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*Abstract*

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The paper will be presented at the 11th IFAC world congress in Tallin, August 13–17, 1990

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# Substrate Control of Biotechnical Fedbatch Processes Robustness and the Role of Adaptivity

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*Abstract.* Results from experiments on laboratory scale fedbatch processes are presented as well as analysis and design of the control system. The main reason for control is to track the drastic growth in feed demand during a cultivation. Variations in the amount and quality of the inocculum makes precalculated dosage schemes of limited value to obtain reproducible cultivation conditions. Two processes have been studied on a laboratory scale, production of bakers' yeast, and production of the enzyme salicylate hydroxylase using a strain of bacteria. Direct measurement was used to monitor the feed demand. A regulator structure is proposed based on an observer for the exponentially growing feed demand. It can be viewed as a modified PID regulator around a dosage scheme, but it is less sensitive to errors in the dosage scheme than conventional PID control. The a priori knowledge of the feed profile is further relaxed by introduction of adaptation of the growth rate parameter. The obtained non-linear control system has a simple structure and stability is garanteed for a wide range of initial values using the technique of Liapunov function. The linearized system is analysed in the frequency domain and the adaptation is shown to have negligible influence on the loop phase margin. The adaptive regulator is tested in simulation against real feed profiles and shows good results.

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## Introduction

The substrate addition in most biotechnical batch processes of industrial scale is done using fixed precalculated schemes. The substrate demand increases, often exponentially, as the total cellmass grows. Even if all environment conditions are held constant it is difficult to get reproducible batches. The amount and quality of the inocculum may vary, as well as the quality of the substrate feed. In many processes it is of importance for both economy and quality of the product to grow the cells at approximately constant substrate concentrations, and it is thus appropriate to adjust the substrate dosage to the actual demand. New sensors have made such control feasible.

Two laboratory scale examples of substrate control are presented here. The first process is the classical production of bakers' yeast, *Saccharomyces cerevisiae*, growing on molasses, and the second process is production of salicylate hydroxylase using *Pseudomonas cepasia* growing on salicylate acid. The bakers' yeast consumes/produces ethanol in case of under/over feeding and this by-product can be measured on-line using a semipermeable membrane in combination with a cheap semiconductor gas sensor. In the second process the substrate concentration is directly measured using a flow spectrofotometer on a liquid filtrate (Sjövall, 1989).

In previous work, (Axelsson et al., 1988; Axelsson, 1989), is described laboratory experience with PID-control of the yeast process. Other studies utilizing an on-line ethanol sensor (Yamané et al., 1981; Dairaku et al., 1981) also report better results than those previously obtained using exhaust gas analysis of oxygen and carbondioxide, eg (Wang et al., 1977).

This paper discusses the similarity in control of the two processes, and how an increased complexity of the controllers can be explored to obtain an improved performance and robustness. After a description of the practical experience of process dynamics and disturbance properties the limitation of PID control is discussed. Then it is found appropriate with an observer for the exponential load disturbance (Axelsson, 1988b). Finally a very simple adaptation of the growth rate in the observer is shown to improve robustness and performance still maintaining good over all stability properties.

### Description of the two processes

Both processes are performed in a well-mixed fermentor, where pH and temperature are kept constant by conventional control. Stirrer speed and air flowrate are adjusted to keep a sufficient oxygen level. Massbalances give the following equations

$$\frac{dV}{dt} = F$$
$$\frac{dVX}{dt} = \mu VX \qquad (1a)$$
$$\frac{dVS}{dt} = -q_S VX + S_{in} F$$

where $V$ is the volume, $X$ the cell concentration, $S$ the substrate concentration, $F$ the manipulated substrate feedrate, and $S_{in}$ its concentration. The two parameters $\mu$, the specific growth rate, and $q_S$, the specific substrate uptake rate, have a quite complicated dependence of mainly the substrate concentration.

In case of the baker's yeast process the ethanol concentration $E$ could be described by the massbalance

$$\frac{dVE}{dt} = q_B VX \qquad (1b)$$

where $q_E$ is the specific ethanol production rate. In (Axelsson, 1988a) and (Sjövall, 1989) parameter estimation experiments are described that verify, that in case the substrate level is kept reasonably constant a linearized approximation suffices

$$\mu = \text{constant}$$
$$\Delta q_S = \alpha \Delta S$$
$$\Delta q_E = \beta \Delta S$$

giving

$$\frac{d\Delta S}{dt} = -\alpha X \Delta S + \frac{S_{in}}{V}(F - F^{\circ}) \qquad (2a)$$
$$\frac{d\Delta E}{dt} = \beta X \Delta S \qquad (2b)$$
$$F^{\circ}(t) = F_0 e^{\mu t} \qquad (3)$$

For the yeast process (2b) means an integrator, while (2a) introduces a timeconstant that typically changes from $10\,min$ to about $1\,min$ during a cultivation. For the salicylate the experiments show a very small dependence of $S$ in $q_S$, i.e. $\alpha = 0$, and (2a) means an integrator. The growth rates are $\mu = 0.2\,h^{-1}$ and $\mu = 0.4\,h^{-1}$ respectively.

The sensor systems introduce a transport delay $T_d$ and a timeconstant $T_s$. The ethanol sensor system has $T_d = 2\,min$ and $T_s = 2\,min$, while $T_d = 3\,min$ and $T_s = 0.5\,min$ for the salicylate sensor.

Both systems to be controlled are thus integrators with some additional rather fast dynamics

$$G_p(s) = \frac{K}{s(Ts+1)} \frac{e^{-sT_d}}{T_s s + 1} \qquad (4)$$

and the main requirement for the controller is to match the exponentially growing substrate feed demand $F^{\circ}$ (3). This feed demand can thus be viewed as a drastically increasing load disturbance.

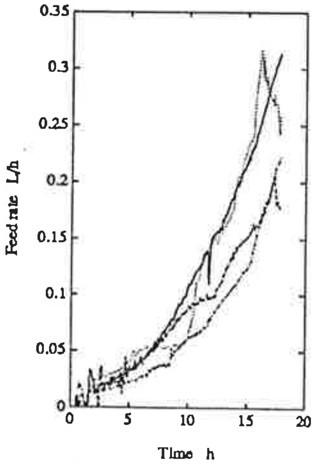


**Figure 1.** The actual feed rate in five cultivations of bakers' yeast.

## Practical experience

A number of cultivations have been done using substrate control of fedbatch cultivations (Axelsson et al., 1988) and (Sjövall, 1989). Crucial parts of the control system are of course the sensor system and the feed pump. Calibration of the sensors before and after a cultivation has been routine. However, it is difficult to check the calibration during a cultivation. The demands of the feed pump are high during a fedbatch cultivation. It should maintain high precision over a 30-fold range of pump rates. However, it has been easy to check the calibration of the pump on-line by using a load cell.

Several substrate controlled cultivations of bakers' yeast have been done using PID-control. Despite the fact that cultivation conditions were kept similar, the feed rate profiles could differ considerably, see Figure 1.

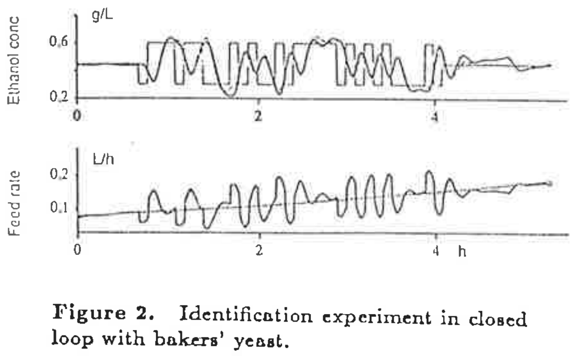


**Figure 2.** Identification experiment in closed loop with bakers' yeast.

A possible reason for the variation is the quality of the inoculum and differences in the introductory batch, but some decrease in the oxygen concentration has been inevitable, and that would influence the substrate demand.

The experience with *Pseudomonas cepasia* is that a well-tuned PI regulator suffices in most cases, but cultivations have occured where low oxygen concentrations lowered the substrate demand but also resulted in a slight increase in gain that actually resulted in unstable control.

The dynamical response of the cell culture to small variations in the feed rate was found to be highly deterministic in both cases. An example of an identification experiment (Axelsson, 1988a) is shown in Figure 2.

## Limitation of PID control

Previous experiments were performed with a PID-regulator

$$G_r(s) = K_p\left(1 + \frac{1}{T_I s} + \frac{T_D s}{1 + sT_D/N}\right)$$

around an estimate of the substrate demand, a basic dosage scheme. For design first approximate the process (4) as an integrator. A reasonable choice of parameters are $KK_p = 10\,h^{-1}$ and $T_I = 0.4\,h$ giving two poles in $-5$. The resulting load rejection, when the actual growth rate decreases, is found in Figure 4. The error in the basic dosage scheme gives an exponential load disturbance, and an ethanol error despite the integral term in the controller.

The neglected dynamics reduces the phase margin from about 75° to about 25° already for $T = 2\,min$, which is clearly too low, but a D-part with high-frequency gain $N = 4$ and $T_D = 0.07\,h$ brings it back up to about 55°. Figure 3 shows the Bode diagram for the corresponding three loop transfer functions.

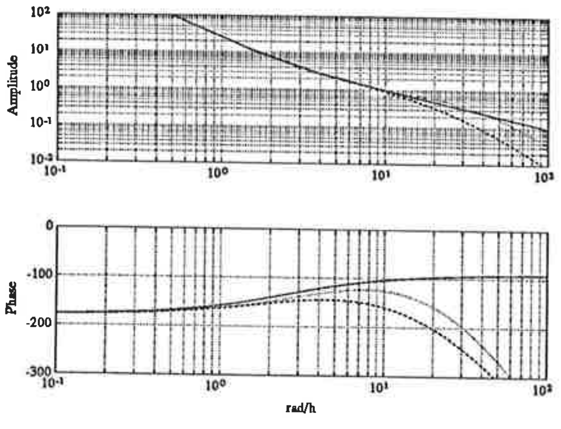


**Figure 3.** Bode diagram for the loop transfer of the process (4) approximated as an integrator with a PI-regulator (—), the full process model (4) with a PI-regulator (- - -), and the full model with a PID-regulator (···).

The low-frequency part of the loop could be retuned to give slightly better disturbance rejection, but the major problem remains, an error in the basic dosage scheme, either in $F_0$ or in $\mu$, gives rise to an exponential load disturbance, and that is hard for a PID-regulator.

## Observer for the exponential load

A regulator with an internal model of the disturbance has a potential to eliminate its influence. Actually one could regard the basic dosage as a model of the load. If that model is supplemented with feedback, i. e. an observer is used, the requirement of good estimates of the initial feed demand could be relaxed.

### Derivation of a reduced order observer

The feed demand, $F^\circ(t) = F_0 e^{\mu t}$, grows slowly, and a simple observer can be based on $G_p(s) = K/s$ as the process model.

$$\begin{cases} \dot{E} = -KF^\circ + KF \\ \dot{F}^\circ = \mu F^\circ \end{cases}$$
$$\begin{cases} \dot{\widehat{E}} = -K\widehat{E} + KF + K_{O_1}(E - \widehat{E}) \\ \dot{\widehat{F}}^\circ = \mu \widehat{F}^\circ + K_{O_2}(E - \widehat{E}) \end{cases} \tag{5}$$

where $K_{O_1}$ and $K_{O_2}$ are the observer gains. The ethanol measurement signal has a low noise level, and it is natural to use a reduced order Luenberger observer instead of the full state observer. The estimate $\widehat{F}^\circ$ of the feed demand could then be obtained as

$$\begin{aligned} \widehat{F}^\circ &= \xi + K_O y \\ \dot{\xi} &= \mu\xi + \mu K_O y + KK_O(\xi + K_O y) - KK_O F \\ y &= E - E_r \end{aligned} \tag{6}$$

A state feedback

$$F = \widehat{F}^\circ - K_R y \tag{7}$$

would then give perfect disturbance elimination, when $\widehat{F}^\circ$ is equal to the actual feed demand $F^\circ$. The two input form (6) and (7) of the regulator is valuable for the design of features for anti-windup and manual/automatic mode changes.

Neglecting such nonlinear effects the internal regulator feedback would give the observer based compensator as

$$L\{F\} = -\left(K_R - K_O - K_O \frac{KK_R + \mu}{s - \mu}\right) L\{y\} + \frac{1}{s - \mu}\widehat{F}_0$$

The regulator thus consists of three parts, a proportional feedback, an unstable dynamical part, and one part that could be interpreted as an exponential basic dosage scheme.

### Load disturbance rejection

Analysis shows that the observer based regulator has good possibilities to track the exponentially increasing feed demand (Axelsson, 1988b). Errors in the estimate of the initial feed demand $F_0$ are eliminated and the regulator is also robust against

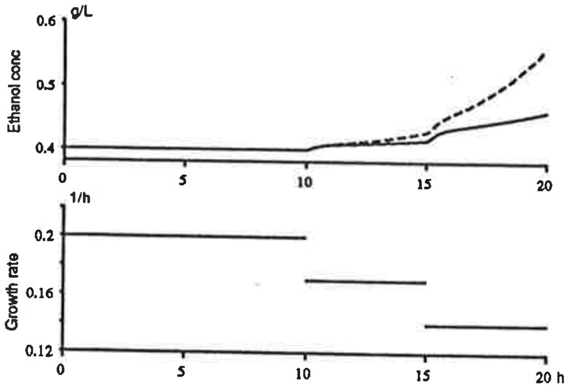


**Figure 4.** Simulation showing the disturbance rejection of the observer based regulator (—) in comparison with PI control (- - -) for changes in the actual $\mu$.

variations in $\mu$. A typical example is shown in Figure 4.

### Stability margins and phase advance

Comparison of the PID-regulator in Figure 3 and the solid line in Figure 7 describing the observer based regulator, shows that the improved load rejection was obtained without sacrificing the properties around the cross-over frequency. It would be straight forward to improve the phase margin by addition of a D-part with the same parameters as for the PID-regulator. Such a D-part could be interpreted as state feedback from an observer with one more state estimating the substrate concentration.

## Adaptation of the observer

The linear observer was based on a priori knowledge of the growth rate $\mu$ while $F^\circ(t)$ was estimated. Further, the closed loop system was found robust against certain variations in the actual growth rate, as well. However, late in the cultivation at high cell concentrations, deviations from the nominal growth rate becomes more and more difficult to meet. Since the growth rate may decrease at high cell densities due to various limitations, like for instance in the oxygen supply, it is important that the control system can handle such variations. Therefore it is interesting to try to make an estimate $\widehat{\mu}$ of the actual growth rate and use that in the regulator.

### Modification of the regulator

A simple way to adapt to variations in the growth rate is to slowly update $\widehat{\mu}$ with the deviation in the measurement signal $y = E - E_r$. The estimated parameter $\widehat{\mu}$ then replaces $\mu$ in the observer, i. e. the idea of extended Kalman filter is used. The regulator thus becomes

$$\begin{cases} F = \widehat{F}^\circ - K_R y \\ \widehat{F}^\circ = \xi + K_O y \\ \dot{\xi} = \widehat{\mu}\xi + K_O(KK_R + \widehat{\mu})y \\ \dot{\widehat{\mu}} = K_A y \end{cases} \tag{8}$$

This modified regulator was tested in simulation and showed good behaviour. In Figure 5 is shown how the regulator adapts to a step change in the actual growth rate at time 10 hours and 15 hours. For comparison it is also shown how $y$ slowly drifts away when there is no adaptation in the observer, cf Figure 5.

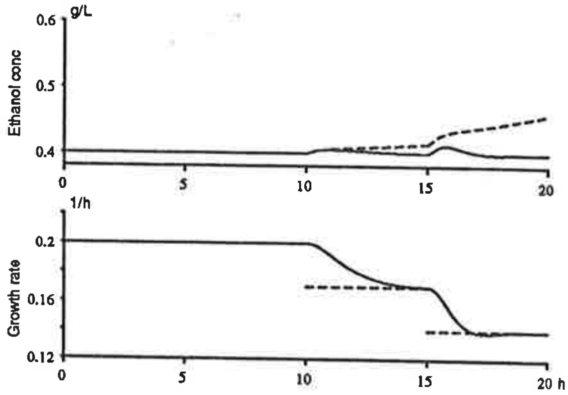


**Figure 5.** The regulator with adaptation of the observer ($K_A = -2$) tested in simulation. The top panel shows a comparison of the observer based regulator with (—) and without (- - -) adaptation, and the lower panel shows how $\widehat{\mu}$ (—) converges to the actual $\mu$ (- - -).

In Figure 6 is shown a simulation where the actual growth rate is $\mu = 0.15\ h^{-1}$ while the initial estimate is $\widehat{\mu}_0 = 0.20\ h^{-1}$. The adaptation is turned on after 5, 10 and 15 hours. Note, that during the first hours the deviation in the growth rate estimate is of little significance. Is is also seen that the convergence of the estimator is more rapid late in the cultivation.

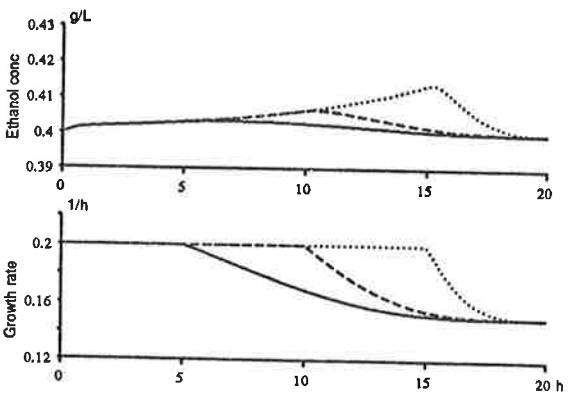


**Figure 6.** Adaptation ($K_A = -2$) is switched on after 5 (—), 10 (- - -) and 15 h (···).

The good results from simulation are encouraging. However, adaptation makes the system nonlinear and analysis of robustness properties are important.

### State space analysis of the stability region

It is convenient to study the dynamics of the closed loop system around a nominal feed demand $F^\circ(t) =$

$F_0 e^{\mu t}$. Introduce the states

$$\begin{cases} x_1 = E - E_r \\ x_2 = \xi - F^o(t) \\ x_3 = \widehat{\mu} - \mu \end{cases}$$

The system equations then becomes

$$\dot{x} = Ax + (\widehat{\mu} - \mu)Bx \qquad (9)$$

where

$$A = \begin{pmatrix} -K(K_R - K_O) & K & 0 \\ K_O(KK_R + \mu) & \mu & F^o(t) \\ K_A & 0 & 0 \end{pmatrix}$$

$$B = \begin{pmatrix} 0 & 0 & 0 \\ K_O & 1 & 0 \\ 0 & 0 & 0 \end{pmatrix}$$

The linearized system $\dot{x} = Ax$, has the characteristic equation

$$\det(\lambda I - A) = (\lambda + KK_R)(\lambda - KK_O - \mu)\lambda - KK_A F^o$$

The closed loop poles are real for the adaptation gain $K_A = 0$. When the magnitude of $K_A$ increases, two poles become complex conjugated and eventually the poles become unstable. The value of $K_A$ should be tuned for good local performance late in the cultivation. The time dependence of $F^o$ means that for constant $K_A$ the adaptation is slower early in the cultivation.

The non-linear character of the closed loop system calls for an investigation of the region of stability in the state space. Introduce the Liapunov function candidate $V(x) = x^T P x$, where $A_0^T P + PA_0 = -I$ and $A_0$ is the constant A matrix for $F^o = 0.2$ L/h, then

$$\begin{aligned} \dot{V} &= \dot{x}^T P x + x^T P \dot{x} = \\ &= x^T\big(A_0^T P + PA_0\big)x + \\ &\quad x^T\big((x_3 B^T + \Delta A^T)P + P(x_3 B + \Delta A)\big)x = \\ &= x^T\big(-I + x_3(bp^T + pb^T)\big)x \end{aligned}$$

where the time variation in $F^o$ is included as $\Delta F^o$ in $\Delta A$. The simple structure of $B + \Delta A$ implies that the second term is a sum of two dyads, where $b = \begin{pmatrix} K_O & 1 & \Delta F^o \end{pmatrix}^T$ and $p$ is the second column of $P$. The two non-zero eigenvalues are $\lambda_{1,2} = p^T k \pm \sqrt{k^T k p^T p}$. Since the matrix is symmetric, the eigenvectors form an ortogonal coordinate transformation $Qz = x$, that gives

$$\dot{V} = -(1 - x_3\lambda_1)z_1^2 - (1 - x_3\lambda_2)z_2^2 - z_3^2$$

and $\dot{V} < 0$ provided $x_3(t) \in [1/\lambda_1, 1/\lambda_2]$.

A reasonable choice of $K_A$ is in the range $[-1, -3]$. This guarantees a stable system for a wide range of initial values. The region of stability is typically $\widehat{\mu} \in [0, 0.7]$ for $\mu = 0.20$.

## Frequency domain analysis of robustness

The discussion so far, has been based on a simplified process model. The nonlinearity structure (9) utilized in the Liapunov analysis remains for more detailed process models and estimators. The robustness of the control system against time delay and neglected dynamics can be described in terms of phase- and amplitude margins in the Bode diagram of the loop transfer function the linearized system. It is especially interesting to see to what extent the adaptation gain $K_A$ influences the stability. The linearized regulator becomes

$$\begin{aligned} \dot{x} &= \begin{pmatrix} \mu & F^o \\ 0 & 0 \end{pmatrix} x + \begin{pmatrix} -K_O(\mu + KK_R) \\ -K_A \end{pmatrix} y \\ u &= \begin{pmatrix} 1 & 0 \end{pmatrix} x + \begin{pmatrix} K_R - K_O \end{pmatrix} y \end{aligned} \qquad (10)$$

Note that the $\widehat{\mu}$ adaptation introduces an extra integrator in the loop. The Bode diagram for the loop transfer function for the full process model is shown in Figure 7. Curves are given for the regulator without adaptation, $K_A = 0$, and for two different values of the adaptation gain, $K_A = -1$ and $K_A = -2$.

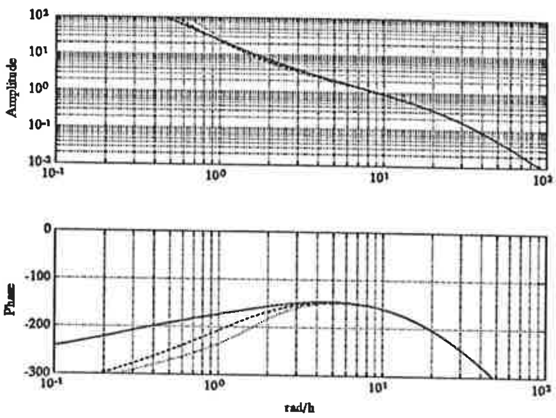


**Figure 7.** Bode diagram of the loop transfer function for the full process model. The frequency response is shown for $K_A = 0$ (—), $K_A = -1$ (- - -) and $K_A = -2$ (···) at $F^o = 0.2$ L/h.

The Bode diagram shows that for a reasonable choice of $K_A$, adaptation has a negligible influence on the frequency response around the bandwidth. However, the phase and amplitude margin at low frequencies decreases considerably when the adaptation gain is increased. Thus, the control system is sensitive to a drop in loop gain. The phase margin around the bandwidth could be increased by adding a D-part to the regulator and the tuning parallells that of the PID-regulator.

## Test in simulation

The adaptive regulator is tested in simulation on the full process model. In order to make the simulation realistic, logged feed rates during a substrate controlled cultivation of bakers' yeast, is used as $F^o(t)$.

The results from such a simulation is shown in Figure 8. The growth rate decreased slowly. After about 9 hours, the growth rate increased for a couple of hours and then went down again. The sudden increase of the growth rate late in cultivation was due to increased aeration. The adaptive regulator successfully tracked these variations in the growth rate and managed to keep the ethanol concentration close to the set-point.

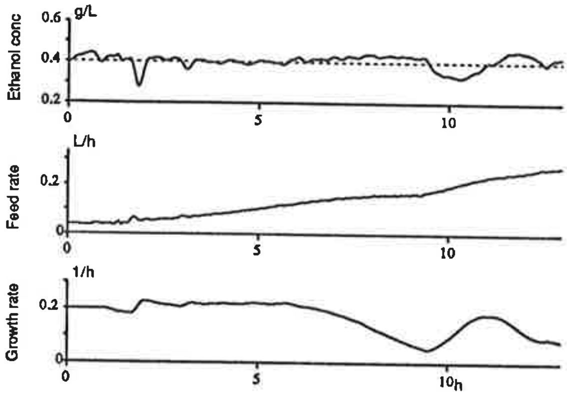


**Figure 8.** Test of the regulator in simulation using a $F^{\circ}(t)$ from a laboratory cultivation of bakers' yeast.

## Conclusion

Two different biotechnical processes are shown to pose a similar control problem, an integrator and an input load disturbance, that grows approximately exponentially. A substantial performance improvement is obtained by the load-observer as compared to a PID-regulator around a nominal exponential dosage scheme, and the simple adaptation enhances the robustness against growth rate changes. The price paid is that the controller is unstable by itself, and a considerable safety-net should be included to handle input saturation and sensor failures. The choice of the controller parameters has to be done with also the downwards gain-margin in mind.

Compared to other reports on adaptive controllers for similar processes, eg. (Pons and Engasser, 1989), (Dekkers and Voetter, 1985), and (Verbruggen et al., 1985), the use of an on-line sensor for substrate or by-product greatly facilitates the control task. It is also found possible to analyse the properties of the resulting systems. The performance robustness is good for reasonable parameter variation, but slower sensors would require a controller that relies more on process knowledge and parameter scheduling or adaptivity.

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## References


AXELSSON, J. P. (1988a): "Characterizing the substrate control problem of ethanol monitored fed-batch yeast production," in D. Seborg: *Adaptive control of chemical processes - ADCHEM'88/IFAC symposium, preprints.*, IFAC, Pergamon Press, pp. 94–99.

AXELSSON, J. P. (1988b): "On the role of adaptive controllers in fed-batch yeast production.," in D. Seborg: *Adaptive control of chemical processes - ADCHEM'88/IFAC symposium, preprints.*, Pergamon Press, pp. 115–120.

AXELSSON, J. P. (1989): "Modelling and Control of Fermentation Processes," Ph. D. thesis TFRT-1030, Department of Automatic Control, Lund Institute of Technology, Lund, Sweden.

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

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

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

PONS, M. N. and J. M. ENGASSER (1989): "Comparison between adaptive and model based extended Kalman filters," *American Control Conference 1989*, pp. 1989–1993.

SJÖVALL, N. (1989): "Reglering av salicylathydroxylasproducerande bakterie," Master thesis TFRT-5399, Department of Automatic Control, Lund Institute of Technology, Lund, Sweden.

VERBRUGGEN, H. B., G. H. B. EELDERINK and P. M. VAN DEN BROECKE (1985): "Multiloop controlled fed-batch fermentation process using a selftuning controller," in A. Johnson: *Modelling and control of biotechnological processes - 1st IFAC symposium, preprints.*, IFAC, Pergamon Press, pp. 91–96.

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

YAMANÉ, T. M. MATSUDA and E. SADA (1981): "Application of porous teflon tubing method to automatic fed-batch culture of microorganisms. II. Automatic constant-value control of fed substrate (ethanol) concentration in semibatch culture of yeast," *Biotechnol. Bioeng.*, **23**, 2509–2524.