.3g/ℓ; $FeCl_3 \cdot 6H_2O$, 25.1 mg/ℓ; $CaCl_2 \cdot 2H_2O$, 0.99 mg/ℓ; $ZnSO_4 \cdot 7H_2O$, 0.27 mg/ℓ; $CoCl_2 \cdot 6H_2O$, 0.27 mg/ℓ; $CuSO_4 \cdot 5H/2/O$, 0.24 mg/ℓ; $MnSO_4 \cdot 4H_2O$, 0.24 mg/ℓ.

The fed-batch media

The feed (2000 mℓ) contained glucose (250 g/ℓ) and $(NH_4)_2SO_4$ (75 g/ℓ).

APPENDIX 2 - THE ETHANOL SENSOR SYSTEM

Ethanol was monitored on-line using a membrane gas sensor. 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. 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. The ethanol sensor is linear in the actual range and is charaterized by a time constant and a time delay. The time delay is due to in gas phase transport through the dilution system to the detector. At a nitrogen flow rate of 180 ml/h the time delay is 3 min and the time constant is 3 min. A detailed description of the system and its performance is given in Mandenius (83).

APPENDIX 3 - COMPARISON OF SOME DATA BETWEEN CULTIVATIONS ON GLUCOSE

Experimental week		8346	8347	8405	8406	8411	8412	8413	8414	8415
Batch time	[h]	-	20	16.5	15	19	15	18.5	14	12.5
Pause before fed-batch	[h]	1	4	1.5	5	9	17	2	1	6
Onset of oscillations in E	[h]	5?	3?	1	-	4	10	7	4	5
Measurements of G and E					x				x	
Added substances						x	x	x	x	
Period with lack of NaOH				x						x
Max feed rate	[mℓ/min]			1	1	1.5	1	3.0	4.2	1
E level (average)	[g/ℓ]			0.3	0.1-0.3	0.1-0.3	0-0.2	1?	0.1-0.2	0.1
DO-control - (m is manual rpm scheme)		rpm-reg	air-reg	m	m	m	m	m	m	m
Poor documentation		x	x					x		x
Published data								*	*	