# Optimal Operation of Fed-Batch Fermentations via Adaptive Control of Overflow Metabolite

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**Abstract**

The maximization of biomass productivity in the fed-batch fermentation of *Saccharomyces cerevisiae* is analyzed. Due to metabolic bottleneck, often attributed to limited oxygen capacity, ethanol is formed when the substrate concentration is above a critical value, which results in a decrease in biomass productivity. Thus, to maximize the production of biomass, the substrate concentration should be kept at the critical value. However, this value is unknown *a priori* and may change from experiment to experiment. A way to overcome this lack of knowledge is to allow the cells to produce a very small amount of ethanol. This way, the problem of maximizing the production of biomass is converted into that of regulating the concentration of ethanol, for which cell growth can be viewed as a perturbation. A novel adaptive control methodology based on the internal model principle is used to maintain the desired ethanol setpoint and reject the perturbation. Only a single parameter needs to be estimated on-line. Experimental results demonstrate the effectiveness of the proposed control methodology.

*Key words:* Fed-batch fermentation; Baker's yeast; Optimization; Disturbance rejection; Adaptive control; Disturbance rejection; Internal model principle.

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## 1 Introduction

For centuries, man has employed biochemical reactions to his benefit. He has used microorganisms to obtain products such as bread, cheese, wine, beer, and yoghurt, to mention only a few. Due to the importance of these products, scientists have ventured into understanding how these reactions take place inside the microorganisms and have exploited their capabilities to perform more complex and more useful transformations (Bailey and Ollis, 1986).

Though the results in this paper are generally applicable to fermentation systems with microorganisms that present an overflow metabolism, the study concentrates on the yeast *Saccharomyces cerevisiae* that is commonly used for making bread, alcohol and, recently, recombinant proteins. Numerous models have been proposed to describe the behavior of *S. cerevisiae* under different growth conditions (Bailey and Ollis, 1986; Lee, 1992). The model used in this work was proposed by Sonnleitner and Käppeli (1986).

The contributions of this paper are twofold:

- **Mathematical analysis of the optimal operation of biofermenters.** Nielsen and Villadsen (1994) argued that optimal productivity corresponds to operating at the critical substrate concentration, This will be by and large confirmed by the present analysis.

  However, it is difficult to maintain the substrate concentration at its critical value since the latter changes from experiment to experiment and from strain to strain (van Hoek *et al.*, 1998). Different methods have been proposed in the literature to circumvent this problem, *e.g.*, by tracking the respiratory coefficient (Cooney *et al.*, 1977), the oxygen uptake rate (Åkesson, 1999), or the ethanol concentration (Axelsson, 1989). This paper will show that a theoretically-sound proposition is to track the total amount of ethanol rather than the ethanol concentration. However, to implement this strategy, all liquid additions and withdrawals need to be accounted for accurately, which is not very practical. So, the option of regulating the concentration of the ethanol will be used in this paper.
- **Adaptive control of the overflow metabolite.** For the control of ethanol concentration, two simple linear models that are valid around the desired operating point are derived from the global nonlinear model of Sonnleitner and Käppeli (1986). The first model depicts the relationship between the substrate feed and the overflow metabolite concentration, while the second model describes the cell growth. Thus, as far as the regulation of the overflow metabolite is concerned, the cell growth can be considered as a disturbance to be rejected.

  A novel disturbance rejection methodology will be developed based on the internal model principle (Francis and Wonham, 1976) which states that

a model of the disturbance needs to be included in the controller denominator to be able to reject the disturbance. The proposed approach follows Tsypkin (1997) in the sense that it enables disturbance rejection without modifying the closed-loop behaviour. Since the disturbance is considered to be a growing exponential of unknown coefficient, an adaptive version of the disturbance rejection methodology is used. For this particular application, only a single parameter, which is a function of the cell growth rate, needs to be estimated on-line.

The paper is organized as follows. A macroscopic description of the behavior of *S. cerevisiae* in a fed-batch fermentation is presented in Section 2. The optimal operation of *S. cerevisiae* fed-batch fermentation is given in Section 3. Section 4 has two main parts. First, the derivation of two simple linear models used for control design is presented. Then, an adaptive controller for the regulation of the ethanol concentration is designed. Implementation issues and experimental results are given in Section 5, while Section 6 concludes the paper.

## 2 Modeling of *S. cerevisiae* Fermentation

### *2.1 Reaction stoichiometry*

The metabolism of *S. cerevisiae* can be described by the following three basic stoichiometric equations [2]:

$$\text{Oxidation of glucose: } S + a_1 O_2 \overset{r_1}{\rightarrow} b_1 X + c_1 CO_2 \tag{1a}$$

$$\text{Reduction of glucose: } S \overset{r_2}{\rightarrow} b_2 X + c_2 CO_2 + d_2 P \tag{1b}$$

$$\text{Oxidation of ethanol: } P + a_3 O_2 \overset{r_3}{\rightarrow} b_3 X + c_3 CO_2 \tag{1c}$$

The reaction components are the microorganisms, $X$, the substrate consumed by them, $S$, the product that results from their metabolism, $P$, the oxygen, $O_2$, and the carbon dioxide, $CO_2$. The constants $a_1$, $a_3$, $b_1$, $b_2$, $b_3$, $c_1$, $c_2$, $c_3$, and $d_2$ are the stoichiometric coefficients, *i.e.*, the yields of the three reactions, and $r_1$, $r_2$, and $r_3$ the rates at which the reactions take place. Experimental values for the stoichiometric coefficients are given in Table 1.

[2] A macroscopic description of the interactions that take place in a bioreactor between the cells and the environment can be written in terms of chemical reactions.

The most widely accepted model for describing the growth of *Saccharomyces cerevisiae* was proposed by Sonnleitner and Käppeli (1986) (see also Nielsen and Villadsen (1994)). It is known as the overflow metabolism or the bottleneck principle model. It assumes a limited respiratory capacity of the cells and is based on the three reactions presented in (1). Figure 1 shows a graphical description of the overflow metabolism. The arrows represent the flux of glucose metabolism, *i.e.*, the glucose uptake rate:

$$r_s = k_s \frac{S}{S + K_s} \qquad \left[\frac{g \text{ of } S}{g \text{ of } Xh}\right] \tag{2}$$

An increase in glucose concentration in the reactor results in an increase in glucose flux into the cells. This is shown as an increase in the width of the arrows. It is assumed that, as soon as the glucose enters the cells, it is metabolized via Reaction (1a) and possibly also Reaction (1b).

Each cylinder in Figure 1 can be seen as a bottleneck representing the maximum rate of the oxidative metabolism. This rate depends on the oxidative capacity of the cells, $r_o$:

$$r_o = k_o \frac{O_2}{O_2 + K_o} \qquad \left[\frac{g \text{ of } O_2}{g \text{ of } Xh}\right] \tag{3}$$

The parameters of the Monod-type equations (2) and (3) are given in Table 2. For aerobic growth, the oxygen concentration in the reactor is normally much higher than $K_o$, thus resulting in $r_o = k_o$, i.e., a constant bottleneck size.

Reaction 1a takes place as long as glucose and oxygen are sufficiently available in the reactor. Its rate is determined by the smallest of the rates at which glucose and oxygen are taken up by the cells, $r_s$ and $k_o/a_1$, respectively:

$$r_1 = \min\left(r_s, \frac{k_o}{a_1}\right) \tag{4}$$

The coefficient $a_1$ in (4) is a simple conversion factor to express the rate of the oxidative metabolism in terms of g of S. The glucose concentration at which the bottleneck saturates is defined as $S_{crit}$ for which $r_s = k_o/a_1$, i.e., $S_{crit} = k_o K_S/(a_1 k_s - k_o)$. When the glucose flux is too large to fit through the bottleneck, *i.e.*, $S > S_{crit}$, the excess will overflow into the reductive metabolism resulting in ethanol production (Reaction 1b). This is in fact what

gives this metabolism its name. The rate at which this reaction takes place is given by:

$$r_2 = \max\left(0, r_s - \frac{k_o}{a_1}\right) \tag{5}$$

If the glucose flux does not use up the whole oxidative capacity of the cell, the ethanol present in the reactor may be oxidized simultaneously with glucose via Reaction (1c). The excess oxidative capacity is given by $k_o - a_1 r_s$ and the rate at which ethanol is oxidized is therefore:

$$r_3 = \max\left(0, \min\left(r_p, \frac{k_o - a_1 r_s}{a_3}\right)\right) \tag{6}$$

where $r_p$ is given by:

$$r_p = k_p \frac{P}{P + K_p} \qquad \left[\frac{g \text{ of } P}{g \text{ of } Xh}\right] \tag{7}$$

The biomass growth rate, $\mu$, is obtained by adding the biomass production rate of each reaction:

$$\mu = b_1 r_1 + b_2 r_2 + b_3 r_3 \qquad [h^{-1}] \tag{8}$$

### *2.3 Model of a fed-batch fermenter*

With the biomass growth rate defined in (8), the mass balances for a fed-batch reactor are:

$$\frac{d(VX)}{dt} = (b_1 r_1 + b_2 r_2 + b_3 r_3)VX \tag{9a}$$

$$\frac{d(VS)}{dt} = -(r_1 + r_2)VX + FS_{in} \tag{9b}$$

$$\frac{d(VP)}{dt} = (d_2 r_2 - r_3)VX \tag{9c}$$

$$\frac{dV}{dt} = F \tag{9d}$$

where $F$ is the substrate feed, $V$ the reactor volume, and $S_{in}$ the substrate concentration in the feed. The initial conditions are given by $X_o$, $S_o$, $P_o$, and $V_o$.

## 3 Optimal Feeding Strategy

### *3.1 Optimization problem and numerical solution*

The optimal operation of a fed-batch fermenter can be formulated as follows: given operational constraints, determine the feeding strategy that optimizes a productivity objective (Nielsen and Villadsen, 1994). In the case of *S. cerevisiae*, the optimization problem can be expressed mathematically as follows:

$$\max_{F(t),t_f} \mathcal{P} = \frac{1}{t_f}\left[\frac{V_f X_f - V_o X_o}{S_{in}(V_f - V_o)}\right] \tag{10}$$

subject to dynamic equations (9):

$$\begin{aligned} V(t_f) &= V_f \\ 0 \leq F(t) &\leq F_{max} \end{aligned}$$

where $t_f$ is the final time, $V_f$ the desired final volume and $F_{max}$ the maximum value at which the substrate can be fed. The term within the brackets represents the amount of biomass produced per quantity of substrate fed into the reactor and is termed the biomass to substrate yield, $Y_{X/S}$. This term can be maximized ($Y_{X/S,max} = b_1$) with any feed that verifies $S \leq S_{crit}$. This non-uniqueness in the optimal solution results from not having a notion of time in the cost function. Thus, a cost function that includes the final time is chosen: $\mathcal{P} = Y_{X/S}/t_f$.

Srinivasan *et al.* (2001) showed that the optimal feed rate for a *S. cerevisiae* fed-batch fermentation lies either on the bounds of $F$, *i.e.*, $F_{min}$ or $F_{max}$, or on $F = F_{crit}$, where $F_{crit}$ corresponds to keeping $S = S_{crit}$ and will be derived next. At $S = S_{crit}$, the biomass growth rate $\mu$ is given by $b_1 r_{1,max} = b_1 k_o/a_1$. Since this rate is maintained constant, equation (9a) can be integrated to give the exponential cell growth:

$$VX = V_e X_e \exp\left[b_1 k_o (t - t_e)/a_1\right] \tag{11}$$

where supscript $e$ represents quantities at the beginning of the exponential growth phase. In the same way, expressing $d(VS)/dt = VdS/dt + SdV/dt$ and setting $S = S_{crit}$ and $d(VS)/dt = 0$ in equation (9b) gives:

$$F_{crit}(t) = \frac{k_o}{a_1(S_{in} - S_{crit})} V_e X_e \exp(b_1 k_o (t - t_e)/a_1) \tag{12}$$

As seen in Figure 2, the optimal feeding policy consists of various intervals which correspond to:

(1) $F = F_{max}$.
To maximize cell growth, it is important to increase the value of $V_e X_e$ before the exponential feed is started. To do this, it is necessary to quickly increase the substrate concentration in the reactor, which is done by applying $F_{max}$ up to $t_1$. However, during this time $S > S_{crit}$, which is not optimal with respect to the yield $Y_{X/S}$. Thus, the value of $t_1$ has to be chosen so as to balance these two effects.

(2) $F = F_{min}$.
The objective of this arc is to consume the substrate introduced in Arc 1 so as to reach $S = S_{crit}$ as quickly as possible. This arc terminates at time $t_2$ when $S = S_{crit}$. Since $S > S_{crit}$ during this arc, ethanol is produced.

(3) $F = F_{crit}$.
As soon as $S = S_{crit}$, $F_{crit}$ is applied. This feeding policy guarantees constant substrate concentration at $S_{crit}$ and no ethanol formation. Cells will grow exponentially until the volume reaches $V_f$ at time $t_3$. The biomass evolution is given by (11).

(4) $F = F_{min}$.
Since ethanol and some glucose are left in the reactor, a final switch to $F_{min} = 0$ allows their consumption. Increasing $t_f$ increases the amount of biomass in the numerator of the cost (10), but also increases its denominator. Thus, $t_f$ is a parameter to be optimized.

The optimal input is characterized by four intervals, the switching times $t_1$, $t_2$, $t_3$, and the final time $t_f$. The feed rate in Arcs 1, 2, and 4 is either $F_{max}$ or $F_{min}$. The switching times $t_2$ and $t_3$ are determined upon reaching $S_{crit}$ and $V_f$, respectively. Thus, the optimization problem consists of determining the times $t_1$ and $t_f$ so that the biomass productivity $\mathcal{P}$ is maximized. The main difference between the optimal results presented here and those available in the literature is the presence of Arcs 1, 2, and 4. Also, a large quantity of ethanol is present in the reactor in Arc 3, when the substrate concentration is maintained at its critical value. The experimental conditions used to generate the optimal input in Figure 2 are given in Table 3. The expression for the reaction rates $r_1$, $r_2$, and $r_3$ are taken from Section 2.2. The optimal state behavior is given in Figure 3. Notice how the ethanol concentration $P$ decreases as a result of increasing volume. However, the product $VP$ remains constant throughout Arc 3. The optimization gives $\mathcal{P} = 0.0244$ [g of $X$/ g of $S$/ h] for the optimal values $t_1^* = 0.29$ h, $t_2^* = 8.4$ h, $t_3 = 19.18$ h, and $t_f^* = 19.73$ h.

### *3.2 Implementation of the optimal strategy*

Maintaining the substrate concentration at $S_{crit}$ is very difficult in practice since its exact value is unknown Some of the approaches that have been used to tackle this problem are presented next. They all involve tracking some

quantity in an effort to implement optimal growth at $S = S_{crit}$.

- **Respiratory quotient:** The most common way of controlling a fed-batch fermenter is by tracking the respiratory quotient (RQ) near a value of 1 (Cooney *et al.*, 1977). RQ is the ratio between the carbon dioxide evolution rate (CER) and the oxygen uptake rate (OUR). These signals are easily obtained on-line. However, it was shown by Chen *et al.* (1995) that RQ is a nonlinear function of the substrate, ethanol and oxygen concentrations. Infinitely many configurations of these concentrations can give the same value of RQ. Furthermore, experimental problems such as foaming make the RQ signal unreliable.
- **Oxygen uptake rate:** Åkesson (1999) used the change in the oxygen uptake rate response to determine $S - S_{crit}$. The shape of the dissolved oxygen concentration indicates whether the substrate concentration in the reactor is above or below $S_{crit}$. A large number of empirical rules have to be followed in order to properly tune the controller, thus making this approach too complex to be used in practice.
- **Total ethanol amount:** As seen in Figure 3, maintaining the product $VP$ constant results in $S = S_{crit}$. In order to maintain $VP$ constant, it is necessary to have an accurate measurement of both ethanol and volume. This means that all liquid additions and withdrawals such as evaporation, sampling, base addition for pH control, antifoam addition, etc. have to be taken into account. Hence, it is simpler to maintain $P$ rather than $VP$ constant.
- **Ethanol concentration:** Another way of tracking the critical growth rate is to allow a small but measurable production of ethanol (Axelsson, 1989; Chen *et al.*, 1995; Rani and Rao, 1999; Preuss *et al.*, 2000). Though this strategy is suboptimal, it comes quite close to the optimal one as the reference value for the ethanol concentration approaches zero.

## 4 Adaptive Control Strategy

### *4.1 Introduction*

In this paper, the optimization of a fed-batch fermentation of *S. cerevisiae* will be implemented *via* the controlled production of a very small amout of ethanol ($S$ slightly above $S_{crit}$, $r_2 > 0$). The key problem is the fact that the biomass, and with it also the feed, grow exponentially.

In the work of Axelsson (1989), the PI controller used for controlling the ethanol production was not able to cope with the exponential cell growth. Chen *et al.* (1995) and Farza *et al.* (1998) tried to tackle this problem with

the estimation of the exponential growth rate (Bastin and Dochain, 1990) together with adaptive nonlinear control. However, these techniques require the tuning of several parameters. In a more recent work, Preuss *et al.* (2000) implemented a predictive controller that requires precise knowledge of certain model parameters as well as on-line measurement of ethanol, carbon dioxide and oxygen concentration.

In this work, an adaptive controller that allows rejecting the unknown unstable time-varying disturbance is derived. This methodology is based on the Internal Model Principle (Francis and Wonham, 1976; Johnson, 1985), which states that an appropiate unstable pole must be included in the feedback controller to cancel out the unstable disturbance. In particular, the work presented here follows Tsypkin (1991) which adds an extra degree of freedom to a two-degree-of-freedom RST controller (Åström and Wittenmark, 1997).

### *4.2 Simplified linear models*

One of the novel contributions of the proposed approach consists of deriving two simple tailor-made models to describe the evolution of the ethanol concentration and the cell growth. It will be assumed here that the fed-batch reactor can be operated in such a way that the substrate concentration is slightly above $S_{crit}$ and thus a small production of ethanol always takes place. The following two assumptions are valid in the neighbourhood of the desired operating point:

**Assumption 4.1** *The ethanol production rate is small.*

This way, Reaction (1a) is saturated ($r_1 = k_o/a_1$). The cell growth rate (8) and the resulting exponential cell growth (11) become:

$$\mu \approx b_1 \frac{k_o}{a_1} = \bar{\mu} \tag{13}$$

$$VX = V_e X_e \exp\left[\bar{\mu}(t - t_e)\right] \tag{14}$$

Note that the main consequence of this assumption is the independence of the cell growth rate $\bar{\mu}$ from the substrate feed rate $F$.

**Assumption 4.2** *The substrate dynamics are fast, i.e., $VS$ is in quasi-steady state.*

This assumption is justified since a small variation in the substrate feed rate $F$ will result in an almost instantaneous change in the amount of substrate

$VS$ present in the reactor. Thus, $d(VS)/dt \approx 0$, and (9b) gives:

$$r_2 VX = FS_{in} - \frac{k_o}{a_1} VX \tag{15}$$

This equation shows that the rate at which glucose is transformed into ethanol is obtained from the difference between the rate at which substrate is fed into the reactor, $FS_{in}$, and the rate at which the cells oxidize it, $k_o/a_1 VX$.

Substituting $r_2 VX$ from (15) into the ethanol mass balance (9c) with $r_3 = 0$ leads to the ethanol production dynamics:

$$\frac{d(VP)}{dt} = d_2 S_{in} \left( F - \frac{k_o VX}{a_1 S_{in}} \right) \tag{16}$$

Since the amount of biomass produced through Reaction (1a) is much larger than the amount produced via Reaction (1b), *i.e.*, $b_1 >> b_2$, biomass is considered to be produced mainly through the oxidative pathway. In fact, as long as $r_2 > 0$, biomass production is not influenced by changes in the substrate feed rate. Thus, with respect to the production of ethanol, substrate oxidation will be considered as an input disturbance for $F$. With (14), equation (16) becomes:

$$\frac{d(VP)}{dt} = d_2 S_{in} \left( F - w \right) \tag{17}$$

where

$$w = \frac{k_o VX}{a_1 S_{in}}$$

In a batch fermenter, the volume varies with time between $V_o$ and $V_f$. Yet, upon considering $d(VP)/dt = VdP/dt + PdV/dt$ and a constant average volume $\bar{V} = (V_o + V_f)/2$, the ethanol dynamics in (17) can be rewritten as:

$$\frac{dP}{dt} = \frac{d_2 S_{in}}{\bar{V}} \left( F - w \right) \tag{18}$$

with the disturbance $w$, corresponding to the exponential substrate oxidation, given by:

$$w(t) = K_w \exp[\bar{\mu}(t - t_e)] \tag{19}$$

where $K_w = k_o V_e X_e / (a_1 S_{in})$.

The disturbance (19) and the ethanol concentration dynamics (18) can be expressed as discrete transfer functions as follows:

$$w(kh) = \frac{K_w}{1 - \gamma q^{-1}} \delta(kh) = \frac{\mathcal{N}(q^{-1})}{\mathcal{D}(q^{-1})} \delta(kh) \tag{20}$$

$$P(kh) = \frac{K_p q^{-1}}{1-q^{-1}}\Big(F(kh) - w(kh)\Big) = \frac{\mathsf{B}(q^{-1})}{\mathsf{A}(q^{-1})}\Big(F(kh) - \frac{\mathcal{N}(q^{-1})}{\mathcal{D}(q^{-1})}\delta(kh)\Big) \quad (21)$$

with $h$ the sampling period, $kh$ the sampling instant, $\delta(kh)$ the unit pulse, $\gamma = \exp(\bar{\mu}h)$ and $K_p = d_2 S_{in} h/\bar{V}$.

The block diagram of Figure 4 shows how the biomass and ethanol production rates have been decoupled. Since the reactor will be operated at constant ethanol concentration, the objective will be to feed glucose into the reactor in order for the cells to grow exponentially and to produce the slight amount of ethanol that is sufficient to keep its concentration constant.

### *4.3 RST controller with $\mathsf{Q}$ parameterization*

The controller used in this work is adapted from Tsypkin (1997) and Holmberg *et al.* (1997). The control law is given by:

$$\mathsf{R}_o(q^{-1})F(kh) = -\mathsf{S}_o(q^{-1})P(kh) + \mathsf{T}(q^{-1})P_{sp}(kh) - \mathsf{Q}(q^{-1})w_B(kh) \quad (22)$$

where $P_{sp}$ is the desired ethanol setpoint. $\mathsf{R}_o$, $\mathsf{S}_o$, $\mathsf{T}$, and $\mathsf{Q}$ are polynomials in the backward-shift operator $q^{-1}$. $\mathsf{Q}$ is a polymonial used for disturbance rejection that can be chosen freely without altering the closed-loop characteristic polynomial. $w_B(kh)$ corresponds to a filtered version of the disturbance, $w_B(kh) = B(q^{-1})w(kh)$, obtained from the feedrate and ethanol measurements:

$$w_B(kh) = \mathsf{A}(q^{-1})P(kh) - \mathsf{B}(q^{-1})F(kh) \quad (23)$$

The dependency of the operators and the signals on $q^{-1}$ and $kh$, respectively, will be omitted for brevity, unless needed for clarity.

From the plant equation (21) and the controller equations (22) and (23), and upon defining the closed-loop characteristic polynomial $\mathsf{A}_c = \mathsf{A}\mathsf{R}_o + \mathsf{B}\mathsf{S}_o$, the closed-loop response becomes:

$$P = \frac{\mathsf{B}\mathsf{T}}{\mathsf{A}_\mathrm{c}}P_{sp} - \frac{(\mathsf{R}_o - \mathsf{Q}\mathsf{B})}{\mathsf{A}_\mathrm{c}}\frac{\mathsf{B}\mathcal{N}}{\mathcal{D}}\delta \quad (24)$$

The second term in the RHS of (24) represents the effect of the disturbance:

$$e_d = -\frac{(\mathsf{R}_o - \mathsf{Q}\mathsf{B})}{\mathsf{A}_c}\frac{\mathsf{B}\mathcal{N}}{\mathcal{D}}\delta = -\frac{(\mathsf{R}_o - \mathsf{Q}\mathsf{B})}{\mathsf{A}_c}w_B \quad (25)$$

The corresponding closed-loop diagram is shown in Figure 5. The poles of $e_d$ are given by the roots of $\mathsf{A}_\mathrm{c}$ and $\mathcal{D}$. The roots of $\mathsf{A}_\mathrm{c}$ are stable by construction

whereas $\mathcal{D}$ has one unstable root, $\gamma = \exp(\bar{\mu}h)$. For large enough $kh$, the effect of the stable roots of $\mathsf{A}_\mathrm{c}$ disappears. The disturbance can then be rejected asymptotically by solving the Diophantine equation:

$$\mathsf{R}_o - \mathsf{Q}\mathsf{B} = \mathsf{M}\mathcal{D} \tag{26}$$

with $\mathsf{M}$ being a polynomial in $q^{-1}$. Algorithms for solving a Diophantine equation are given by Kučera (1979). If $\mathcal{D}$ is known, the choice of $\mathsf{Q}$ that solves (26) will cancel the unstable terms in the denominator of (25), thereby making the disturbance effect go to zero asymptotically. Note that $\mathsf{Q}$ is a scalar since there is only a single unstable pole in $e_d$.

The difficulty arises when only an imperfect and/or incomplete disturbance model is available. This is the case for the fed-batch fermentation under study, since the exponential growth rate is unknown *a priori*. Thus, an approach to adapt the $\mathsf{Q}$ polynomial on-line is proposed. The expression for the disturbance effect given in (25) can be rewritten as:

$$e_d(kh) = -\epsilon_1(kh) + \epsilon_2(kh)\mathsf{Q} \tag{27}$$

where the signals $\epsilon_1$ and $\epsilon_2$ are defined as:

$$\epsilon_1(kh) = \frac{\mathsf{R}_o}{\mathsf{A}_\mathrm{c}} w_B(kh) \tag{28a}$$

$$\epsilon_2(kh) = \frac{\mathsf{B}}{\mathsf{A}_\mathrm{c}} w_B(kh) \tag{28b}$$

Equation (27) corresponds to a linear regression problem (Ljung, 1987). An on-line estimate of $\mathsf{Q}$ can be obtained by minimizing the following criterion:

$$\min_{\mathsf{Q}} J(\mathsf{Q}, k) = \frac{1}{2} \sum_{i=1}^{k} \lambda^{k-i} \left[\epsilon_1(ih) - \epsilon_2(ih)\mathsf{Q}\right]^2 \tag{29}$$

where $\lambda \leq 1$ is known as the forgetting factor. The on-line adaptation of the optimal solution, $\mathsf{Q}^*(kh)$, can be done using the following recursive least-squares algorithm (Ljung, 1987):

$$H(kh) = \frac{H(kh-h)}{\lambda + H(kh-h)\epsilon_2^2(kh)} \tag{30a}$$

$$\mathsf{Q}^*(kh) = \mathsf{Q}^*(kh-h) + H(kh)\epsilon_2(kh)\left[\epsilon_1(kh) - \epsilon_2(kh)\mathsf{Q}^*(kh-h)\right] \tag{30b}$$

It has been shown in Valentinotti (2001) that $\mathsf{Q}^*(Mh) \to \mathsf{Q}_D$, and $e_d \to 0$ as $M \to \infty$, where $\mathsf{Q}_D$ is the solution of the Diophantine equation. Thus, the unknown disturbance can be rejected asymptotically.

The overall adaptive control procedure can be summarized as follows:

- With the new measurement $P(kh)$, (30a)-(30b) are used to estimate $\mathsf{Q}^*(kh)$.
- The estimated $\mathsf{Q}^*(kh)$ is then used together with $\mathsf{R}_o$ and $\mathsf{S}_o$ in (22)–(23) to calculate the control signal $F(kh)$ that is applied to the fermentation system.

## 5 Experimental Results

### *5.1 Experimental setup*

A 16-liter stirred tank bioreactor (Bioengineering AG, Wald, Switzerland) was used to cultivate *S. cerevisiae* (W303-1A) in defined medium (see Cannizzaro (2002) for the detailed medium composition). Temperature, dissolved oxygen concentration, and pH were controlled by the reactor control box. Air was sparged into the reactor at the rate of 10 l/min. The fed-batch process was started with 7 l of medium containing 3 g/l of glucose. Initial biomass concentration was 0.85 g/l. The feed contained 5 l of medium with 300 g/l of glucose. A customized bioprocess management and control environment, BioOPT, was developed with LabVIEW (National Instruments, Austin, TX) to run the process.

### *5.2 Measurement system*

Ethanol concentration was measured with a mid-infrared spectrometer (ASI Applied Systems, Millerville, MD) equipped with an ATR diamond probe. The spectroscopic detector provided BioOPT with ethanol concentration measurements every six minutes, *i.e.*, $h = 0.1$ h. The calibration of the mid-infrared spectrometer showed an error of 0.07 g/l (Rhiel *et al.*, 1999). Thus, the smallest ethanol setpoint that could be chosen, considering that the calibration error should not exceed 10% of the measured signal, was $P_{sp} = 0.7$ g/l.

### *5.3 Adaptive control*

The only non-operational parameter needed for controlling the fed-batch fermentation of *S. cerevisiae* using (22), (23), and (30) is $d_2$. The experimental value of this parameter can be found in the literature. Sonnleitner and Käppeli (1986) proposed $d_2 = 0.48$ g of P/ g of S. With the sampling period $h = 0.1$ h, the feed substrate concentration $S_{in} = 400$ g/l, and the initial and final

volumes of 7 and 12 liters respectively, giving $\bar{V} = 9.5$ l, the gain for the transfer function (21) is $K_p = 2.02$. With $\mathsf{A} = 1 - q^{-1}$, $\mathsf{B} = K_p q^{-1}$ and the choice $\mathsf{A}_c = 1 - 0.95q^{-1}$, the following proportional controller is found: $\mathsf{R}_o = 1$, $\mathsf{S}_o = 0.025$. $\mathsf{T}$ is chosen as the scalar value that provides zero steady-state error, *i.e.*, $\mathsf{T} = \mathsf{A}_c(1)/\mathsf{B}(1) = 0.025$. From (20) and (26) with $\mathsf{M} = 1$, it follows that $\mathcal{D} = 1 - \gamma q^{-1} = \mathsf{R}_o - \mathsf{QB}$, then $\gamma = \mathsf{Q}K_p$.

### *5.4 Results and Discussion*

The experiment was started by adding a small amount of substrate to the fermenter to increase the substrate concentration up to 3 g/l. This corresponds to Arc 1 in the optimization procedure described in Section 3.1. Cells started to grow and produce ethanol which reached a concentration of 2 g/l. In this case, and in contrast to the optimal solution, the ethanol formed was allowed to be consumed. The reason for this, as explained before, was the lack of a reliable volume measurement. When the ethanol concentration reached a value of 0.7 g/l, at $t \approx 7$ h, the controller was started.

As shown in Figure 6, the proposed control algorithm was able to maintain the ethanol concentration around the setpoint $P_{sp} = 0.7$ g/l throughout the experiment. Figure 7 shows a plot of the oxygen uptake rate, OUR, and the carbon dioxide evolution rate, CER. The quotient CER/OUR is generally referred to as the respiratory quotient, RQ. RQ $> 1$ indicates that ethanol is being formed. Figure 7 shows how the CER signal oscillates around the OUR signal. Saturation of the oxygen uptake capability of the cells can be inferred from the smooth OUR signal.

The controller response to changes in the operating conditions was tested at $t \approx 17$ h when the pressure in the reactor was abruptly released. This caused the dissolved oxygen concentration to decrease, with the consequence that the production of ethanol increased (see Figure 6). An increase in ethanol production is also reflected by an abrupt increase in the CER as shown in Figure 7. The controller reacted by reducing the feed rate until the ethanol concentration returned to setpoint. The pressure in the reactor went back to its original value and the situation normalized after a short time. At $t \approx 21$ h, the dissolved oxygen in the reactor became limiting, resulting in a shift in the value of the critical growth rate. As seen in Section 2.2, the lack of oxygen reduces the bottleneck size. The available oxygen was insufficient to oxidize all of the glucose present and, as a consequence, some ethanol was produced. The controller reacted by adjusting the feed rate which was no longer exponential.

A plot of the natural logarithm of the total biomass ($VX$) is given in Figure 8. A curve was fitted through the linear part of this plot. The slope of this

line corresponds to the exponential growth rate and was calculated to be $\bar{\mu} = 0.27\ \mathrm{h}^{-1}$, which is in agreement with the literature values (Nielsen and Villadsen, 1994). Oxygen limitation at the end of the experiment results in a decrease of the exponential growth rate.

## 6 Conclusions

The optimal operating strategy for the fed-batch fermentation of *S. cerevisiae* has been determined. The optimal feeding policy suggests maintaining a constant value of $VP$ during the exponential growth. However, due to practical reasons, this was not possible. Instead, a suboptimal solution, which consists of maintaing a constant product concentration while feeding exponentially, was used.

The fed-batch fermentation of *S. cerevisiae* was modeled as two simple linear models that capture the main macroscopic processes taking place in the reactor: 1) exponential substrate uptake for cell growth, and 2) very small production of ethanol. The first process is taken as a disturbance to be rejected, while the second one represents the plant to be controlled.

An adaptive control strategy for the rejection in closed-loop of unstable disturbances has been proposed. An extra degree of freedom, which can explicitly take into account disturbances, is added to a two-degree-of-freedom controller. This way, the disturbance model is implicitly included into the controller denominator, thereby allowing to reject the disturbance.

The proposed control methodology was implemented in a fed-batch fermentation of *S. cerevisiae*. Ethanol concentration was maintained constant while biomass grew exponentially. Only one parameter needed to be estimated on-line. The ethanol concentration was inferred from on-line spectroscopic measurements. Tuning of the controller required the knowledge of only one stoichiometric coefficient.

The optimization, modeling, and control methodologies presented in this paper can also be used for the regulation of other quantities related to the exponential cell growth in fed-batch fermentations. Glucose and/or dissolved oxygen are just two other examples. Furthermore, the proposed approach can also be used in fed-batch fermentations of other microorganisms presenting an overflow metabolite metabolism.

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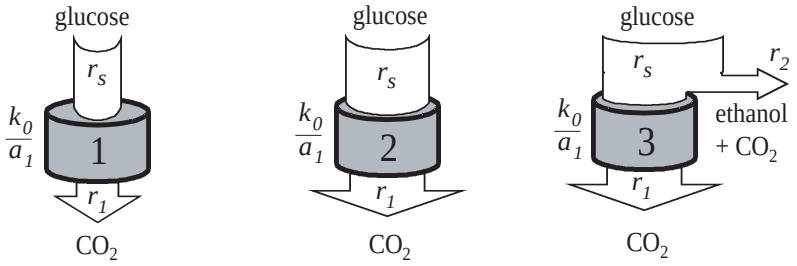


Fig. 1. Overflow metabolism (Sonnleitner and Käppeli, 1986). Three metabolic flux models where the substrate passes through a limitation or "bottleneck" in the metabolism are shown. For Case 1, there is excess capacity and no metabolite is formed. Case 2 represents growth at the critical rate where any excess substrate would "overflow" to ethanol. In Case 3, the excess growth above the critical rate is represented by the ethanol flux.

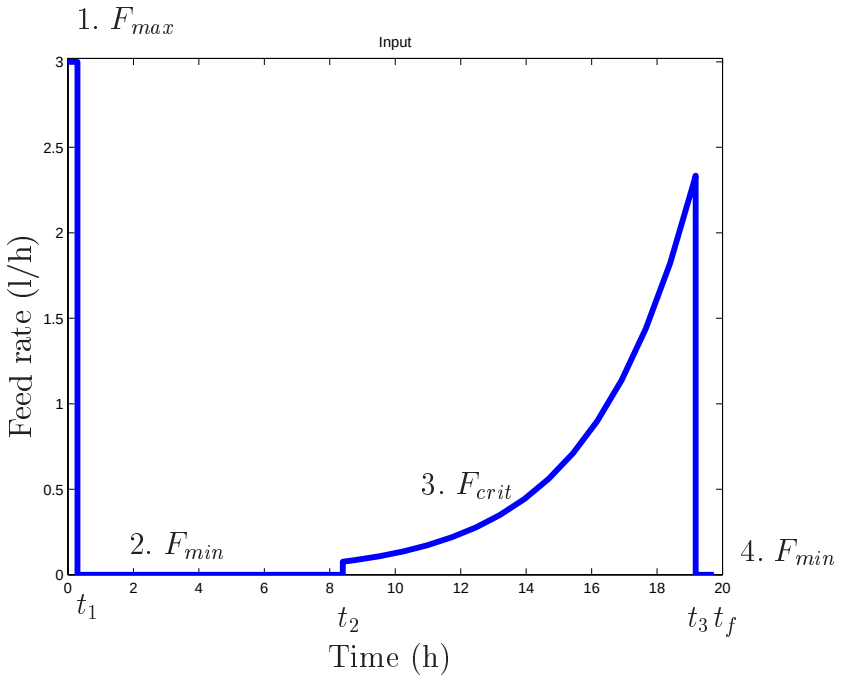


Fig. 2. Optimal feeding policy for the fed-batch fermentation of *S. cerevisiae*. Four arcs are needed: 1. $F_{max}$, 2. $F_{min}$, 3. $F_{crit}$, 4. $F_{min}$.

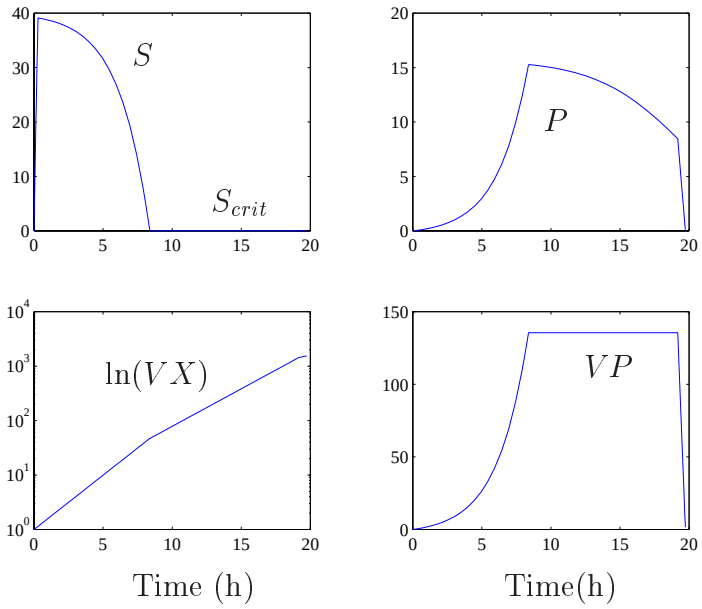


Fig. 3. The optimal operation corresponds to initially increase quickly the substrate concentration in the fermenter. At some optimal time $t_1$, the feed is stopped and the cells are allowed to consume the substrate until $S = S_{crit}$. The product VP is then kept constant by feeding the substrate exponentially. When the maximal volume is reached, the substrate and ethanol remaining in the reactor are allowed to be consumed until the optimal final time $t_f$.

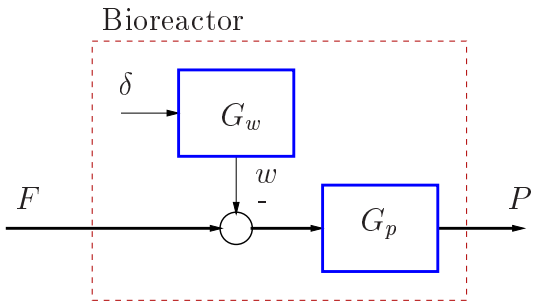


Fig. 4. Schematic representation of the simplified fed-batch fermentation model. The transfer functions $G_w$ and $G_p$ correspond to the exponential substrate uptake for cell growth and ethanol production, respectively

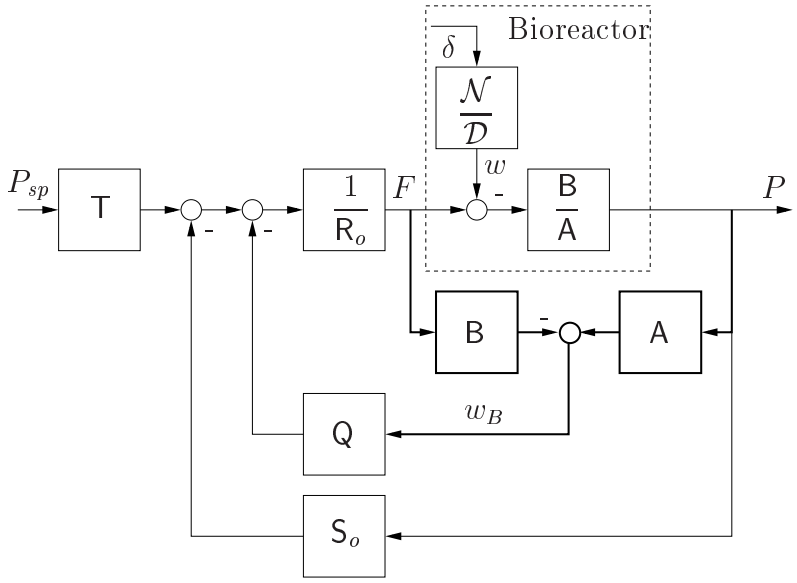


Fig. 5. Closed-loop diagram for the bioreactor characterized by $\mathsf{A}$, $\mathsf{B}$, $\mathcal{N}$, and $\mathcal{D}$, and using the control law (22). It is seen (in bold) how an internal estimate of the disturbance is implicitly constructed and used by the $\mathsf{Q}$ parametrization.

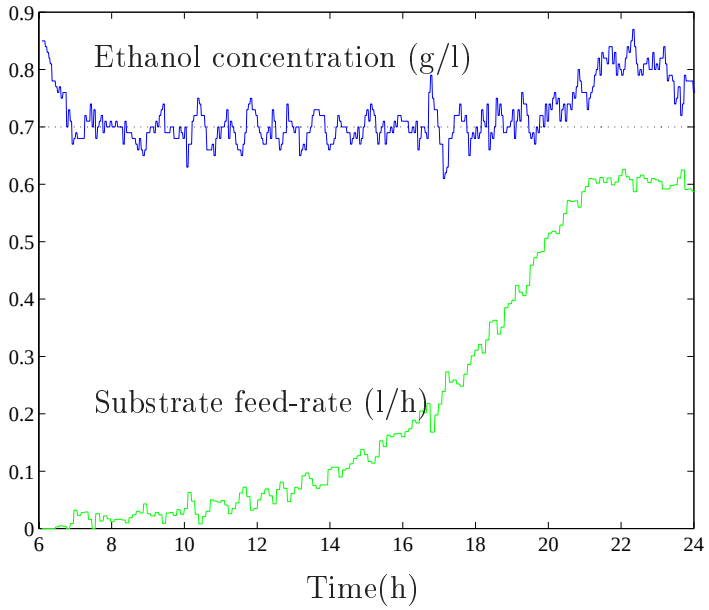


Fig. 6. Fed-batch fermentation of *S. cerevisiae*. The controller was started when the ethanol concentration reached 0.7 g/l. At $t \approx 21$ h, the oxygen concentration in the reactor was depleted and the cells stopped growing exponentially.

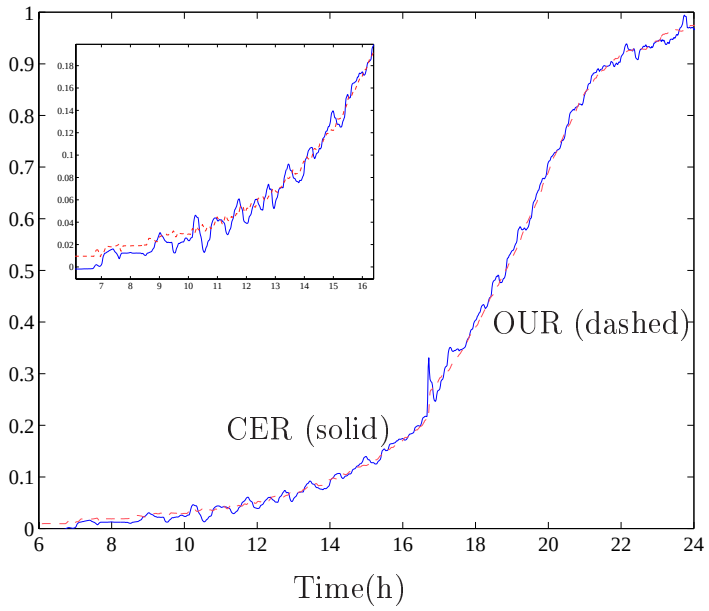


Fig. 7. On-line gas analysis. The dashed and solid lines show the OUR and CER, respectively. Notice that the OUR is a smooth line while sustained oscillations are present in the CER signal. This is more clearly seen in the inset which shows a magnification of the first 16 hours.

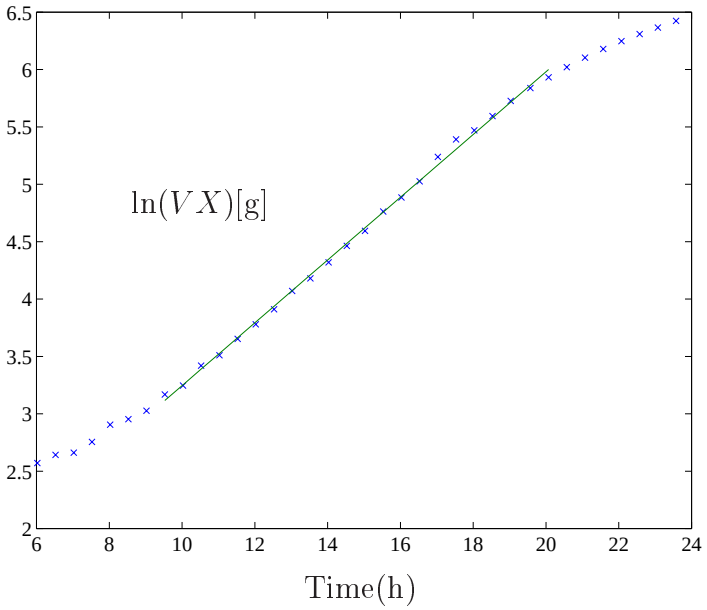


Fig. 8. Logarithm of the total biomass ($VX$) *vs.* time. A linear fit of the biomass during the exponential growth phase gives the growth rate $\bar{\mu} = 0.27$ h$^{-1}$.

| parameter | value | units |
|---|---|---|
| $a_1$ | 0.396 | g of $O_2$/g of $S$ |
| $b_1$ | 0.490 | g of $X$/g of $S$ |
| $c_1$ | 0.590 | g of $CO_2$/g of $S$ |
| $b_2$ | 0.050 | g of $X$/g of $S$ |
| $c_2$ | 0.462 | g of $CO_2$/g of $S$ |
| $d_2$ | 0.480 | g of $P$/g of $S$ |
| $a_3$ | 1.104 | g of $O_2$/g of $P$ |
| $b_3$ | 0.720 | g of $X$/g of $P$ |
| $c_3$ | 0.625 | g of $CO_2$/g of $P$ |

Table 1
Stoichiometric coefficients of Reaction mechanism (1).

| parameter | value | units |
|---|---|---|
| $k_s$ | 3.500 | g of $S$/g of $X$ h |
| $k_o$ | 0.256 | g of $O_2$/g of $X$ h |
| $k_p$ | 0.170 | g of $P$/g of $X$ h |
| $K_s$ | 0.100 | g of $S$/l |
| $K_o$ | 0.100 | mg of $O_2$/l |
| $K_p$ | 0.100 | g of $P$/l |

Table 2
Kinetic parameters for the rates $r_s$, $r_o$, and $r_p$.

| | |
|---|---|
| $V_o$ | 8 l |
| $V_oX_o$ | 1 g |
| $V_oP_o$ | 0 g |
| $V_oS_o$ | 0 g |
| $S_{in}$ | 400 g/l |
| $V_{max}$ | 16 l |
| $F_{max}$ | 3 l/h |

Table 3
Experimental conditions and bounds for the fed-batch optimization.