0273-2289/04/114/0601–0617/$25.00

Controlled Fed-Batch Fermentations of Dilute-Acid Hydrolysate in Pilot Development Unit Scale

Andreas Rudolf,* Mats Galbe, and Gunnar Lidén

Chemical Engineering, Lund University,
Box 124, SE-221 00 Lund, Sweden,
E-mail: andreas.rudolf@chemeng.lth.se

Abstract

Inhibitors formed during wood hydrolysis constitute a major problem in fermenting dilute-acid hydrolysates. By applying a fed-batch technique, the levels of inhibitory compounds may be held low, enabling high ethanol productivity. In this study, a previously developed fed-batch strategy was modified and implemented for use in pilot development unit (PDU) scale. The rate of total gas formation, measured with a mass flow meter, was used as input variable in the control algorithm. The feed rate in the PDU-scale experiments could be properly controlled based on the gas evolution from the reactor. In fed-batch experiments utilizing TMB 3000, an inhibitor-tolerant strain of *Saccharomyces cerevisiae*, close to 100% of the hexoses in the hydrolysate was converted.

Index Entries: Pilot development unit scale; dilute-acid hydrolysate; fed-batch fermentation; feed rate; carbon dioxide evolution rate.

Introduction

Hydrolysis of lignocellulose is necessary to enable its use for ethanol production. However, when lignocellulosic materials are hydrolyzed with acid, compounds toxic to the yeast cells are released. The inhibitors are of three main types: aldehydes, organic acids, and phenolic compounds. Among the aldehydes, furfural and hydroxymethylfurfural (HMF) are typically found in high concentrations—particularly in dilute-acid hydrolysates *(1,2)*. These compounds have been shown to inhibit certain enzymes in the catabolism necessary for cell growth *(3–9)*.

The amount of inhibitory compounds can be decreased by detoxification. Several methods have been suggested, of which overliming is probably the most well studied *(10,11)*. One major drawback with

*Author to whom all correspondence and reprint requests should be addressed.

overliming is the necessity of handling the precipitates formed in the process. The potential loss of fermentable sugars is another important drawback *(10,11)*. An alternative strategy is to use the capacity of the yeast itself to convert the inhibitors, and thereby obtain in vivo detoxification. However, this only works if the concentrations of inhibitors are rather low. In a batch process, it is likely that the concentrations of inhibitors will be so high that cellular metabolism will completely stop *(12–14)*. High concentrations of inhibitors may be avoided if a fed-batch procedure is applied provided that there is an accurate control of the feed rate in order to keep the level of inhibitors low *(12–14)*.

Feed rate control based on the carbon dioxide evolution rate (CER) has been shown to be an efficient method. Taherzadeh et al. *(14)* increased the feed rate stepwise as long as the relative increase in CER was at least 50% of the relative increase in feed rate. Nilsson et al. *(13)* developed the method further and used the derivative of the CER to control the feed rate. This control principle was very successful in keeping the hexoses at low levels and preventing inhibition from toxic compounds and was the chosen control algorithm in the present work.

In small-scale fermentations, the most common way to measure the CER is to have a controlled flow of nitrogen through the reactor and monitor the outlet gas concentration with a gas analyzer. Nitrogen flushing is obviously not an option in large-scale operation, but the total gas evolution is still a measurable quantity.

In the present work, the objective was to develop and implement a control algorithm for addition of hydrolysate, based on the total gas flow from a pilot development unit (PDU)-scale reactor. The control algorithm was tested in fed-batch fermentations of dilute-acid hydrolysate made with two different yeast strains.

Controlling the addition of hydrolysate from the total exhaust gas flow proved successful. With one of the strains tested (TMB 3000), complete conversion of the hexoses present in a strongly inhibiting hydrolysate was obtained.

Material and Methods

Wood Hydrolysate

The hydrolysate used was a dilute-acid hydrolysate produced in Rundvik, Sweden, by a two-stage hydrolysis process performed in a 350-L rebuilt Masonite gun-batch reactor *(1)*. In the first process stage, about 10 kg of dry wood splinter was impregnated with H_2SO_4 and water to obtain an acid concentration of 5 g/L and a solids concentration of 30%. After impregnation the wood was loaded into the reactor and the reaction was started by direct steam injection. After a heat-up period of 50 s, a pressure of 12 bars was held for 10 min, followed by a rapid decompression. The solid material was separated from the liquid by filtration. Further hydrolysis of the remaining solid was carried out at a pressure of 21 bars for 7 min. The hydrolysates from

Table 1
Compositions of Dilute-Acid
Hydrolysates Used in PDU Experiments

Components	Hydrolysate A Concentration (g/L)	Hydrolysate B Concentration (g/L)
Glucose	24.3	19.9
Mannose	11.8	15.9
Xylose	5.7	7.4
Galactose	3.4	4.0
Arabinose	1.5	1.7
Acetate	1.7	1.7
HMF	2.1	1.8
Furfural	0.4	0.5

the two stages were mixed together. Two different batches of hydrolysate were used (Table 1).

Yeast Strains and Medium

Two different strains of *Saccharomyces cerevisiae* were used: CBS 8066 (Centralbureau voor Schimmelcultures, Delft, The Netherlands) and TMB 3000 (Division of Applied Microbiology, Lund University, Sweden). TMB 3000 is a strain originally isolated from spent sulfite liquor, and therefore it has a high tolerance to inhibitors. The strains were maintained on agar plates (20 g/L of agar, 20 g/L of soya peptone, 20 g/L of glucose, and 10 g/L of yeast extract). Before the fermentations, inoculum cultures were grown in 250-mL conical E-flasks. The flasks were placed in a shaker bath (30°C, 140 rpm) for 24 h.

Cultivation Procedures

Fed-Batch Fermentations

The fed-batch fermentations in PDU scale were performed in a 22-L reactor (NLF 22; Bioengineering AG, Wald, Switzerland). The pH was maintained at 5.0 by the addition of 2 *M* NaOH. The stirrer speed was 600 rpm and the fermentation temperature was 30°C. The fed-batch phase was preceded by a batch phase for production of cell mass. The initial glucose concentration in the medium was 64 or 45 g/L. To the medium was added 28.5 g/L of $(NH_4)_2SO_4$, 12.1 g/L of KH_2PO_4, and 2.6 g/L of $MgSO_4$ as sources for nitrogen, phosphorus, magnesium, and sulfur. Trace metal solution, Ergosterol/Tween-80, and vitamin solution were also added in accordance with Taherzadeh et al. *(15)*. The outlet gas flow was measured with a mass flow meter calibrated for CO_2 at a flow of 10–500 mL/min (F 111C; Bronkhorst High-Tech B.V., Ruurlo, The Netherlands). After consumption of the glucose in the batch medium, feeding of the hydrolysate was started.

In total, about 15 L of dilute-acid hydrolysate was pumped into the reactor using a peristaltic pump (U1-M; Alitea AB, Stockholm, Sweden). Before addition the pH of the hydrolysate was increased from 1.8 to 3.0 by the addition of about 22 mL of 2 *M* NaOH/L of hydrolysate. The reason for the pH adjustment was to avoid disturbance of the CO_2 signal owing to large base additions during the fed-batch fermentation.

Batch Fermentations

Batch fermentations of dilute-acid hydrolysate were done to compare the performance between batch and fed-batch procedures. Since the amount of hydrolysate available from each hydrolysis batch was limited, the batch fermentations were made in a 2.5-L fermentor (Biostat A, B. Braun Biotech, Melsungen, Germany). Scaling effects could potentially affect the comparison between batch and fed-batch fermentations. However, comparisons between batch and fed-batch fermentation of dilute-acid hydrolysates have been made repeated times, and our results were in good agreement with previous findings, showing a strong inhibition in batch fermentation *(12,13)*.

The pH was maintained at 5.0 by the addition of 2 *M* NaOH (Micro DCU-300; B. Braun Biotech). The fermentation temperature was 30°C and the stirrer speed was 500 rpm (MCU-200; B. Braun Biotech). Cell mass was produced in an initial batch phase using a glucose concentration of 64 g/L. The concentrations of mineral salts, trace metals, vitamins, and Ergosterol/Tween-80 were the same as in the fed-batch experiments. When the glucose in the batch medium was completely consumed, 1.9 L of dilute-acid hydrolysate was pumped into the reactor at maximum pump speed, by using a peristaltic pump (U1-M; Alitea AB). The ratio between batch volume and the final volume (i.e., after all the hydrolysate had been added) was similar to the ratio in the PDU fed-batch experiments, approx 1:4. The reactor medium was sparged with nitrogen (600 mL/min). The CO_2 content in the exhaust gas was measured with a gas analyzer (TanDem; Adaptive Biosystems, Luton, UK).

Analytical Methods

Metabolite Analysis

During the batch experiments, samples were withdrawn every hour. Samples were taken less frequently during the fed-batch experiments to avoid disturbances in the CO_2 signal. The samples were centrifuged in Eppendorf tubes at 12,000*g* for 5 min (Z 160 M; Hermle Labortechnik, Wehingen, Germany). Afterward, the supernatant was filtered with 0.2-µm sterile filters and the filtered samples were stored in a freezer. Analysis of the most common metabolites was done using high-performance liquid chromatography. The concentrations of glucose, mannose, xylose, and galactose were determined using a polymer column (Aminex HPX-87P; Bio-Rad, München, Germany) at 85°C. Ethanol, glycerol, acetate, HMF, and furfural were analyzed using an Aminex HPX-87H column (Bio-Rad) at

60°C. The compounds of interest were detected with a refractive index detector (Waters 2410; Milford, MA).

Biomass

Dry weight was determined from duplicate 10-mL samples. The samples were centrifuged for 4 min at 1000*g* (Z 200 A, Hermle). The supernatant was then discarded and the pellet washed. After a second centrifugation the pellet was dried at 105°C for 24 h.

CO_2 Evolution

The analog signals from the mass flow meter (during the fed-batch fermentations) and from the gas analyzer (during the batch fermentations) were transformed to digital signals by 12-bit data acquisition cards (PCI-6024E; National Instruments, Austin, TX). Labview 5.1 software from National Instruments was used for data logging and feed rate control.

Cell Viability

Samples for cell viability tests were taken three times during the different batch and fed-batch fermentations. A sample of 1 mL was diluted 10^4–10^5 times and 0.1 mL of diluted sample was applied to an agar plate. Five to six plates were prepared for every sample, and the plates were incubated for 24 h at 30°C. The total cell concentration in the reactor sample was determined with microscopy using a Bürker counting chamber. The viability, expressed as the fraction of cells able to form colonies, was calculated by dividing the concentration of colony-forming cells by the total cell concentration.

Theory

Feed Rate Optimization

Measuring the CO_2 evolution, CER, is an excellent way to monitor the ethanol production since the dominant source of CO_2 production in anaerobic fermentations is directly linked to ethanol formation *(13)*. One of the main goals of the present project was to maximize ethanol production. The feed rate, $F(t)$, which maximizes the overall ethanol production, $I(F)$ (Eq. 1), for a given time, t, should therefore be found *(16)*:

$$I(F) = \int_t R_p\left[F(t)\right] dt \tag{1}$$

The productivity, R_p (g/h), is composed of three factors (Eq. 2):

$$R_p = q_p(t)X(t)V(t) \tag{2}$$

in which q_p (g/g·h) is the specific productivity, X (g/L) is the biomass concentration, and V (L) is the volume (Eq. 3):

$$V(t) = \int_t F(t)dt + V_0 \tag{3}$$

The composition of the hydrolysate will affect growth rate, specific substrate uptake rates, and yield coefficients in a not easily predictable way *(14)*. It is thus not possible to use a predetermined feed profile, but instead a closed-loop procedure should be used. One approach is to maximize the productivity at every point in time, and this can relatively easily be implemented in a closed-loop control. This approach was used by Taherzadeh et al. *(14)*, who applied step changes in the feed rate and gradually increased the feed rate as long as there was a relative response in CER corresponding to at least include 50% of the relative increase in feed rate. The major drawbacks of this control were that the increase in feed rate was fixed and that the CER-response ratio was somewhat sensitive to measurement errors.

Control Strategy

The control algorithm in the present work was based on using the derivative of the CER (Eqs. 4 and 5) as the variable on which to base the control *(13)*:

$$\nu = \frac{d\,\mathrm{CER}}{dt} \tag{4}$$

ν expressed in a discretized form is given in Eq. 5:

$$\nu = \frac{\left(\mathrm{CER}_t - \mathrm{CER}_{t-1}\right)}{\left(t_t - t_{t-1}\right)} \tag{5}$$

The derivative of the CER was continuously calculated during the length of a step, and the maximal value of ν, denoted ν^{max}, was the basis for determining the increase in feed rate, ΔF. The increase in feed rate was determined by the ratio between the maximal value of the CER derivative following a step change, ν^{max}, and the maximal CER derivative obtained after the first step, ν_0^{max}. In the beginning of the fed-batch fermentation, the levels of inhibitors are low and the response to the first increase in feed rate thus represents a noninhibited system. During the fed-batch fermentation, if the cell growth is strongly inhibited, there will be a dilution of the cells resulting in a lower *X* (cell mass concentration). Nilsson et al. *(13)* showed that a decreasing *X* will lead to a lower ν. This fact was taken into consideration in the control program by introducing a correction factor, $V/\Delta F$ (Eq. 6). This factor compensates for the dilution of biomass, as well as the magnitude of the increase in feed rate in the preceding step. The expression of ΔF as a function of the corresponding ν^{max} is given by Eq. 6:

$$\Delta F_i = \frac{\left(\nu_{i-1}^{max} \times \frac{V_{i-1}}{\Delta F_{i-1}}\right)}{\left(\nu_0^{max} \times \frac{V_0}{\Delta F_{max}}\right)} \tag{6}$$

If the concentration of inhibitors increases to such levels that the yeast metabolism is affected, the numerator in Eq. 6 will decrease. In case the ratio on the right-hand side of Eq. 6 decreases below a threshold value, no further increase in feed rate will be made.

Disturbances in the measured signal of only a few percent can affect the CER derivative (ν) considerably. Therefore, ν must be calculated from an average value of the CER, obtained within a certain time span.

Results

Fed-batch fermentations in PDU scale were made with two different *S. cerevisiae* strains, CBS 8066 and TMB 3000. Batch fermentations in a 2.5-L scale were done to compare the performance of batch and fed-batch procedures.

Comparison of Strain Performance

Fed-Batch Fermentations

There was a significant difference in the ability of the two strains to ferment the hydrolysates in fed-batch cultivation, as seen in the measured CER profiles of the two strains (Fig. 1). This is in accordance with observations made by A. Nilsson, (Chemical Engineering, Lund University, personal communication). During fed-batch fermentations with TMB 3000, there was a clear and significant response to the steps in feed rate throughout the whole fed-batch phase (Fig. 1A). This was in contrast to fed-batch fermentations with CBS 8066, in which responses to the changes in feed rate decreased after 1.30 h and ceased completely at 2 h (Fig. 1B). Even though the feed rate remained constant the CER continued to decrease. When CBS 8066 was used, only 75% of the hexoses (glucose plus mannose) was fermented whereas close to 100% of the hexoses in the hydrolysate was fermented when TMB 3000 was used. In addition, very significant differences in cell viability after the fed-batch phase were observed. The cell viability of CBS 8066 at the end of feeding was merely 15%, whereas it was close to 100% for TMB 3000 (Table 2).

Batch Fermentations

After a 500-mL batch phase with an initial glucose concentration of 64 g/L, 1.9 L of dilute-acid hydrolysate was added using the maximum pump speed. Batch experiments with both TMB 3000 and CBS 8066 were concluded (Figs. 2 and 3). In addition, TMB 3000 was tested with two different batches of dilute-acid hydrolysate (Table 1).

Compared with the fed-batch fermentations the performance of the batch fermentations was poor. Only a small fraction of the hexoses was fermented (Table 3), compared with almost 100% during the fed-batch fermentations with TMB 3000. As a consequence, the specific ethanol productivity was very low in the batch experiments. The levels of HMF did not decrease significantly during the batch fermentations. On the other hand, furfural was completely converted. The cell viability was close to 0% at the end of all three batch fermentations.

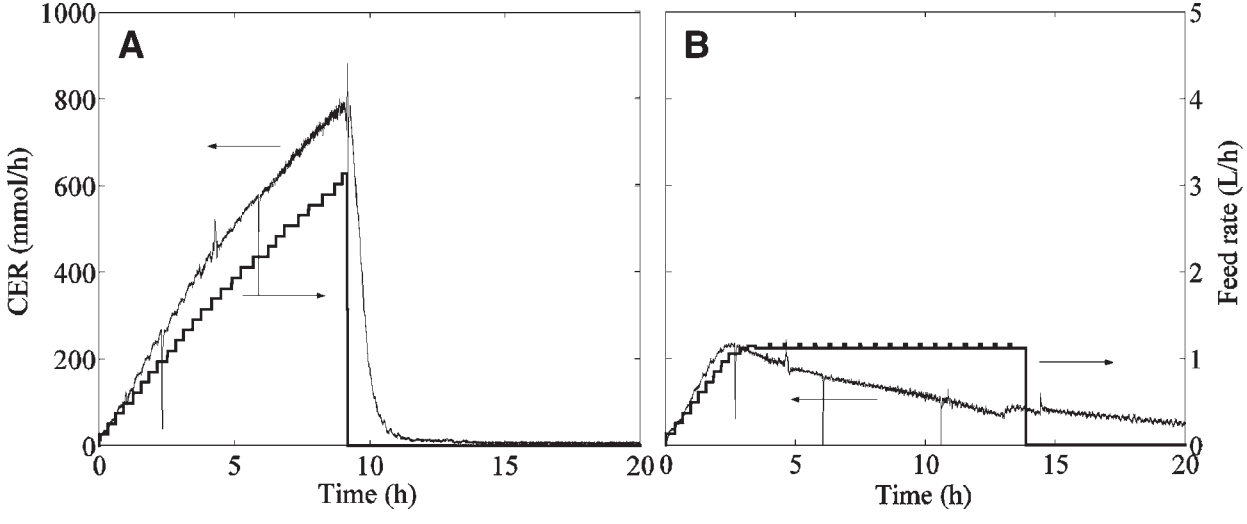

Fig. 1. CER and feed rate during fed-batch fermentations in PDU-scale. After 2 h the CBS 8066 culture was severely inhibited and the increase in feed rate was stopped. Dilute-acid hydrolysate B was used. **(A)** TMB 3000; **(B)** CBS 8066.

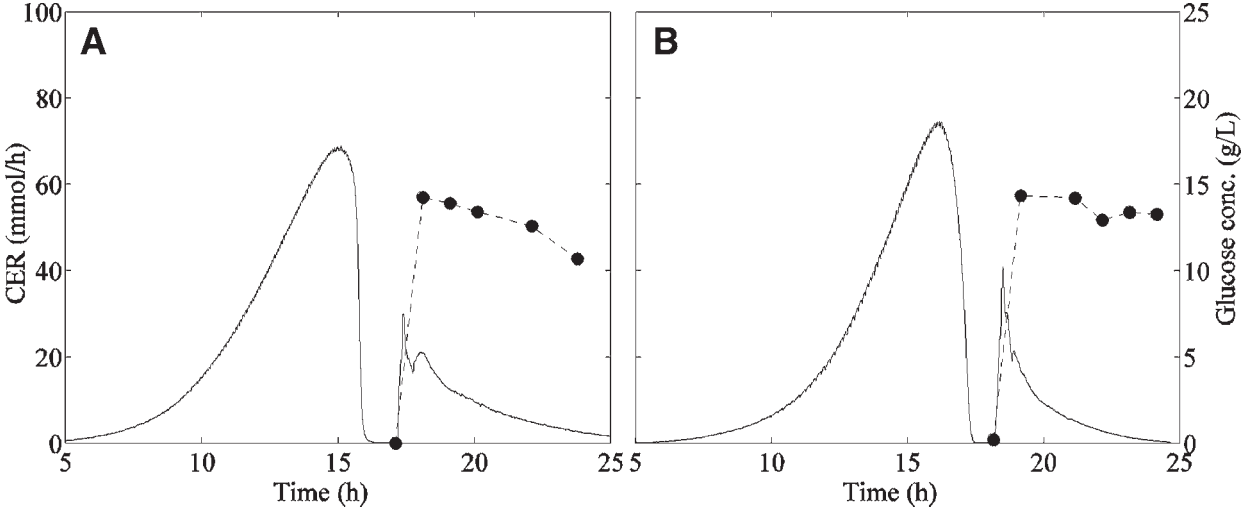

Fig. 2. CER (—) and glucose concentration (– –●– –) during batch fermentations with dilute-acid hydrolysate B. Batch fermentations (0.5 L) on glucose were made during the first 15–17 h to produce cell mass. **(A)** TMB 3000; **(B)** CBS 8066.

Table 2
Cell Viability After Fed-Batch Phase

Strain	Hydrolysate	Viability at end of fed-batch phase (%)
CBS 8066	B	15
TMB 3000	B	~100
TMB 3000	A	93
TMB 3000	A	95

Table 3
Conversion of Glucose and Mannose in Small-Scale Batch Fermentations

Strain	Hydrolysate	Conversion of mannose (%)	Conversion of glucose (%)
CBS 8066	B	9	8
TMB 3000	B	28	30
TMB 3000	A	20	20

Productivity and Yields

The productivity of the strain TMB 3000 was clearly superior to that of CBS 8066, and therefore strain TMB 3000 was analyzed further. Two fed-batch fermentations with dilute-acid hydrolysate A (Table 1) were run using two different glucose concentrations in the batch phase.

The overall specific ethanol productivity, calculated on the whole fed-batch fermentation, was 0.72 g/(g·h) when the glucose concentration in the batch phase was 45 g/L and 0.58 g/(g·h) when the glucose concentration in the batch phase was 64 g/L. The specific ethanol productivity in fact reached a value as high as 0.9 g/(g·h) at the end of the fed-batch fermentations (Fig. 4). The levels of HMF were low during the fed-batch experiments (Fig. 5), and the levels of furfural were too low to be detected, indicating a successful *in situ* detoxification. The total cell mass was doubled (Fig. 6) during the two fed-batch fermentations with TMB 3000, but since the volume during the same time increased fivefold, the cell mass concentration decreased substantially. The ethanol yield on hexoses (glucose and mannose) was 0.45 g/g in the two fed-batch fermentations. The biomass yield on hexoses in the mentioned fed-batch fermentations was about 0.05 g/g (Table 4). This value can be compared with the value of 0.10 g/g obtained for growth on glucose in synthetic medium (i.e., the value obtained in the anaerobic batch phases preceding the fed-batch phases). A cell yield of 0.05 g/g is rather low, but it is well known that inhibitors in the dilute-acid hydrolysate affect cell growth negatively *(1,3,4,6–8,17)*.

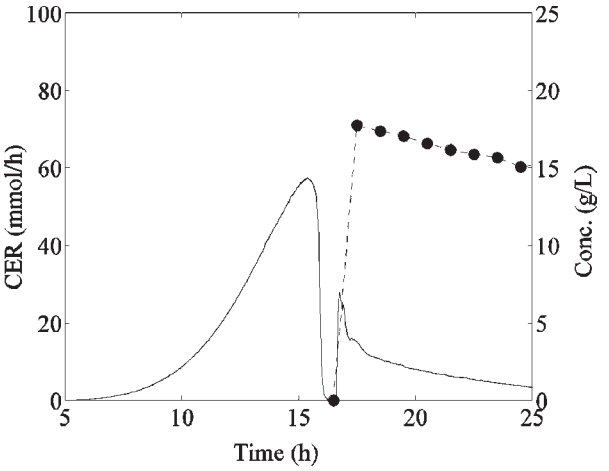

Fig. 3. CER (—) and glucose concentration (– – ● – –) during batch fermentation with dilute-acid hydrolysate A. The strain used was TMB 3000. Batch fermentations (0.5 L) on glucose were made during the first 15–17 h to produce cell mass.

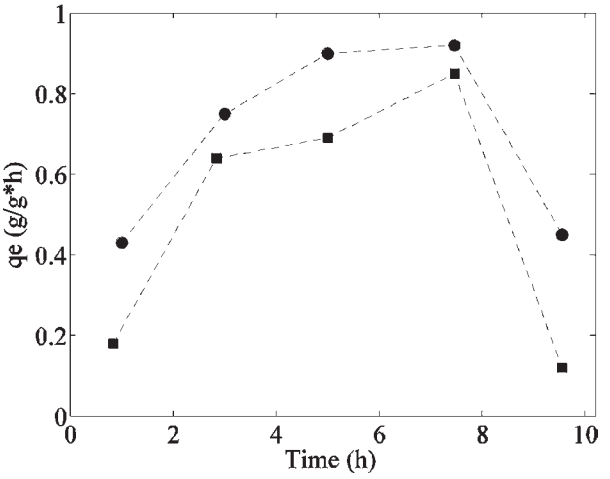

Fig. 4. Specific ethanol productivity in two fed-batch fermentations with TMB 3000. Dilute-acid hydrolysate A was used. (– – ● – –) *qe* for the fed-batch fermentation with 45 g/L of glucose in the batch phase; (– – ■ – –) *qe* for the fed-batch fermentation with 64 g/L of glucose in the batch phase.

Performance of Control

There was initially a fast and significant response in CER after a step change in the feed rate. During the first 3 h, the stepwise increases in feed rate gave sharp and smooth peaks in ν. However, during the last 5 h, the noise in the input signal created a more irregularly shaped ν profile (Fig. 7A).

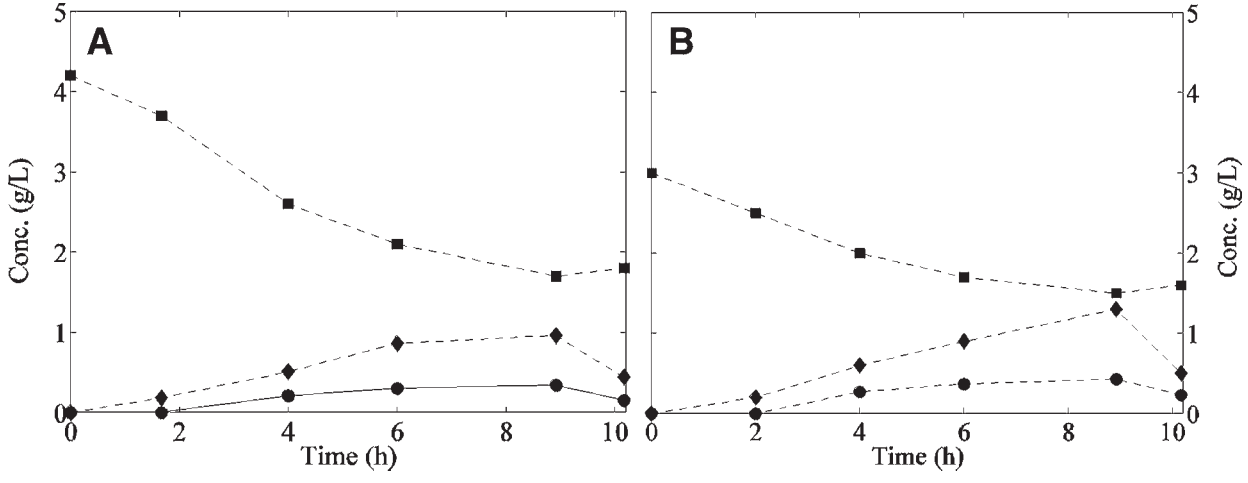

Fig. 5. Concentrations of glycerol (--■--), glucose (--◆--), and HMF (--●--) during two fed-batch fermentations with TMB 3000. Dilute-acid hydrolysate A was used. **(A)** 64 g/L of glucose in the batch phase; **(B)** 45 g/L of glucose in the batch phase.

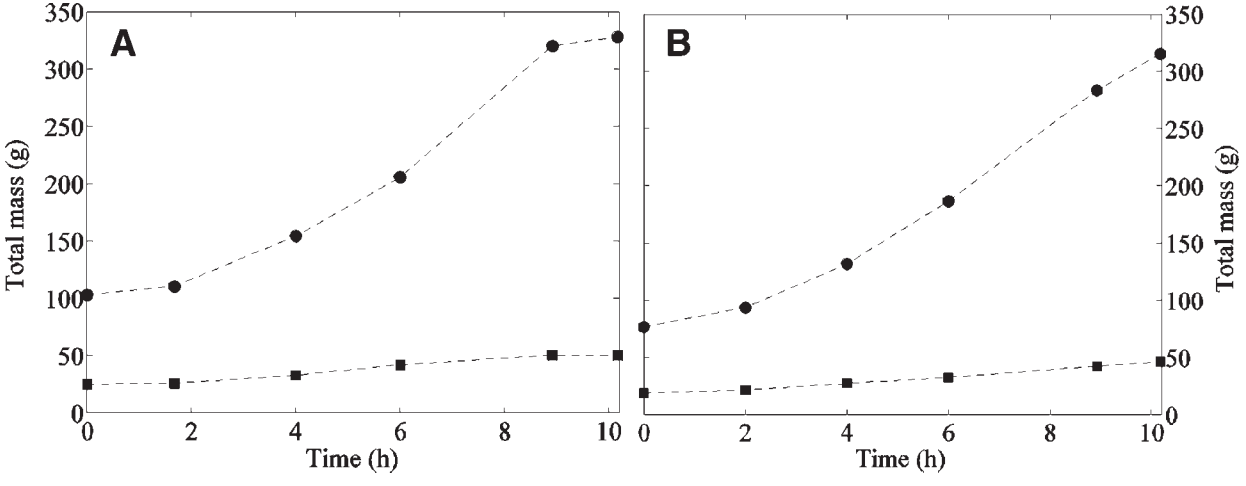

Fig. 6. Total amount of ethanol (––●––) and cells (––■––) produced during two fed-batch fermentations with TMB 3000. Dilute-acid hydrolysate A was used. **(A)** 64 g/L of glucose in the batch phase; **(B)** 45 g/L of glucose in the batch phase.

Table 4
Yield Coefficients from Fed-Batch Fermentation with TMB 3000 and Dilute-Acid Hydrolysate A[a]

		Batch phase		Fed-batch phase	
Yield coefficients	Explanation	(C-mol/C-mol)	(g/g)	(C-mol/C-mol)	(g/g)
Y_{SE}	Yield of ethanol on glucose and mannose	0.47	0.36	0.57	0.45
Y_{SX}	Yield of cell mass on glucose and mannose	0.12	0.10	0.064	0.053
Y_{SG}	Yield of glycerol on glucose and mannose	0.066	0.068	0.036	0.037
Y_{SC}	Yield of CO_2 on glucose and mannose	—	—	0.27	0.40

[a] The initial glucose concentration in the batch phase was 64 g/L. Y_{SC} could not be calculated during the batch phase because the gas bulk in the fermentor initially consisted of air and not CO_2, as when the fed-batch was started.

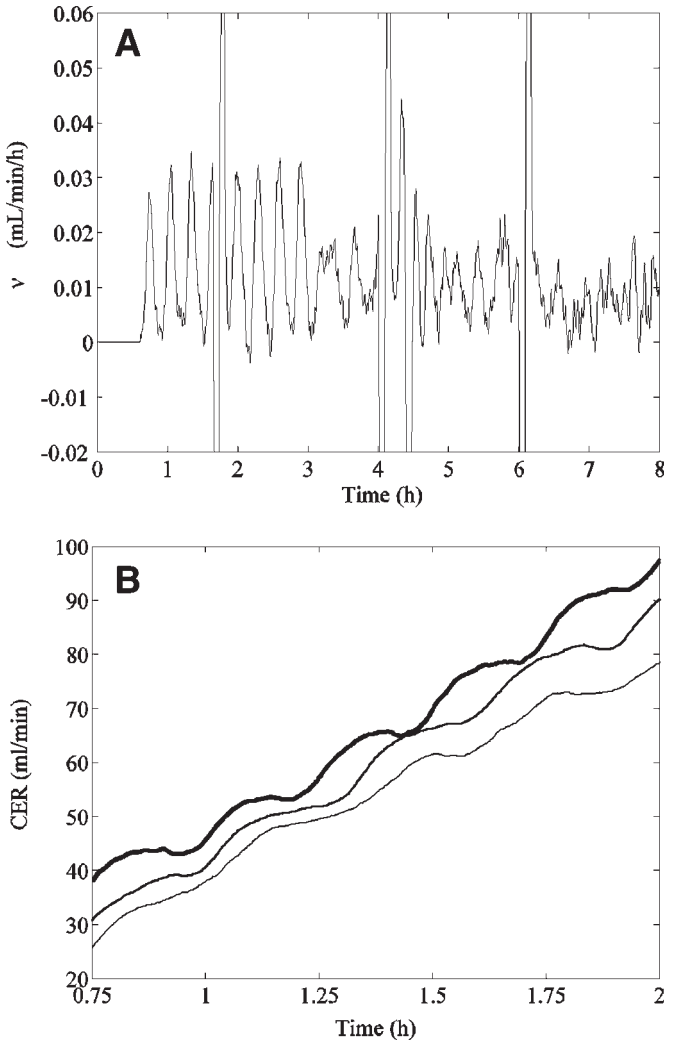

Fig. 7. Dynamics of CER responses during fed-batch fermentations in PDU scale. **(A)** CER derivative, ν, plotted during fed-batch fermentation with TMB 3000. The peaks at 1.45, 4, and 6 h are disturbances owing to sampling. **(B)** Comparison of step responses in CER: (▬)CBS 8066, 35 g/L of glucose solution; (—) TMB 3000, dilute-acid hydrolysate B; (—) CBS 8066, dilute-acid hydrolysate B.

The step length was important for a successful control. It was found that the dynamics of CER was different depending on which carbon source was used. When a 35 g/L glucose solution was used as feed, a step length of 15 min was enough for the CER to stabilize again within the pulse length (Fig. 7B). However, when dilute-acid hydrolysate was used as a substrate, a step length of 18 min had to be applied for both TMB 3000 and CBS 8066 (Fig. 7B) in order to get a $\nu < 37\%$ of ν^{max} (no increases in feed rate were made before $\nu < 37\%$ of ν^{max}).

Discussion

Problem of Measurement Noise

Using the total mass flow from the reactor as input signal in the control algorithm was overall found to work quite satisfactorily. The main improvement to be made relates to handling measurement noise. The measurement noise was not much of a problem during the first hours of the fed-batch fermentations, but it increased continuously and became problematic in the end of the fed-batch fermentations. The reason for this increased noise was not quite clear. As expected, the base addition, necessary for the pH control, contributed to the noise somewhat but was not the sole explanation. Possibly, the noise level can be explained by the changed headspace volume. With increasing liquid volumes, the headspace volume decreased. The pressure changes caused by a changed CER thereby progressively increased, taking away the "buffering capacity" of the headspace to CER fluctuations.

The used control algorithm should be modified to improve the performance in the presence of measurement noise. The time span during which the average CER is calculated could be further prolonged. This has been tested and it has a great impact on the ν profile. However, if the time span is too long, the delay time will demand increased pulse lengths in order to allow ν to decrease below 37% of ν_{max}. Another approach to moderate the effects of the noise could be to implement some kind of signal filter into the algorithm. However, the frequency and amplitude of the noise seems to be very irregular, which would probably hamper signal filtering.

Advantage of Comparing Strains in Batch and Fed-Batch Fermentations

No major difference in performance of the strains was found in the batch cultivations, but the performance was quite different during fed-batch operation. This shows the importance of using not just batch cultivations for comparing strains. Presumably, the toxicity levels in the batch case were so high that both strains were completely inhibited. However, at intermediate levels, as obtained in fed-batch cultures, the strain performances were quite different.

Acknowledgments

This work was financially supported by the Swedish National Energy Administration.

References

1 Taherzadeh, M. J., Eklund, R., Gustafsson, L., Niklasson, C., and Lidén, G. (1997), *Ind. Eng. Chem. Res.* **36,** 4659–4665.

2 Larsson, S., Palmqvist, E., Hahn-Hägerdal, B., Tengborg, C., Stenberg, K., Zacchi, G., and Nilvebrant, N.O. (1999), *Enzyme Microb. Technol.* **24,** 151–159.

3. Banerjee, N. and Viswanthan, L. (1976), *Proc. Annu. Conv. Sugar Technol. Assoc. India* **41,** G75–G80.
4 Sanchez, B. and Bautista, J. (1988), *Enzyme Microb. Technol.* **10,** 315–318.
5 Taherzadeh, M. J., Gustafsson, L., Niklasson, C., and Lidén, G. (2000), *J. Biosci. Bioeng.* **90,** 374–380.
6 Taherzadeh, M. J., Gustafsson, L., Niklasson, C., and Lidén, G. (2000), *Appl. Microbiol. Biotechnol.* **53,** 701–708.
7. Sárvári Horváth, I., Taherzadeh, M. J., Niklasson, C., and Lidén, G. (2001), *Biotechnol. Bioeng.* **75,** 540–549.
8 Palmqvist, E., Almeida, J. S., and Hahn-Hägerdal, B. (1999), *Biotechnol. Bioeng.* **62,** 447–457.
9 Modig, T., Lidén, G., and Taherzadeh, M. J. (2002), *Biochem. J.* **363,** 769–776.
10 Nilvebrant, N.-O., Persson, P., Reimann, A., de Sousa, F., Gorton, L., and Jönsson, L. J. (2003), *Appl. Biochem. Biotechnol.* **105,** 615–628.
11 Millati, R., Niklasson, C., and Taherzadeh, M. J. (2002), *Process Biochem.* **38,** 515–522.
12 Nilsson, A., Taherzadeh, M. J., and Lidén, G. (2002), *Bioprocess Biosyst. Eng.* **25,** 183–191.
13. Nilsson, A., Taherzadeh, M. J., and Lidén, G. (2001), *J. Biotechnol.* **89,** 41–53.
14 Taherzadeh, M. J., Niklasson, C., and Lidén, G. (2000), *Biotechnol. Bioeng.* **69,** 330–338.
15 Taherzadeh, M. J., Lidén, G., Gustafsson, L., and Niklasson, C. (1996), *Appl. Microbiol. Biotechnol.* **46,** 176–182.
16. Lidén, G. (2001), *Bioprocess Biosyst. Eng.* **24,** 273–279.
17. Chung, I. S. and Lee, Y. Y. (1985), *Biotechnol. Bioeng.* **27,** 308–315.