MODELLING AND CONTROL OF BIOTECHNOLOGICAL PROCESSES

Edited by

A. JOHNSON

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FOREWORD

Following in the footsteps of the very successful IFAC Workshop held in Helsinki in 1982, the first IFAC symposium on modelling and control of biotechnical processes took place in Noordwijkerhout, The Netherlands, in December 1985. It was attended by two hundred control engineers, mathematicians, biotechnologists and microbiologists interested in control. There is no doubt in my mind that the participation of so many industrialists and academics was stimulated by the cosponsorship of the European Federation of Biotechnology, which has organised similar meetings in the past. Another factor contributing to the high attendance was also the public and commercial interest in biotechnology as one of the technologies of the future.

This volume contains all the papers presented during the symposium, which consisted of five sessions of parallel lectures. In addition about thirty posters were displayed by their authors in poster sessions. The papers were selected from nearly eighty which were submitted, and each was subjected to a full paper review by the International Program Committee.

To the interested observer it will be apparent that a considerable change has taken place since the first workshop three years ago. I refer to the widespread application of the computer in controlling biotechnological processes; one may now safely assume that a control scheme or strategy will be implemented in a digital computer of some shape or size. Other controllers are the exception, rather than the rule, nowadays. An interesting question is how has this change been brought about so quickly? Perhaps the advent of the microcomputer is the most relevant factor, but I believe that more important has been the revolution in personal computers that has occurred. Being able to experiment with their own home computer, many people have discovered not only that programming is not so difficult but that neither is interfacing a computer with a process, or implementing a simple control algorithm. There must already be hundreds, if not thousands, of small fermentors today which are under "home" computer control or which are controlled thanks to experience gained from personal computers.

This change, which has also stimulated interest in the theory of control amongst those not normally thought of as control engineers, has meant the demise of papers whose content is only a description of process and computer hardware. It has, and will increasingly, free the authors to concentrate on the development of more robust and optimal control strategies and algorithms. The time is hopefully at hand, thank goodness, when control can no longer be dismissed in one sentence - usually concerning a three-term controller!

As will become apparent when reading this volume, the time varying parameters of most biotechnological processes cause particularly difficult control problems. Many authors propose adaptive or self-tuning controllers as the answer. These proposals sometimes neglect to attempt to answer the important questions such as the convergence, initialization and robustness of the controller. This can lead to the application of controllers which perform reasonably when simulated but poorly in practice.

Another and equally popular topic is that of estimation. The lack of robust sensors to measure variables such as biomass concentration quickly and reliably has led to the development of estimators, often based on the Kalman-Bucy filter, to do the job. Since information concerning the whole state of the biotechnological process is essential for controller design, this may be a profitable way into the field of control for many non-specialists. The papers of Session 4a on Kalman Filtering Techniques provided interesting reading for anyone interested in estimation.

The principle which guided the National Organising Committee when it was faced with the task of creating the Symposium program was to divide the available time between the topics of control, measurement, modelling, estimation (including parameter identification) and optimization. This volume has been compiled in a similar fashion, with only the placement of the three plenary papers on control, modelling and estimation as exceptions. Although it is the first IFAC symposium on the biotechnological aspects of control, I feel sure that it will not be the last.

A Johnson
Delft, January 1986

CONTENTS

THE CONTROL OF FERMENTATION PROCESSES

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Abstract. A survey of the control of fermentation processes operated in fed-batch mode is presented. Truely optimal conditions can only be found by minimizing (or maximizing) a cost criterion subject to the constraints imposed by a mathematical model of the process. This approach leads to the design of a tracking controller, or servomechnism as it is also called. A different approach uses a physiological model of the process to determine a control strategy, which usually results in keeping a measured or estimated variable at its setpoint by means of a regulator.

Keywords. Biocontrol; fermentation; fed-batch processes; modelling; estimation.

1. INTRODUCTION

Most biotechnological processes consist of the fermentation, oxidation and/or reduction of feedstuff by microorganisms such as yeasts or bacteria. These processes may be conveniently classified according to the mode chosen for process operation: either batch, fed-batch or continuous. During batch operation of a process no feedstuff is added to the initial change nor is product removed until the end of the process. Some pharmaceutical preparations are made in this way but generally batch operation is not commercially attractive. More economic is continuous operation where feedstuff is continually added and product continually removed. Examples are the continuous fermentation of milk in the production of margarine and the biological purification of waste-water.

From the control engineer's viewpoint it is the fed-batch processes, however, which present the greatest challenge. In fed-batches, the feed rate may be changed during the process but no product is removed until the end. Baker's yeast and antibiotics such as penicillin are made in fed-batches commercially, and there is an enormous economic incentive to optimize such processes. Such activity has been confined in the past to improving equipment design and, of course, to microbiological investigations. Thus the efficiency of penicillin production at Gist Brocades, The Netherlands, has been increased three to four times during the last 15 years (v.d. Meer and Valkema, 1985). Recently, attention has turned towards the optimization of the feed rate and better control during the fed-batch.

It must be said from the outset that the quest to maximize productivity is not the only reason for the control engineer's interest in the fed-batch process. Not only are these processes commercially important but they are technically challenging: the process variables are difficult to measure, the "quality" of the product plays an important role, the process model usually contains strongly time-varying parameters etc. etc. But above all the technical challenge arises from the nature of the process optimization since it is a *dynamical* problem.

Two approaches have emerged to solve this dynamic optimization problem and the principal contribution of this survey is to compare and contrast both approaches. The approach termed here physiological control attempts to emulate the routine process control of, say, the chemical industry where process variables are held at set-points by means of controllers called regulators. Of paramount interest here is not the design of the regulator (about which so much has been written in the control engineering literature) but rather how the set-points are chosen.

The other approach is to minimize (or maximize) a cost criterion while not violating the dynamic behaviour of a mathematical model describing the process. This text book approach, which is henceforth referred to simply as dynamic optimization, results in a number of variables being required to follow non-steady optimal trajectories. This tracking of the variables is accomplished using a servomechanism, or tracking controller as it is sometimes called. Here, it is not the design of the servomechanism which is of particular interest since again a substantial body of literature exists on the subject, rather the choice of mathematical model, cost criterion and optimization technique.

In constrast to previous surveys (e.g. Halme and Holmberg, 1978; Zabriskie, 1979) only fed-batch biotechnological process control has been reviewed. By this means a complete in-depth study has been possible. Moreover, if control engineers are confronted by problems concerning continuous processes the technique of physiological control described in this paper may offer an attractive solution, without the need for mathematical modelling.

2. MODELLING FOR CONTROL

We may distinguish three different types of model used for control. The first is the physiological model where knowledge of the physiology of the biological growth process is expressed in consequent, but usually non-mathematical, statements. The second is the structured model where

a system of partial differential equations (sometimes simplified to ordinary differential equation) is used to characterize the age distribution, for example, of the growing cells in the culture. Thirdly comes the unstructured model where the fermentation is assumed to be dominated by a single, homogeneously growing organism. Of the three types of model, the first and the last are most frequently encountered in controller synthesis. Since it is usual to control the environmental variables (temperature, pH, aeration and agitation) to be constant the models given will be understood to be valid only in these circumstances.

2.1. Control without a mathematical model

The earliest attempts at control of the fed-batch/batch fermentation process used no model at all. Successful state trajectories from previous batches which had been stored in the process computer were tracked using open-loop control (Yamashita, Hoshi and Inagaki, 1969). Many industrial fermentations are still operated using this method.

Constructing an accurate mathematical model of the fermentation process is a formidable task; moreover suitable values must be found for the model's parameters. It is not surprising, therefore, that considerable attention has been paid to developing controllers without the need for a mathematical model. Physiological models based upon an understanding of the Pasteur, Crabtree etc. (see e.g. Roels, 1983) effects are important here. Consider the aerobic cultivation of baker's yeast using molasses, which is a commercially important fed-batch fermentation. From physiological models, (at least) four control strategies can be envisaged viz:

A) Cell growth rate is maximal without byproduct (ethanol) formation if substrate concentration is held at its critical value.
B) Cell growth is optimal when the specific growth rate follows a predetermined trajectory.
C) Cell growth is optimal when the ethanol concentration is constant.
D) Maximum cell growth occurs when there is pure respiratory growth without ethanol production and simultaneously maximum molasses feed.

Obviously, refinements and hybrids of these strategies are possible. All need direct measurements or estimates to produce the required variables for feedback purposes. Estimators and the implementation of control strategies derived from physiological models are discussed in Sections 3 and 4, respectively.

Another promising approach, subject to much current research activity, is that of fuzzy control theory (Tong, Beck and Latten, 1980; Nakamura, Kuratani and Morita, 1985) where no formal model is required but where the process operator's experience can be exploited in the controller design.

2.2. Control with a structured model

The most mathematically complex fermentation models are those describing the effects of the internal state of the organisms on process operation. The model variables are classified or structured according to the age, volume, mass, species of the organism or the different organic carbon compounds in the growth medium (Alberghina, Martegani and Mariani, 1982; Holmberg and Ranta, 1982). The most important special case of this type of model is the cell age distribution model because the growth, decay, production of secondary products and consumption of oxygen of the organisms is known to be age dependent.

By introducing various assumptions regarding the cell metabolism, it is possible to reduce the distributed parameter cell age model to a lumped parameter (or finite dimensional) model of tractable size for controller design (Dairaku, Izumoko, Morikawa, Shioya and Takamatsu, 1982b; Ranta, 1982; Nestaas and Wang, 1983). However, apart from this approach there does not yet appear to be any application of distributed parameter models for control purposes.

2.3. Control with a homogeneous, single organism model

The last approach to controller design is to use an unstructured fermentation model where the culture is assumed to consist of a single, homogeneously growing organism. Moreover, it is often assumed that there is a single growth limiting substrate. An expression for the growth rate of the micro-organism and the consumption rate of the substrate is given by the equations

$$r_x(t) = \mu(t)x(t) - k_d(t)x(t) \tag{2.1}$$

$$r_s(t) = -\frac{\mu(t)}{Y(t)}\,x(t) \tag{2.2}$$

where $x(t)$ is the biomass concentration (kg/m^3), $\mu(t)$ is the specific growth rate (hr^{-1}), $k_d(t)$ is the biomass decay rate (hr^{-1}) and $Y(t)$ is the yield factor. For a perfectly mixed fermentor to which substrate of concentration $s_i(kg/m^3)$ is fed, diluting the culture at a rate $D(hr^{-1})$, then a mass balance using the above equations gives

$$\dot{x}(t) = \mu(t)x(t) - D(t)x(t) - k_d(t)x(t) \tag{2.3}$$

$$\dot{s}(t) = -\frac{\mu(t)}{Y(t)}\,x(t) + D(t)[s_i(t) - s(t)] \tag{2.4}$$

$$x(t_o) = x_o \tag{2.5}$$

$$s(t_o) = s_o \tag{2.6}$$

Here, $s(t)$ is the substrate concentration (kg/m^3) and the dilution rate $D(t) = F(t)/V(t)$, where $F(t)$ is the volumetric feed flowrate (m^3/hr) and $V(t)$ the volume of the culture (m^3). In some circumstances such as antibiotic production it is necessary to augment the above equations with an equation describing the product (e.g. antibiotic) concentration. An equation for the concentration of dissolved oxygen may also be required.

There are many analytical expressions for $\mu(t)$; Spriet (1982) lists nine possibilities, although more exist. The most popular is the Monod equation (Monod, 1942)

$$\mu(t) = \frac{\mu_m . s(t)}{k_s + s(t)} \tag{2.7}$$

with μ_m the maximum specific growth rate and k_s the Monod constant for growth on the substrate. An alternative approach is to assume that no analytical expression is available for $\mu(t)$, which leads to the design of an adaptive controller (Dochain and Bastin, 1984).

Much the same can be said for the yield factor, $Y(t)$. In controller design it is often assumed to be constant

$$Y(t) = Y \tag{2.8}$$

However, this neglects the influence of other factors, e.g. the synthesis of (by-)products. Dekkers (1983) has shown that when changes are

occuring slowly the yield factor for baker's yeast should be expressed as a relatively simple function of the specific growth rate and the critical specific growth rate, $\mu_c(t)$.

The model is not complete, of course, without the output equations relating the measured variables to the two (or more) state variables. Sensors meauring the biomass and substrate concentrations would be most advantageous but as yet there are no such commercially available sensors suitable for industrial applications. Discussion of the output equations is therefore deferred to Section 3 on estimation.

We consider next some properties of the unstructured fermentation model presented. For control purposes it is important to distinguish between two situations. Firstly (because it is easier), we investigate what happens when small deviations occur in the desired, or reference, trajectories of x(t) and s(t). For instance, how long does it take before such perturbations are damped out? This sort of question is vitally important to setpoint and tracking control. Secondly, the dynamical behaviour of the model must be understood "in the large", when deviations can no longer be considered to be small. This understanding is essential for dynamic optimisation. Note that wherever numerical values of parameters have been used they relate to the aerobic cultivation of baker's yeast using molasses.

The perturbation analysis may proceed by the linearization of eqns. (2.3) and (2.4). However, we choose to transform these equations first into equations in terms of M(t), the weight of biomass (kg), and W(t) the weight of substrate (kg):

$$\overset{o}{M}(t) = \mu(t)M(t) \tag{2.9}$$

$$\overset{o}{W}(t) = \frac{-\mu(t)}{Y(t)} M(t) + s_i(t)F(t) \tag{2.10}$$

$$\text{and} \quad \overset{o}{V}(t) = F(t) \tag{2.11}$$

$$\text{with } M(t_o) = M_o \tag{2.12}$$

$$W(t_o) = W_o \tag{2.13}$$

$$V(t_o) = V_o \tag{2.14}$$

It has been assumed that $k_d(t)$ is so small compared with $\mu(t)$ that it may be neglected. Note that if $\mu(t)$ and $Y(t)$ were known a priori the above two equations would be linear. Moreover, if $\mu(t)$ was known to be a constant, any perturbation in the biomass from a desired trajectory would never decay away, nor could it be controlled away (i.e. the system is unstabilizable). More realistically, let both $\mu(t)$ and $Y(t)$ be known linear functions of the substrate concentration:

$$\mu(t) = \alpha s(t) + \beta \tag{2.15}$$

$$Y(t) = \gamma s(t) + \delta \tag{2.16}$$

Linearization of eqns. (2.9) - (2.16) about some not necessarily steady state gives

$$\begin{bmatrix} \overset{o}{\Delta M}(t) \\ \Delta W(t) \\ \Delta V(t) \end{bmatrix} = \begin{bmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} \Delta M(t) \\ \Delta W(t) \\ \Delta V(t) \end{bmatrix} + \begin{bmatrix} 0 \\ s_i \\ 1 \end{bmatrix} \Delta F(t) \tag{2.17}$$

where $\Delta M \triangleq M(t) - M_r(t)$ etc. Consideration of the numerical values of the parameters of the above equation shows that the eigenvalue corresponding to the volume is zero while the eigenvalues of the biomass and substrate are widely separated. The biomass responds very slowly when perturbated from its reference value (and never returns to it) while the substrate feed is more or less immediately consumed. There are two important conclusions to be drawn from this analysis. Firstly that regulatory control is necessary for maintaining the biomass trajectory at its desired value in the face of disturbances, and secondly (Dekkers, 1983) that because of the separation of the eigenvalues the two major problems of control, that of optimization and that of regulation, may be separated.

It is worthwhile noting here that it would not be prudent to use the continuous-time, linearized model, equation (2.17) to design a controller. Rather, we would prefer to use a discrete-time model so that we could easily implement the controller in a digital computer and also so that stochastic (noise) processes corrupting the system could easily be modelled. The type of discrete-time model required is one whose behaviour at the sampling instants exactly matches that of eqn. (2.17); such a model has been termed the *equivalent discrete system*. Once the sampling period, T, is known the equivalent discrete system is easily derived (Johnson, 1985):

$$\begin{bmatrix} \Delta M(k+1) \\ \Delta W(k+1) \\ \Delta V(k+1) \end{bmatrix} = \begin{bmatrix} 1+a_{11}T & a_{12}T & a_{13}T \\ a_{21}T & 1+a_{22}T & a_{23}T \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} \Delta M(k) \\ \Delta W(k) \\ \Delta V(k) \end{bmatrix} + \begin{bmatrix} 0 \\ s_i T \\ T \end{bmatrix} \Delta F(k) + n(K) \tag{2.18}$$

Here, k = 0,1,2,..., $\Delta M(k)$ is the value of $\Delta M(t)$ after kT and n(k) is a vector of zero-mean discrete white noise.

For large changes the above perturbation analysis no longer holds. Some insights into the global dynamic behaviour of the fermentation model now follow. Dochain and Bastin (1984) have proved under certain dynamic conditions that the variables x(t) and s(t) of the model (2.3) - (2.6) are bounded for any dilution rate. To be precise, if the following assumptions are made:

(A1) $Y(t) = Y$, constant

(A2) $k_d(t) \equiv 0$

(A3) $\mu(t) = 0$ if $s(t) = 0$

(A4) $\mu(t) \geq 0$

(A5) $0 \leq s_i(t) \leq s_i^{max}$

(A6) $\frac{x_o}{Y} + s_o \leq s_i^{max}$

then for any nonnegative, but not necessarily bounded, dilution rate,

$$0 \leq x(t) \leq Y s_i^{max} \tag{2.19}$$

$$0 \leq s(t) \leq s_i^{max} \tag{2.20}$$

The upper bounds on the state variables are, of course, very conservative and the assumption of a constant yield factor is not very realistic, but this result is nevertheless important for the convergence analysis of adaptive control and estimation schemes.

If dynamic optimization (section 5) is implemented by a computer then the controlled variable F(t) will be piecewise-constant. It is possible to show that the dynamic reponse of the model (2.9) - (2.16) between sampling instants is in that case governed by a Bernoulli-type equation. For fast sampling rates the linear model, equation (2.17), would be an adequate approximation to reality and its response to a piecewise-constant control signal is readily calculated.

3. ESTIMATION

Many different types of sensors and monitoring systems for fermentors have been described or postulated in the literature (see e.g. Proceedings 1st IFAC Workshop on Modelling and Control of Biotechnical Processes, Helsinki, 1982; Proceedings Symposium on Analytical Methods and Problems in Biotechnology, Noordwijkerhout, 1984). Some of the more common types of sensor are listed in Table 1.

TABLE 1

VARIABLE	SENSOR	REFERENCE
biomass growth	filtration probe	Nestaas et al (1983)
	optical density	Shioya et al (1982)
	fluorescence	Shioya et al (1982)
dissolved oxygen	polarographic	
	PTFE tubing	
	galvanic cell	
gaseous oxygen	paramagnetic	
	zirconia	Flynn (1982)
	mass spectro-meter	Flynn (1982)
gaseous carbon dioxide	infra-red	Flynn (1982)
	mass spectro-meter	Flynn (1982)
substrate concentration	affinity	Shioya et al (1982)
	microbial	Shioya et al (1982)
	enzyme electrode	Shioya et al (1982)
	enzyme thermis-tor	Axelson et al (1982)
ethanol concentration	teflon tubing	Shioya et al (1982)
air flow rate	flow meters	
pH	glass electrode	
temperature	resistance thermometer	
agitated speed	counter	
volume	differential pressure cell	
foam	contact electrode	

If we exclude variables that are usually encountered in process control (e.g. temperature, pressure etc.) then many of the remaining sensors which register directly biomass, byproduct or substrate concentrations are not robust enough or are too expensive for routine industrial control applications. Sometimes measurements are available but they must be analysed in a laboratory before a value of the variable of interest can be obtained. Therefore it is common practice to use measurements of related variables, such as gaseous oxygen and gaseous carbon dioxide, to estimate the unobtainable variables.

The simplest, but least accurate, way to obtain the estimate is to neglect all measurement errors, instrument noise etc. and to correlate experimentally obtained measurements of the desired and related variables. A more sophisticated approach is to model the neglected errors and noise and to employ an estimator. If linearity and additivity assumptions on the model are justified, the optimal estimator is the Kalman Filter; if not, recourse is normally made to a suboptimal estimator such as the Extended Kalman Filter. For a description of these estimators and their properties the reader is referred to one of the many texts on the subject (e.g. Anderson and Moore, 1979; Johnson, 1985), while applications of both optimal and suboptimal estimators to fermentation processes have been well documented (Svrcek, Elliott and Zajic, 1974; Dekkers, 1982; Nihtila, Harmo and Perttula, 1984). In the remainder of this section attention is focussed on just one aspect of estimation for control, namely the theoretical and experimentally derived relationships between measured and unobtainable variables that we wish to estimate. The relationships serve as the output equations for the models discussed in the previous section.

We begin by defining three intermediate variables OUR, CER and RQ. It is assumed that we measure the partial fraction, λ_O(mol/mol), of gaseous oxygen and the partial fraction, λ_C(mol/mol), of gaseous carbon dioxide in the dried effluent gas, together with the total dry air inlet flowrate, ϕ(mol/hr). From these three measurements the *oxygen uptake rate*, OUR(mol/hr), and the *carbon dioxide evolution rate*, CER(mol/hr) can be calculated (Cooney, Wang and Wang, 1977):

$$OUR(t) = \phi(t)\left[\frac{0.20945\lambda_C(t)+0.99967\lambda_O(t)-0.20945}{\lambda_C(t)+\lambda_O(t)-1.0}\right] \tag{3.1}$$

$$CER(t) = \phi(t)\left[\frac{0.79055\lambda_C(t)+0.00033\lambda_O(t)-0.00033}{1.0-\lambda_C(t)-\lambda_O(t)}\right] \tag{3.2}$$

Dekkers (1982) has calculated additional terms for the above expressions to account for the influence of various dynamical accumulation effects. The order of magnitude of the correction terms depends on the process circumstances. For control purposes it may be desirable to use as the controlled variable the *respiratory quotient*, RQ(t), which is the ratio of the amount of carbon dioxide produced to the amount of oxygen consumed, i.e.

$$RQ(t) = \frac{CER(t)}{OUR(t)} \tag{3.3}$$

$$= \frac{0.79055\lambda_C(t)+0.00033\lambda_O(t)-0.00033}{0.20945-0.20945\lambda_C(t)-0.99967\lambda_O(t)} \tag{3.4}$$

3.1. Estimating the biomass

It has been found experimentally that the behaviour of OUR(t), CER(t) and RQ(t) is very dependent upon the type of fermentation, and upon the operating conditions. For fed-batch baker's yeast fermentation where the critical specific growth rate is exceeded, OUR(t) varies only slowly and is correlated to the biomass growth; CER(t) or RQ(t), by contrast, change more quickly and are correlated to the uptake of the substrate feed (Dekkers, 1985). This is not true with many other organisms, where the RQ(t) is more

or less constant during the fermentation; in such cases the biomass growth may be correlated with either the OUR(t) or CER(t).

Using the stochiometric equation for the growth of biomass, Dekkers (1982) has shown that for baker's yeast fermentation

$$BPR(t) = 2.17\ OUR(t) - 3.26\ CER(t) + 6.52\ GFR(t) \tag{3.5}$$

is a good approximation to reality, with BPR(t) the biomass production rate (mol/hr) and GFR(t) the glucose feedrate (mol/hr). Equation (3.5) could also be written as

$$\dot{M}(t) = 0.06\ OUR(t) - 0.09\ CER(t) + 297.75\ F(t) \tag{3.6}$$

Subject to knowledge of the reaction mechanism being available, the above approach may be applied to any fed-batch fermentation. In certain circumstances, when the fermentation proceeds with a near constant respiratory quotient for example, the correlation may be considerably simplified. For cell growth in fed-batch penicillin production, Mou and Cooney (1983) have found that the amount of biomass is accurately estimated from the relation

$$M(t) = K \int_0^t CER(\lambda)d\lambda \tag{3.7}$$

where K is an experimentally determined constant.

Because of measurement noise and the uncertainty of the initial estimate of the biomass in the inoculum it is not usually acceptable (Stephanopoulos and San, 1981) to obtain the estimate of the biomass from the estimate of the biomass production rate by straightforward integration, such as is done in equation (3.7). A much better approach, see Fig. 1, is to augment output equation (3.6) or similar with a model having two states, M(t) and $\dot{M}(t)$, and to use the Extended Kalman Filter to produce estimates of both the amount of biomass and its production rate (Dekkers, 1982). For baker's yeast the state model is simply

$$\dot{M}(t) = BPR'(t) \tag{3.8}$$

$$\dot{BPR}'(t) = [BPR'(t)]^2/M(t) \tag{3.9}$$

where BPR'(t) is BPR(t) expressed in kg/hr.

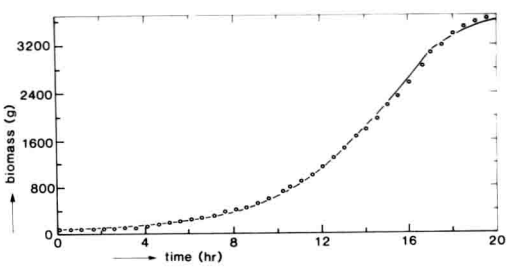

fig.1. Biomass estimation (Dekkers, 1982).
—— on-line estimates
• • • off-line measurements.

3.2. Estimating the substrate concentration

This topic has received little attention in the literature. The only noteworthy approach is based on equation (2.10) which models the substrate accumulation during the fed-batch. It is assumed that the composition of the exit gas is in some way correlated to the amount of substrate consumed by the growing biomass; for example in the case of fed-batch fermentation of glutanic acid that OUR(t) is directly proportional (Hong et al, 1984) to the substrate consumption $\mu(t)x(t)/Y(t)$. If s_i is constant and since $W(t) = V(t)s(t)$, where $d/dt(V(t)) = F(t)$, integration of eqn. (2.10) gives

$$s(t) = \frac{V(o)s(o)}{V(t)} + \left[1 - \frac{V(o)}{V(t)}\right]s_i - \frac{K}{V(t)} \int_0^t OUR(\lambda)d\lambda \tag{3.10}$$

It would appear that the estimate of the substrate concentration obtained in this way, even with exactly known initial concentrations and volumes, would be subject to an accumulation of errors as mentioned in the previous section for biomass estimation.

3.3. Estimating the specific growth rate

If estimates of both the biomass production rate, $\dot{M}(t)$, and the amount of biomass, M(t), are available then an estimate of the specific growth rate can be made from equation (2.9).
When both estimates of M(t) and $\dot{M}(t)$ have been produced by an Extended Kalman Filter using a two state model, this approach gives quite satisfactory results (Dekkers, 1982) for a fed-batch baker's yeast fermentation. See Fig. 2.

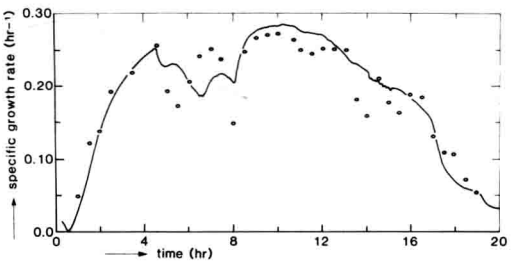

fig.2. Specific growth rate estimation (Dekkers, 1982).
—— on-line estimate
• • • off-line measurements.

Sometimes it is possible to regard the specific growth rate as a state variable in a model of the type

$$\dot{\mu}(t) = 0 \tag{3.11}$$

$$\dot{M}(t) = \mu(t)M(t) \tag{3.12}$$

and to estimate $\mu(t)$ directly by means of the Extended Kalman Filter, using measurements of CER(t) and OUR(t). The suboptimal estimates can once again be quite satisfactory (Nihtila, Harmo and Pettula, 1984).

If only one estimate of the amount of biomass is available, the derivative in equation (2.9) could be approximated by a backward-difference term to give (the biomass decay rate is zero)

$$\mu(t) \approx \frac{1}{\Delta t} \ln[M(t)/M(t-\Delta t)] \tag{3.13}$$

Mou and Cooney (1983) have applied this formula to estimate the specific growth rate in a penicillin fermentation. The approach is, however, very sensitive to small errors in the estimates of the biomass.

4. PHYSIOLOGICAL CONTROL

In section 2.1 we indicated that a control strategy could be formulated without a mathematical model, and we gave four examples of strategies derived from physiological models. The examples illustrate what has recently been termed *physiological control* (Mou and Cooney, 1983) although such control strategies have been in use since the fifties (see e.g. Dietrich, 1959). The two stages of the synthesis of a physiological controller are (i) the identification, by means of a physiological model, of one or more variables that must either be held constant or required to follow specified time-trajectories (ii) the development of a controller to ensure that those variables are, indeed, maintained at their constant (setpoint) values, or track the specified time trajectories without error. This second stage is the usual setpoint or servomechanism (tracking) control problem usually involving the estimation of one or more unmeasurable variables; the many different approaches to the controller design will not be discussed further here. We only remark that to thisend, the controller parameters may either be adjusted on-line using a technique such as that known as the Ziegler-Nichols method or be calculated offline with the aid of a mathematical model. A third possibility, exploited in adaptive controllers, is to automate the on-line adjustment of the controller parameters.

Physiological control has the advantage that the mathematical relationships between the process variables need not be known; at most a linearised model expressing the dynamics of perturbed variables is required. As a result it is possible in some circumstances to control the product quality. Moreover, physiological control is usually computationally less demanding than other approaches.
Its chief disadvantage is that it may not produce truly optimal control. It may also require special sensors or costly offline laboratory analyses, which can introduce a variable time delay into the system and thus complicate the control.

Some aspects of the physiological control of a fed-batch baker's yeast fermentation are now briefly explained. Other fermentations are usually more complex and less well understood. Starting point is the assumption that temperature and pH are controlled to be constant; the two manipulated variables are then the air supply and the feedrate of sugar. In general, the growth rate of the biomass (dM(t)/dt) can be assumed to be limited by the available amount of sugar. Recalling equation (2.9) we see that the rate of growth of yeast is proportional to the value of the specific growth rate, $\mu(t)$: the larger $\mu(t)$ is, the more yeast is produced. But $\mu(t)$ is controlled by the substrate concentration in the broth, which is in turn determined by the sugar feedrate, so that increasing the sugar feedrate increases the specific growth rate, producing more biomass. Two complications upset this straightforward view of the physiology, however. Firstly, if the substrate concentration in the broth is too high, ethanol will be produced (the glucose or negative Pasteur effect). Secondly, if not enough oxygen is supplied to combust the substrate, ethanol will again be produced. Ethanol is unwanted since it represents wasted sugar (although the quality of the yeast may be improved if some alcohol is present in the broth at the end of the fermentation). From stochiometric considerations it can be shown that when ethanol is produced CER(t) increases while OUR(t) remains constant. This has the effect of increasing RQ(t), see equation (3.3), so that the respiratory quotient is a good indicator of ethanol production.

The above outline of the physiology of yeast growth is by no means complete. However, it is comprehensive enough to explain nearly all the physiological control strategies which have been proposed to date in the literature, some of which are now described. Unless otherwise stated, all refer to baker's yeast fermentation.

4.1. Substrate control

Assume that there is sufficient oxygen dissolved in the broth to ensure the combustion of the sugar present. Then control, in some way, the substrate concentration to be constant during the whole fermentation. This will ensure that the specific growth rate is constant and, if it is less than some critical value, $\mu_c(t)$, no ethanol will be produced, leading to maximum productivity (Peringer and Blachere, 1978). According to equation (2.9) there will be an exponential growth of yeast resulting from this substrate control:

$$M(t) = M_0 e^{\mu t} \tag{4.1}$$

How can both the dissolved oxygen concentration and the substrate concentration be held constant? The dissolved oxygen concentration may be measured and its value used by a feedback setpoint controller to adjust the air flow rate and/or the stirring speed. However, one of the major constraints in a yeast fermentation is the oxygen transfer capacity of the fermentor (Wang, Cooney and Wang, 1979) so constant dissolved oxygen concentration control may not be feasible throughout the fermentation.

Various techniques have been proposed to keep the substrate concentration constant. The most obvious is to use a feedback controller where the sugar in the broth is measured and the sugar feedrate is varied accordingly (Axelson et al, 1982). If both the substrate concentration and the biomass production rate are measured, the adaptive minimum variance control algorithm proposed by Dochain and Bastin (1984) could be used to keep the substrate concentration constant.

If a reliable sensor is not available then an estimate of the substrate concentration could be used in its place (see section 3.2). Failing this, open-loop control could be used: from equation (2.4) the feedrate to achieve a constant substrate concentration is given by

$$F(t) = \frac{\mu M_0 e^{\mu t}}{Y(s_i - s)} \simeq \frac{\mu M_0 e^{\mu t}}{Y s_i} \tag{4.2}$$

However, open-loop control cannot compensate for any disturbances occurring in the process conditions during the fermentation. To counteract this, Cooney and Swartz (1982) proposed incorporating equation (4.1) in (4.2) to give

$$F(t) = \frac{\mu M(t)}{Y s_i} \tag{4.3}$$

Based on stochiometric balances and measurements of CER(t) and OUR(t) the biomass, μ and Y can be estimated from an expression similar to equation (3.6) so that feedback control can be used. With the specific growth rate kept just below 0.22 hr^{-1} and the substrate concentration constant and near zero (< 0.15 g/l) the authors claim high productivities and yields approaching the maximum theoretically achievable of 0.5 g cell/g sugar.

Hong et al (1984) exploited the correlation between substrate concentration and OUR(t) to maintain a constant substrate level during glutamic acid fermentation. Their predictions of increased productivity were verified by experiments using a 30 l fermentor.

4.2. Specific growth rate control

As we have seen, the philosophy of substrate control implies that the specific growth rate is held at some constant value, μ_c, during the whole fermentation. Recently an adaptive controller has been proposed (Wu, Chen and Chiou, 1985) to accomplish this for a fed-batch baker's yeast fermentation. The actual specific growth rate is estimated from measurements of OUR(t). If the heuristic Certainty Equivalence approach is valid, which the experimental results of the authors seem to imply, then good control around the chosen specific growth rate setpoint could be expected; no proof of convergence is given, however.

The optimization of comprehensive mathematical models of fed-batch fermentations subject to realistic cost criteria indicate that maintaining the specific growth rate at a constant value seldom or never will lead to optimal productivities or yields (Dekkers, 1983). Instead, the specific growth rate must follow a time-dependent trajectory since the critical specific growth rate (above which ethanol is produced) is not constant as had hitherto been assumed but time-varying. In fact, it appears that for baker's yeast fermentation, the value of the critical specific growth rate depends upon whether the fermentation is at the stage of (i) start-up or exponential growth, or (ii) limited oxygen supply (Dekkers, 1984). Anyway, since the desired specific growth rate is time dependent, the controller for specific growth rate control is not of the set-point type, but of the tracking variety.

For the fed-batch culture of baker's yeast Dairaku and co-workers (1982a) have calculated the glucose feedrate necessary to achieve time optimal transfer from one specific growth rate to another. Using a measurement of the ethanol concentration by means of a porous teflon tube, and a feedback controller also regulating the glucose feedrate, it could be assumed that there was no ethanol consumption or production during the fermentation. The resulting optimal control strategy consisted of firstly applying the maximum feedrate until the critical substrate concentration was achieved and then maintaining that critical concentration until the desired specific growth rate is reached.

The principle of specific growth rate control has found primarily application in the fed-batch fermentation of penicillin. The objectives there are to maximize the specific growth rate during the fast-growth period and then to keep the production-phase specific growth rate at a lower, fixed value (Mou and Cooney, 1983). The specific growth rate is calculated from an on-line estimate of the cell mass, found by either stochiometric balances or from the correlation with CER(t). Refinements to the control, such as provision to ensure no substrate accumulation in the broth or to ensure that the dissolved oxygen never falls below a certain value (Nelligan and Calam, 1983) have been proposed and verified experimentally.

4.3. Ethanol control

According to the physiological model, any ethanol which is produced during a baker's yeast fermentation represents a wasted conversion of sugar. Using a porous teflon tubing ethanol sensor, a control system to regulate the ethanol concentration about a setpoint has been proposed (Dairaku et al, 1983). The objective of the controller is to maintain a maximum feedrate of sugar without ethanol production, and it is basically a three-term (PID) controller. The controller parameters have been determined by a series of experiments, and are tuned on-line according to the biomass concentration, estimated from a simple mathematical model.

It may be desirable to allow some alcohol to be produced during the last stage of the yeast fermentation. This is because it has been noted (Reed and Peppler, 1973) that the baking power of yeast is diminished if significant alcohol consumption takes place at the end of the batch.

4.4. RQ control

The respiratory quotient can be easily measured. If the RQ is regulated at 1.03 - 1.06 during the yeast fermentation while at the same time the sugar feed rate is maximized then there will only be pure respiratory growth without ethanol production, and hence maximum productivity. These two factors have made RQ control very popular over the years.

Nearly all the examples of RQ control in the literature employ the setpoint, feedback type of controller (Aiba et al, 1976; Wang, Cooney and Wang, 1979; Pons, Bordet and Engasser, 1982). There has been little attention paid to choosing the optimal parameter values of the controller (i.e. the PID action coefficients). This is a pity, since the RQ is very often hardly controlled at all during the start or the end of the batch, due to the different stages during a fermentation requiring different controllers. See Fig. 3.

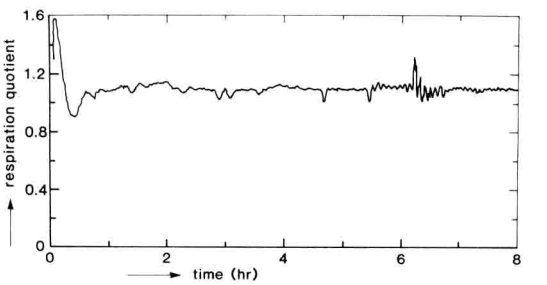

fig.3. RQ control (Dekkers, 1984).

Recently, studies of the optimal control of a baker's yeast fermentation have shown that the optimal RQ(t) trajectory will not, in general, be a constant straight line (Dekkers, 1983). That is if an economically realistic cost criterion is used for the optimization (see section 5). Two adaptive RQ controllers which can, if necessary, follow a predetermined RQ trajectory have been proposed and tested experimentally (Dekkers and Voetter, 1985). Both appear to offer much better RQ regulation than was previously considered possible.

4.5. Environment and quality control

Often the environmental variables such as temperature, pH, agitation and aeration are controlled to be constant. If this is not so, or indeed if process conditions are deliberately changed during the fermentation the consequences can be far-reaching and may be beneficial (Biryukov, 1982). For example, it is commonplace during some fermentations to control the pH of the broth to increase from about 3 initially (so reducing the chance of infection of the microorganism during the