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A Case Study of Adaptive Nonlinear Regulation of Fed-batch Biological Reactors*

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A general methodology for the design of adaptive regulators for fed-batch biological reactors is presented with the aid of an illustrative case study, the regulation of the ethanol concentration in a fed-batch yeast production process.

Key Words—Nonlinear biological systems; adaptive control; nonlinear control; process control.

Abstract—Fed-batch biological reactors are good candidates for the application of adaptive nonlinear techniques because they involve kinetic parameters that are highly uncertain and slowly time-varying. In this paper, the issue is illustrated and discussed with the aid of an industrial application: the ethanol regulation in yeast production processes.

1. INTRODUCTION

Dynamics of fed-batch biological stirred tank reactors are commonly described by a set of ordinary differential equations arising from mass balances of the biological species (microorganisms, enzymes, substrates, etc.) involved in the process. A basic difficulty for the application of modern control science to these processes lies in the fact that the models usually include kinetic parameters which are highly uncertain and slowly time-varying. For this reason, fed-batch reactors are good candidates for the application of adaptive nonlinear control (ANC) techniques in order to robustify the systems performances against parametric uncertainties.

The aim of this paper is to present a general methodology for the design of adaptive regulators for fed-batch biological reactors with the aid of an illustrative case study which has given rise to a genuine industrial application. The application under consideration is the feedback optimization of yeast fermentations that are of particular interest because yeasts are among the most widely used microorganisms in genetic engineering for the production of high added value metabolites. This case study is representative of a wide class of control problems in biotechnology where the regulation of some substrate or metabolite concentration helps to solve yield/productivity conflicts and to enhance product quality.

In a fed-batch culture, the reactor is progressively fed with substrates necessary for the fermentation. No substance in liquid form is removed from the reactor during the culture. A fed-batch culture has the advantage of avoiding substrate overfeeding which can inhibit the growth of microorganisms. The fact that no substance is withdrawn from the reactor during the culture helps the process to work in good sterilized conditions. For these reasons, this mode of production is often preferred to batch and continuous modes in many processes.

Industrial fed-batch production of yeasts is traditionally carried out in open loop conditions using precalculated glucose feeding profiles of dosage schemes (which are often considered as manufacturing secrets). The determination of optimal feeding profiles for fed-batch processes has therefore been an attractive subject of research for a long time (see Ohno *et al.*, 1976; Aiba *et al.*, 1976; Parulekar *et al.*, 1985; Modak and Lim, 1987 and the review paper of Johnson, 1987). During the last decade, numerous laboratory studies have however shown that improved production is obtained with feeding profiles that are calculated on-line in a feedback loop using data from exhaust gas analysis (O_2 and CO_2) and/or ethanol sensors

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(see Dairaku *et al.*, 1982; Dairaku *et al.*, 1983; Williams *et al.*, 1984; Wu *et al.*, 1985; Dekkers and Voetter, 1985; Verbruggen *et al.*, 1985; Axelsson, 1989; Hagander *et al.*, 1990; Queinnec *et al.*, 1991; Chen *et al.*, 1991; Pomerleau and Viel, 1992 and Keulers, 1993).

The paper is organized as follows. We first describe the yeast production process in Section 2 and we derive the state-space model which is the basis of the control study. In Section 3, we present a review of the literature on the subject. The various control objectives that have been proposed in the literature are reviewed and the main control applications that have given rise to experimental results are extensively examined. This review allows us to emphasize and to motivate the original features of our approach with respect to the previous contributions on the subject. In Section 4, which is the core of the paper, we describe in detail the adaptive nonlinear regulation strategy. Firstly, using a qualitative knowledge about the reaction kinetics of the system, it is shown that a biomass estimator can be derived. Secondly, the nonlinear model of the system is reduced to a simple first order input–output model by a singular perturbation method. Thirdly, the adaptive nonlinear controller is obtained by using a feedback linearizing control action combined with a reference model and a classical Lyapunov design for adaptive systems. The industrial application is finally presented in Section 5.

2. DESCRIPTION OF THE YEAST PRODUCTION PROCESS

The mechanism of yeast growth on glucose with ethanol production is described by the following set of microbiolgoical reactions (see Sonnleitner and Kappeli, 1986):
Respiratory growth on glucose:

$$k_1 S + k_3 C \rightarrow X + k_5 P$$

Fermentative growth on glucose:

$$k_2 S \rightarrow X + k_8 E + k_6 P$$

Respiratory growth on ethanol:

$$k_9 E + k_4 C \rightarrow X + k_7 P$$

where S, C, X, P and E represent glucose (substrate), oxygen, yeasts, carbon dioxide and ethanol, respectively. These reactions are autocatalytic since yeasts are self-reproducing microorganisms. The coefficients $k_i > 0$ represent the stoichiometric (or yield) coefficients corresponding to the production of 1 unit of biomass (i.e. yeasts) in each reaction.

The physiological interpretation of the system is the following. The above three reactions represent three metabolic pathways from glucose to yeast. It is known that at low glucose concentrations and sufficiently high oxygen concentrations, the biomass grows primarily on glucose (respiratory growth, first reaction) without ethanol production. This reaction provides a high yield of yeast production with respect to glucose but its rate is rather low. At high glucose concentrations, the respiratory growth is inhibited or repressed (known as the Crabtree effect, Crabtree (1929)), the fermentative growth (second reaction) significantly appears with a high growth rate but a low yield, and ethanol is produced by the consumption of a part of glucose. In the third pathway, ethanol produced by the fermentative reaction can be consumed for the yeast growth in the presence of oxygen. Actually, the three reactions can take place simultaneously and the distribution of glucose among them depends on the level of glucose and oxygen concentrations.

When the process takes place in a fed-batch stirred tank reactor with glucose and oxygen supply (see Fig. 1), the following dynamical model expresses the mass balance of the various components around the reactor:

$$\dot{S} = -k_1\mu_1(\xi)X - k_2\mu_2(\xi)X - DS + DS_{\text{in}} \quad (1)$$

$$\dot{C} = -k_2\mu_1(\xi)X - k_4\mu_3(\xi)X - DC + Q_{O_2} \quad (2)$$

$$\dot{X} = \mu_1(\xi)X + \mu_2(\xi)X + \mu_3(\xi)X - DX \quad (3)$$

$$\dot{P} = k_5\mu_1(\xi)X + k_6\mu_2(\xi)X + k_7\mu_3(\xi)X - DP - Q_P \quad (4)$$

$$\dot{E} = k_8\mu_2(\xi)X - k_9\mu_3(\xi)X - DE - Q_E \quad (5)$$

$$\dot{V} = DV \quad (6)$$

where S, C, X, P and E now denote the concentrations of the components in the liquid phase of the reactor, Q_P is the gaseous CO_2 outflow rate (per unit of volume), Q_E is the gaseous ethanol outflow rate (per unit of volume), D is the dilution rate defined as the ratio between the liquid substrate feed rate, F_{in} and the liquid volume of the reactor, V, S_{in} is the

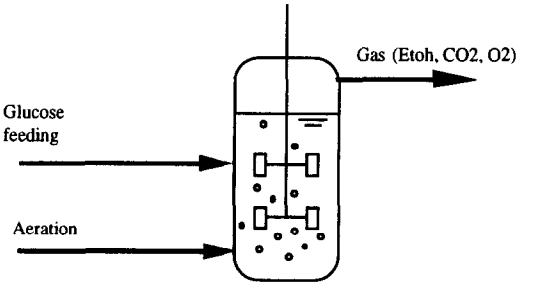

Fig. 1. Fed-batch culture of yeast production.

influent glucose concentration, Q_{O_2} is the oxygen transfer rate (per unit of volume), ξ denotes the state vector: $\xi \triangleq (S, C, X, P, E, V)^T$.

The specific growth rates $\mu_1(\xi)$, $\mu_2(\xi)$ and $\mu_3(\xi)$ are very complex time-varying functions of the state of the process and their analytical modelling is often uncertain. In spite of this, a basic qualitative knowledge is available. It is a well known fact in bioengineering that the growth capacity of a population of microorganisms (like yeasts) is intrinsically limited, irrespective of the environmental conditions. This means that the specific growth rates $\mu_i(\xi)$ can be assumed to be upper bounded as:

$$0 \leq \mu_i(\xi) \leq \mu_i^* \quad \text{for all } \xi \tag{7}$$

with the constants μ_i^* representing the maximal growth capacity of the yeast population in each reaction. This also implies that the specific growth rates can be written, without any loss of generality, in the following form:

$$\mu_i(\xi) \triangleq \mu_i^* \psi_i(\xi) \quad \text{for all } \xi \tag{8}$$

where $\psi_i(\xi)$ are positive bounded functions:

$$0 \leq \psi_i(\xi) \leq 1 \quad \text{for all } \xi. \tag{9}$$

3. REVIEW OF THE LITERATURE

In this section, we present an overview of the scientific literature concerning the feedback control of the yeast production processes, with a special emphasis on the contributions reporting experimental applications on pilot and industrial processes.

Industrial fed-batch production is traditionally carried out in open loop using precalculated substrate feeding profiles. During the last decade, numerous laboratory studies have however shown that improved production is obtained with feeding profiles that are calculated on-line in a feedback loop. There are basically three reasons for the use of advanced control strategies in the fed-batch yeast production.

(1) The conflict between yield and productivity (Aiba *et al.*, 1976; Wang *et al.*, 1977; Peringer and Blachere, 1979 and Woehrer and Roehr, 1981): a high glucose feed rate usually induces high biomass (i.e. yeasts) productivity (at least up to concentrations that may become themselves inhibiting) but with a low yield. The converse is obviously true for low substrate (i.e. glucose) feeding which causes productivity decrease but with a better yield.

(2) The level of ethanol concentration can enhance the production of inhibitory substances (Pons *et al.*, 1986).

(3) The reproducibility of cultivations is an important factor for a good and/or uniform quality of the yeast and is not easy to obtain without efficient feedback control.

3.1. *Control strategies*

Numerous feedback control strategies have been described in the literature to solve these difficulties by using the substrate (i.e. glucose) feed rate as the control action:

(ST1) to set the respiratory quotient RQ close to 1, RQ being the ratio between the carbon dioxide evolution rate (CER) and the oxygen uptake rate (OUR) [see equations (10) and (11) hereafter];

(ST2) to maintain the glucose concentration at a constant (low) level;

(ST3) to track an exponential profile for the amount of biomass;

(ST4) to set the overall specific growth rate $\mu_{tot} = \mu_1(\xi) + \mu_2(\xi) + \mu_3(\xi)$, or OUR or CER at a constant value;

(ST5) to keep the ethanol concentration at a constant level or to track a given ethanol profile.

The most frequently used strategy is (ST1) the control of the respiratory quotient RQ (Aiba *et al.*, 1976; Cooney *et al.*, 1977; Wang *et al.*, 1977; Peringer and Blachere, 1979; Woehrer and Roehr, 1981 and Verbruggen *et al.*, 1985) because it is physiologically well known that RQ ranging between 1 and 1.2 mainly corresponds to the respiratory yeast growth on glucose. Furthermore, the value of OUR and CER can be easily obtained from gaseous inflow–outflow balances and dissolved O_2 and CO_2 concentrations. However, this strategy does not guarantee a high productivity of the yeast production. On the other hand, it can be seen, from the experiments presented in Woehrer and Roehr (1981) and Williams *et al.* (1986), that maintaining RQ around a presumably good set point neither prevents the ethanol production nor even maintains it at a constant level. A straightforward computation from equations (2) and (4) shows that

$$\mathrm{OUR} = \frac{Q_{O_2} V - \frac{d}{dt}(CV)}{V} = k_3 \mu_1(\xi) X + k_4 \mu_3(\xi) X \tag{10}$$

$$\mathrm{CER} = \frac{Q_P V + \frac{d}{dt}(PV)}{V} = k_5 \mu_1(\xi) X + k_6 \mu_2(\xi) X + k_7 \mu_3(\xi) X \tag{11}$$

and

$$RQ = \frac{\text{CER}}{\text{OUR}} = \frac{k_2\mu_1(\xi) + k_6\mu_2(\xi) + k_7\mu_3(\xi)}{k_3\mu_1(\xi) + k_4\mu_3(\xi)}. \quad (12)$$

It appears that RQ is in general a nonlinear function of the state of the system, especially of substrate, ethanol and oxygen concentrations. A constant value of RQ thus corresponds to an infinity of configurations of these concentrations. Its choice is therefore not easy to justify.

The control of the overall growth rate $\mu_{tot} = \mu_1(\xi) + \mu_2(\xi) + \mu_3(\xi)$ (ST4) is used to optimize the biomass productivity (Dairaku *et al.*, 1982) or the biomass yield (Keulers, 1993). The set-points of this controlled variable also appear to be experimentally related to the ethanol production (Wu *et al.*, 1985). However, it is not a directly measurable variable. It is estimated from a model relating it to OUR in Wu *et al.* (1985) and from biomass measurements in Dairaku *et al.* (1982).

The tracking of a desired biomass trajectory (ST3) is studied in Takamatsu *et al.* (1985) with simulations, but, to our knowledge, has never been practically experienced mainly because the biomass is generally not measurable on-line.

The regulation of glucose concentration (ST2) is applied when the process objective is to produce alcohol (Queinnec *et al.*, 1991). However the glucose concentration is also a state variable which is difficult to measure, especially when the value is very small, while in the processes where the objective is to obtain a good yield of biomass production or to avoid substrate inhibition, the glucose concentration is often very low.

Since ethanol production is the main factor observed in the switching from growth with high yield to growth with high productivity, its regulation in yeast fermentations (ST5) has received more and more attention (Woehrer and Roehr, 1981; Dairaku *et al.*, 1983; Williams *et al.*, 1986; Axelsson, 1989; Chen *et al.*, 1991 and Pomerleau and Viel, 1992). Simulation studies have indeed shown that ethanol regulation in the reactor (with glucose feed rate as control action) allows the process to be maintained at operating points that correspond to a good trade-off (from an economic viewpoint) between yield and productivity.

From the control engineering point of view, the choice of one controlled variable is often equivalent to another one in the sense that they all imply, approximately, an exponential biomass growth and an exponentially growing glucose feed rate.

In addition to the above control strategies using the glucose feed rate as control action, the regulation of oxygen concentration by the agitation rate in the liquid medium is generally performed in yeast processes either with a local control or with a multivariable control system (see e.g. Williams *et al.*, 1984).

3.2. *Control algorithms*

Because of the complexity of the fed-batch biological systems, their nonlinearities and modelling uncertainties, modern control science is not easy to apply to these systems. Early attempts of feedback control were made with a variety of PID controllers. It is only recently that adaptive techniques have been used to improve the control performances, most often in laboratory or small pilot scale systems. In the following, a brief review of literature is given on the application of feedback control techniques to fed-batch yeast production systems using glucose feed rate as control action. The review is limited to a set of papers where real life experiments are reported. The main technical characteristics of the experiments are summarized in Table 1.

3.2.1. *PID controllers.* Classical PID controllers with fixed parameters are in general designed for the regulation of time-invariant systems. Therefore, a first ID controller based on a steady state assumption with a low biomass growth rate can be found in Woehrer *et al.* (1981). The objective is to regulate RQ or ethanol concentration with substrate feed rate. The regulation performance is not very satisfactory: oscillations and even a small divergence in the ethanol regulation are observed after two hours of operation.

Since, in any case, the feedback control will produce an exponentially growing glucose feed rate, it is normal to use an open loop precalculated feeding profile F^0 as a nominal function and a PID regulation of the feed rate around this predetermined function. Such a PID controller can be found in Dairaku *et al.* (1982) and Keulers (1993) for the regulation of the overall growth rate μ_{tot}. The latter can be calculated from biomass measurements. A PI controller is tested in Axelsson (1989) for the regulation of ethanol concentration but a divergence of the control system is experimentally observed. The main reason is that the derivation between the real glucose feed rate needed for the system and the precalculated one also increases exponentially with time. This exponentially increasing disturbance on the control action is difficult to handle with a fixed parameter controller. Furthermore, the linearized system between the glucose feed rate and

Table 1. Literature of control applications to real time fed-batch systems

Reference	Controlled variables	Control techniques	Volume V_0–V_f (l)	Biomass X_0–X_f (g/l)	Duration (h)	Sampling period (min)
Woehrer *et al.* (1981)	*RQ*, *E*	ID	3.3	8–13	2	—
Dairaku *et al* (1982)	μ_{tot}	PID around F^0	10	9–24	11	—
Dairaku *et al.* (1983)	*E*	PID around $\hat{F}^0$	10	4–13	5	—
Wu *et al.* (1985)	μ_{tot}	Self-tuning	1.5	4–25	11	1
Williams *et al.* (1984)	O_2, CO_2, *RQ*, *E*	Self-tuning	3.6–4.1	10–31	7	3–5.5
Dekkers and Voetter (1985)	*RQ*	Self-tuning	30–80	5.8–48.7	16	1
Verbruggen *et al.* (1985)	*RQ*	Self-tuning	10	—	—	—
Axelsson (1989)	*E*	PID around F^0	4–6	2.6–66.8	18	0.5
Queinnec *et al.* (1991)	*S*	MRAC + GPC	6–16	—	10	5
Chen *et al.* (1991)	*E*	ANC	60–150	0.5–80	52	2
Pomerleau and Viel (1992)	*E*	ANC	20 (V_f), 60000 (V_f)	—	10, 15	—

$\hat{F}^0$: estimation of F^0; V_0 and V_f: initial and final liquid volumes; X_0 and X_f: initial and final biomass concentrations; F^0: predefined substrate feed rate.

the ethanol concentration around a set point has a time constant inversely proportional to the biomass concentration. This means that the dynamics of the system increase with the biomass growth. The performance of the regulator is once again difficult to ensure with fixed parameters. In his work (see e.g., Axelsson, 1988 and Axelsson, 1989), the use of several linear models is proposed to cover the whole range of the system dynamics. In Dairaku *et al.* (1983), the parameters of the PID controller are adjusted according to some empirical knowledge.

The performance of a classical PID controller around a precalculated profile F^0 can be improved by calculating on-line F^0 in order to reduce the disturbance on the control action. In Dairaku *et al.* (1983), under the assumption that the substrate concentration is well regulated and that the volume variation is negligible with respect to the other terms involved in the dynamics of S, F^0 is calculated from the following equation relating the biomass growth to the glucose consumption:

$$\hat{F}^0 = YF_{in}S_{in} \tag{13}$$

where Y is a yield coefficient between the biomass production and the glucose consumption. The real glucose feed rate F_{in} is composed of $\hat{F}^0$ and F^{PID}, the latter being calculated from the PID controller.

In Axelsson (1989) and Hagander *et al.* (1990), an internal model for glucose demand is derived and used to design a Luenberger observer. The latter is driven by ethanol measurements and provides the on-line estimate of F^0.

3.2.2. *Adaptive linear control techniques.* Motivated by the nonlinearity, the nonstationary and the modelling uncertainties of yeast production systems, several laboratories have tried to develop adaptive controllers based on 'black-box' linear models. These controllers are also based on a linearization of the system around a set point and the nonlinearity of the system is interpreted by the time-varying parameters of the model, which are adapted on-line.

In these applications, the (indirect) self-tuning techniques have been most frequently used. The parameters of the linear model are estimated on-line, in general with a recursive identification algorithm. These estimated parameters are then used to design a linear controller at each sampling time. The design of the controller is based either on a quadratic cost function or on the pole assignment principle. In Williams *et al.* (1984) and Williams *et al.* (1986), LQ (linear quadratic) controllers are used for the regulation of *RQ*, ethanol, oxygen or CO_2 concentrations with substrate feed rate and/or agitation rate. In Dekkers and Voetter (1985), an LQG (linear quadratic Gaussian) controller is designed to regulate *RQ*. In Wu *et al.* (1985), an LQ controller is used to regulate the overall specific growth rate which is estimated from the measurements of oxygen in the gas outlet. Oxygen is the only variable measured in the system. In Verbruggen *et al.* (1985), a self-tuning controller based on the pole assignment principle is used to regulate *RQ*.

Self-tuning techniques have the advantage of not requiring much prior knowledge about the real system. They can cope with the time-varying characteristics of the parameters when the sampling period is chosen to be sufficiently small with respect to the time constants of the system provided measurements are precise enough. However, in Williams and Montgomery (1986), it is pointed out that these techniques should inherently check the accuracy of the parameter estimation. Without this facility, the self-tuning controller may not be adequate to represent the dynamics of the process and optimal control will not be achieved. It is said that the convergence

of the parameters requires a rather long time period of adaptation. The latter is not easy to realize with slowly time-varying systems and when the duration of an experiment is limited. Furthermore, the off-set problem has been encountered by several groups but eliminated by introducing an integral action in the control system (Dekkers and Voetter, 1985 and Verbruggen *et al.*, 1985).

The objective of the model reference adaptive control (MRAC) is to make the system behave like a given reference model with desired dynamics. This kind of controller is only found in Takamatsu *et al.* (1985) to track a predefined biomass trajectory or the overall specific growth rate in simulations. In Queinnec *et al.* (1991), a partial state model reference control is designed based on a generalized predictive control (GPC) criterion to regulate the substrate concentration for an alcohol production system.

3.2.3. *Adaptive nonlinear control (ANC) techniques.* Since fed-batch systems are nonlinear, it is more interesting, in the design of a control system, to exploit the nonlinear structure and the available physical knowledge about the system. On the other hand, in these processes, the models usually include kinetic parameters which are highly uncertain and slowly time varying. For this reason, they are good candidates for the application of adaptive nonlinear control techniques in order to robustify the system performances against parametric uncertainties. Similar applications of these techniques for the ethanol regulation with substrate feed rate can be found in Chen *et al.* (1989), Chen *et al.* (1991) and Pomerleau and Viel (1992). They have given rise to genuine industrial applications and it is reported in Pomerleau and Viel (1992) that the transfer of such a controller from a small scale industrial reactor (20 l) to a large scale industrial reactor (60000 l) within 15 h of operation was possible without changing the tuning parameters.

It is the main purpose of this paper to present a general methodology for the design of such adaptive nonlinear regulators for fed-batch bioreactors through this case study of the ethanol control with substrate feed rate.

4. ADAPTIVE NONLINEAR REGULATION OF THE SYSTEM

4.1. *Statement of the regulation problem*

As mentioned above, ethanol production is the major factor in the switching from growth with high yield and growth with high productivity. Our objective is therefore to regulate the ethanol concentration E at a desired set point E^* all along the fed-batch operation by using the dilution rate D or equivalently, the substrate feed rate F_{in} as control action under the following assumptions.

(A1) The ethanol concentration E, the dissolved oxygen concentration C and the dissolved CO_2 concentration P are measured on-line.
(A2) The gaseous outflow rates Q_P and Q_E and the oxygen transfer rate Q_{O_2} are measured on-line, with an exhaust gas analysis system.
(A3) The influent substrate concentration S_{in} is fixed and known.
(A4) The stoichiometric (or yield) coefficients k_i are known (from a preliminary identification study, see e.g. Chen, 1992)
(A5) The specific growth rates $\mu_i(\xi)$ are unknown.

This control problem will be solved in four steps: (i) design of a biomass estimator independent of the kinetics; (ii) model reduction based on qualitative information about the system; (iii) design of a feedback linearizing control law based on the reduced-order model; and (iv) design of a parameter adaptation law.

4.2. *Biomass estimator*

By an appropriate state transformation, we can obtain a biomass estimator without modelling the growth rates. This biomass estimator is a special case of a general state observer for biological systems given in Bastin and Dochain (1990) and is briefly described in the following. The dynamical model of the process can be partially rewritten in matrix form as follows with one part representing the state variable to be esitmated (i.e. biomass) and the other one related to the measured variables available for the estimation:

$$\dot{X} = \mathbf{b}^T \boldsymbol{\mu}(\xi) X - DX \tag{14}$$

$$\begin{pmatrix} \dot{E} \\ \dot{C} \\ \dot{P} \end{pmatrix} = \mathbf{K}_a \boldsymbol{\mu}(\xi) X - D \begin{pmatrix} E \\ C \\ P \end{pmatrix} + \begin{pmatrix} -Q_E \\ Q_{O_2} \\ -Q_P \end{pmatrix} \tag{15}$$

with the following definition of $\mathbf{b}$, $\boldsymbol{\mu}(\xi)$ and $\mathbf{K}_a$:

$$\mathbf{b}^T = (1\ 1\ 1),\ \boldsymbol{\mu}^T(\xi) = (\mu_1(\xi)\quad \mu_2(\xi)\quad \mu_3(\xi)),$$

$$\mathbf{K}_a = \begin{pmatrix} 0 & k_8 & -k_9 \\ -k_3 & 0 & -k_4 \\ k_5 & k_6 & k_7 \end{pmatrix}.$$

The following auxiliary scalar variable Z is introduced:

$$Z = X - \mathbf{b}^T (\mathbf{K})_a^{-1} \begin{pmatrix} E \\ C \\ p \end{pmatrix}. \tag{16}$$

It is then easily shown from (14) and (15) that the dynamics of Z are written:

$$\dot{Z} = -DZ - \mathbf{b}^{\mathrm{T}}\mathbf{K}_a^{-1}\begin{pmatrix} -Q_E \\ Q_{O_2} \\ -Q_P \end{pmatrix}. \tag{17}$$

It follows that an on-line estimator of the yeast concentration X is trivially obtained by integrating this equation on-line and calculating the estimate as:

$$\hat{X} = Z + \mathbf{b}^{\mathrm{T}}\mathbf{K}_a^{-1}\begin{pmatrix} E \\ C \\ P \end{pmatrix}. \tag{18}$$

Hence the on-line estimator of biomass is made up of equations (17) and (18) from which the reaction rates have disappeared. It is asymptotically convergent to the theoretical value when the dilution rate D is kept positive. It is worth noting that it makes use of all the available on-line measurements: C, P, E, F_0, Q_P, Q_E, D, V.

In practice, the parameters involved in this kind of estimator have to be identified with off-line data and sometimes with sparse measurements of biomass. For this reason, it may be useful to implement the estimate in terms of the total amounts of the components in the reactor instead of their concentrations (Chen, 1992).

4.3. *Model reduction*

When the ethanol concentration is chosen as a controlled variable and the dilution rate or, equivalently, the substrate feed rate F_{in} as control input, equation (5) is actually an input–output equation of relative degree 1. But a linearizing control law based on this equation may result in divergence of the closed loop system when the control input saturates. In order to get a simple and practically applicable adaptive regulator, the design will be based on a feedback linearization of a reduced-order model of the process, obtained by using a singular perturbation of the state-space model (1)–(6). The singular perturbation techniques can be used for systems in which some reactions proceed at much faster rates than the others. In this case, an appropriate state transformation can be applied to transform the state-space model into the so-called two-time-scale (i.e. fast and slow) standard form of the singular perturbation theory in order to explain the model reduction (Kokotovic *et al.*, 1986 for the theory, and Van Breusegem and Bastin, 1991 for the application to reaction systems). For our system, the reduced model is based on the following two conditions that are in full agreement with the experimental reality.

Condition (C1). The process operates essentially under glucose limiting conditions: sufficient aeration and nitrogen supply are assumed as well as good control of pH and temperature. This condition means, in particular, that the fermentative growth is stopped when (and only when) glucose is missing: $\mu_2(\boldsymbol{\xi}) = 0$ if $S = 0$. This may be further formalized by factorizing the normalized specific growth rate $\psi_2(\boldsymbol{\xi})$ [equation (8)] as:

$$\psi_2(\boldsymbol{\xi}) = S\varphi_2(\boldsymbol{\xi}) \tag{19}$$

where the function $\varphi_2(\boldsymbol{\xi})$ is now strictly positive and bounded:

$$0 < \varphi_2(\boldsymbol{\xi}) \leq \varphi_2^* \quad \text{for all } \boldsymbol{\xi}. \tag{20}$$

Condition (C2). The maximal specific fermentative growth rate is larger than the maximal respiratory growth rates (see e.g. Sonnleitner and Kappeli, 1986), i.e.

$$\mu_2^* \gg \mu_1^* \quad \text{and} \quad \mu_3^*. \tag{21}$$

Under this condition, the fermentative reaction is a faster reaction than the two others when it proceeds near its maximal rate. This instantaneously occurs when a large amount of glucose is added with the feeding flow.

According to condition (C2), we can define a small parameter ϵ as the inverse of μ_2^*:

$$\epsilon = 1/\mu_2^*. \tag{22}$$

We also define the following change of coordinates:

$$(\eta_1, \eta_2, \eta_3, \eta_4, \eta_5) = (X, S + k_2X, C, P - k_6X, E - k_8X). \tag{23}$$

Using (22) and (23) together with condition (C1), we transform the dynamical model (1)–(5) as follows:

$$\epsilon\dot{\eta}_1 = \varphi_2(\boldsymbol{\xi})\eta_1(\eta_2 - k_2\eta_1) + \epsilon[\mu_1(\boldsymbol{\xi}) + \mu_3(\boldsymbol{\xi}) - D]\eta_1 \tag{24}$$

$$\dot{\eta}_2 = -(k_1 - k_2)\mu_1(\boldsymbol{\xi})\eta_1 + k_2\mu_3(\boldsymbol{\xi})\eta_1 - D\eta_2 + DS_{\mathrm{in}} \tag{25}$$

$$\dot{\eta}_3 = -k_3\mu_1(\boldsymbol{\xi})\eta_1 - k_4\mu_3(\boldsymbol{\xi})\eta_1 - D\eta_3 + Q_{O_2} \tag{26}$$

$$\dot{\eta}_4 = -(k_6 - k_5)\mu_1(\boldsymbol{\xi})\eta_1 - (k_6 - k_7)\mu_3(\boldsymbol{\xi})\eta_1 - D\eta_4 - Q_P \tag{27}$$

$$\dot{\eta}_5 = -k_8\mu_1(\boldsymbol{\xi})\eta_1 - (k_8 + k_9)\mu_3\eta_1 - D\eta_5 - Q_E. \tag{28}$$

Setting ϵ to zero in equation (24), we get the following relation

$$\varphi_2(\boldsymbol{\xi})\eta_1(\eta_2 - k_2\eta_1) = 0$$

which has two roots with respect to η_1:

$$\eta_1 = 0 \quad \text{and} \quad \eta_1 = \eta_2/k_2.$$

It can be quite easily checked that only the reduced model obtained with $\eta_1 = \eta_2/k_2$ is a valid approximation of equations (24)–(28) in the sense of Tikhonov's theorem (see Van Breusegem and Bastin, 1991). This reduced order model is:

$$\dot{\eta}_2 = \frac{-(k_1 - k_2)}{k_2}\mu_1(\xi)\eta_2 + \mu_3(\xi)\eta_2 - D\eta_2 + DS_{in}$$

$$\dot{\eta}_3 = \frac{k_1}{k_2}\mu_1(\xi)\eta_2 - \frac{k_4}{k_2}\mu_3(\xi)\eta_2 - D\eta_3 + Q_{O_2}$$

$$\dot{\eta}_4 = \frac{-(k_6 - k_5)}{k_2}\mu_1(\xi)\eta_2 - \frac{(k_6 - k_7)}{k_2}\mu_3(\xi)\eta_2 - D\eta_4 - Q_P$$

$$\dot{\eta}_5 = \frac{-k_8}{k_2}\mu_1(\xi)\eta_2 - \frac{k_8 + k_9}{k_2}\mu_3(\xi)\eta_2 - D\eta_5 - Q_E.$$

Finally, we come back to the original coordinates. The ethanol concentration E is readily seen to be

$$E = \eta_5 + \frac{k_8}{k_2}\eta_2 \tag{29}$$

and the corresponding dynamics are given by

$$\dot{E} = D\left(\frac{k_8}{k_2}S_{in} - E\right) - \theta X - Q_E \tag{30}$$

with

$$\theta \triangleq \frac{k_1 k_8}{k_2}\mu_1 + k_9\mu_3. \tag{31}$$

Notice that as a consequence of the model reduction, the glucose concentration S is supposed to be very small (i.e. approximately zero). This means that the model reduction assumes complete glucose conversion. It is also of interest to notice that it is necessary to pass through the change of coordinates (23) to get a valid approximation of the initial model in the sense of the singular perturbation theory. Indeed, if we replace directly $S = 0$ in the initial equations (1) and (2), it is obvious that the reduced model of ethanol, i.e. equation (30) cannot be obtained because when $S = 0$, then $\mu_1 = 0$ and $\mu_2 = 0$ in the initial equations.

4.4. *The feedback linearizing control law*

Remember that the objective is to regulate the ethanol concentration E at the set point E^* by manipulating the dilution rate D or equivalently the substrate feed rate F_{in}. On the other hand, it can be seen from equation (5) that the dynamics of the ethanol concentration accelerate with the biomass concentration. This motivates the choice of the regulation error to be decreasing according to the following stable linear time-varying first-order dynamics:

Reference model:

$$\frac{d}{dt}(E^* - E) = -(\lambda_1 + \lambda_2 X)(E^* - E) \quad \lambda_1, \lambda_2 > 0 \tag{32}$$

which has a time constant decreasing in parallel with the natural acceleration of the biomass growth.

A model reference linearizing control law is then obtained by substituting equation (32) into equation (30):

Control law:

$$F_{in} = DV = \frac{(\lambda_1 + \lambda_2 X)(E^* - E) + \theta X + Q_E}{\left(\frac{k_8}{k_2}S_{in} - E\right)}V. \tag{33}$$

4.5. *The adaptive control law*

Since the biomass concentration X is not measured on-line and the kinetic parameter θ is unknown, the above control law (33) cannot be applied just as it is. An adaptive form is implemented as follows:

Adaptive control law:

$$F_{in} = DV = \frac{(\lambda_1 + \lambda_2 \hat{X})(E^* - E) + \hat{\theta}\hat{X} + Q_E}{\left(\frac{k_8}{k_2}S_{in} - E\right)}V \tag{34}$$

where $\hat{X}$ and $\hat{\theta}$ denote on-line estimates of X and θ, respectively. The on-line estimate of X is calculated with the observer (17) and (18) described above. The parameter adaptation law is obtained by a classical Lyapunov design for adaptive systems (Praly *et al.*, 1991 and the references therein) as follows:

Parameter adaptation law:

$$\dot{\hat{\theta}} = \gamma(E^* - E) \tag{35}$$

with γ a scalar design coefficient.

Finally, the full adaptive controller is made up of equations (17), (18), (34) and (35).

4.6. *Comments*

(1) Besides dealing with the modelling uncertainty associated with the kinetic parameter θ, an additional major advantage of the adaptation law is to insert an integral action into the loop in a very natural way. If Q_E is neglected in equation (34), the controllers (34) and (35) can also be interpreted in terms of a classical PI

controller with time-varying gain P and integral time constant τ_i as follows:

$$P = \frac{\lambda_1 + \lambda_2 \hat{X}}{\frac{k_8}{k_2} S_{in} - E} V, \quad \tau_i = \frac{\lambda_1 + \lambda_2 \hat{X}}{\gamma \hat{X}},$$

i.e. the gain is made increasing with the biomass growth while the integral time constant is slightly decreasing accordingly. In practice, the integral action is provided with an anti-windup mechanism in case of saturation of the control action (see Chen, 1992).

(2) The adaptive control law can be viewed as a combined application of the theories of adaptive nonlinear control as presented in Taylor *et al.* (1989) and Sastry and Isidori (1989): adaptive regulation of a process with unmodelled dynamics (Taylor *et al.*, 1989) and with feedback cancellation of the zero dynamics (Sastry and Isidori, 1989). It is however important to realize that the goal here is not to globally stabilize the plant but rather to keep an unstable (hopefully optimal) trajectory under control. The reason is that a fed-batch process is operated during a finite time. The objective is to accumulate the biomass, which implies that some of the state variables necessarily follow an exponentially growing trajectory which has to be kept under control.

5. APPLICATION TO AN INDUSTRIAL PROCESS

This adaptive control strategy has been successfully applied to an industrial production plant. The process is operated in a stirred tank bioreactor. A simplified flow sheet of the control system is shown in Fig. 2. It can be briefly characterized by the following points:

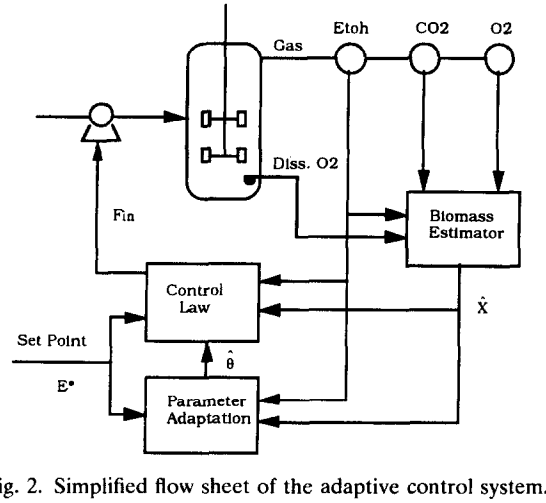

Fig. 2. Simplified flow sheet of the adaptive control system.

— The initial and the final volumes are around 60 and 150 (l), respectively. The initial and final values of the biomass concentration are about 0.55 and 80 (g/l), respectively. The initial value of the glucose concentration is 0.69 (g/l).
— The substrate concentration in the feeding medium is 357.28 (g/l).
— The pH and the temperature are regulated by local control devices. The dissolved oxygen concentration is also kept at a maximum level for as long a time as possible by the agitation. But when the agitator achieves its maximum capacity, the oxygen concentration falls to a lower level.
— The duration of the fermentation is in general around 65 h; the first 4–5 h of the fermentation are in batch mode (without substrate feeding).

The information about the control system is the following:

— Only the ratio $\frac{k_8}{k_2} = 0.3$, the yield between the ethanol production and the glucose consumption in the fermentation growth is needed in the control law and identified from off-line measurements (see Chen, 1992 for further details).
— The parameters needed for the estimation of the biomass are also computed from off-line data of several experiments including some control experiments. The following values are chosen: $\mathbf{b}^T\mathbf{K}_a^{-1} = (0.0572, 0.6445, 0.3930)$ (see Chen, 1992 for further details).
— The control sampling period Δt is 2 (min).
— The design parameters of the controller are calibrated by trial and error during several experiments, their values are as follows: $\lambda_1 = 3$, $\lambda_2 = 0.02$, $\gamma = 0.2$.

A typical experimental result over a period of 60 h is shown in Figs 3 and 4.

In Fig. 3(a), we can appreciate the performance of the biomass estimator, for an overall variation from 25 (g) to 12 (kg) for the total amount or from 0.5 (g/l) to 80 (g/l) for the concentration. The off-line test data are obtained by dry weight measurements.

The adaptive controller is switched on at time $t = 8.2$ h, with an ethanol set point of 1.5 (g/l). The performance of the regulator can be appreciated in Fig. 3(b). During the first 10 h, the achievement of the set point is fairly slow partially due to the saturation of the control input [Fig. 4(a)]. The response to a step change of the set point (from 1.5 g/l to 0.6 g/l) at time

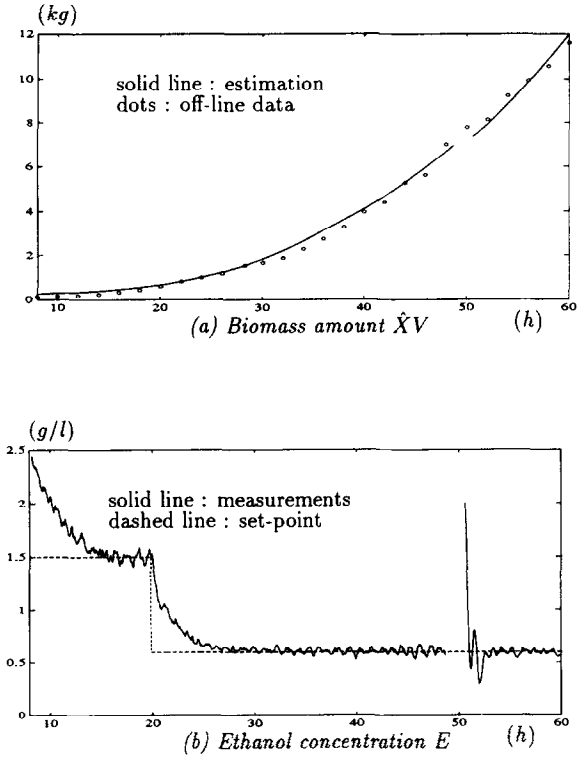

Fig. 3. On-line ethanol control with substrate feed rate. The system is in open loop from 48.7 to 50.6 h.

$t = 20$ h can be observed. At this time, the control action is instantaneously saturated [see Fig. 4(a)], the parameter is reset to a new value

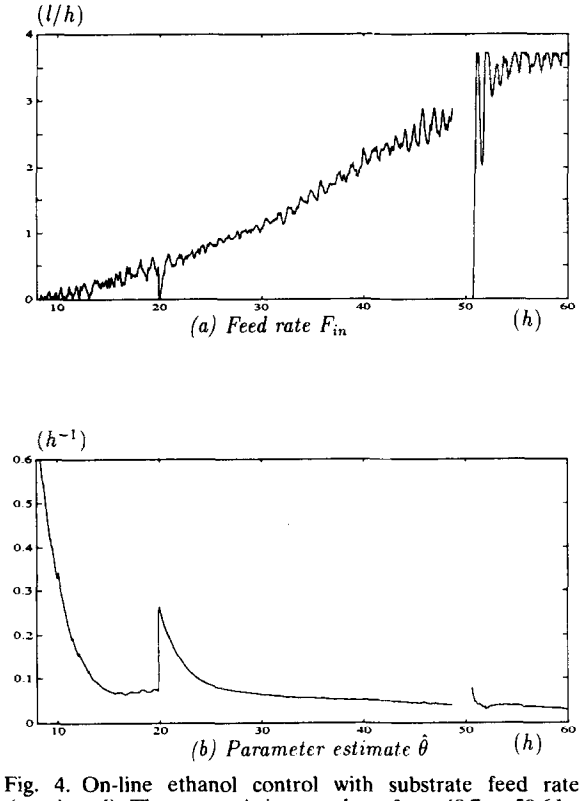

Fig. 4. On-line ethanol control with substrate feed rate (continued). The system is in open loop from 48.7 to 50.6 h.

by the anti-windup mechanism as shown in Fig. 4(b). The ability of the controller in rejecting external disturbances is also assessed. At time $t = 48.7$ h, the regulator is abruptly switched off and the process is set in open loop with a maximum glucose feed rate during two hours. This results in an accumulation of ethanol up to a concentration of 2.2 (g/l). The controller is then switched on at time $t = 50.6$ h and brings the system back to the set point. The anti-windup action can be observed again during the saturation of the control action. It can be seen that, as expected, the response time is faster when the biomass value is higher.

6. CONCLUDING REMARKS

In this paper, we have presented the application of an adaptive nonlinear controller for the ethanol control with substrate feed rate to a fed-batch yeast production system. The derivation of this controller follows a general methodology in several steps: design a state observer for the biomass without modelling the reaction kinetics; find a reduced model between the substrate feed rate and the ethanol concentration under realistic conditions; design a feedback linearizing control law and design a parameter adaptation law. This adaptive control law can be viewed as a combined application of the theories of adaptive nonlinear control, i.e. adaptive regulation of a process with unmodelled dynamics and with feedback cancellation of the zero dynamics. However, the goal here is not to globally stabilize the plant but rather to keep an unstable (hopefully optimal) trajectory under control. The reason is that a fed-batch process is operated during a finite time. The objective is to accumualte the biomass, which implies that some of the state variables necessarily follow an exponentially growing trajectory which has to be kept under control.

Another important feature of this application compared to most of the approaches encountered in the literature is obviously the use of a nonlinear model representing a wide range of system dynamics in the design of the controller. Although this is a simple example of application of adaptive nonlinear techniques, it has really demonstrated its good performance in coping with the system nonlinearities and the parameter uncertainties for biological systems.

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