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Fermentation Monitoring and Control: A Perspective

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Introduction

One of the primary objectives of industrial fermentation research and development is the establishment of economically viable processes through increasing product yields and reduced operating costs. Historically, the most important means of achieving this has been by strain improvement, using a variety of techniques, by growth medium development and improvements in nutrient feeding. In recent years, however, tremendous progress has been made in the measurement of biotechnical parameters, bioprocess instrumentation and bioprocess modelling and control. The 'control' of biotechnological plants is a complex problem since there is a great range of processes. These can be considered from both a biological and an engineering viewpoint. From the biological perspective—different substrates can be used (e.g. pure or synthetic substrates, natural substrates including waste). These are converted by a variety of micro-organisms under anaerobic or aerobic conditions to the desired products (e.g. pharmaceuticals, amino acids, organic acids, enzymes, proteins, biodegradable materials, biogas, alcohol, etc.). The engineering, or technological, aspects include different reactor types—stirred tank type, tube type, tower type, cascaded reactors, etc. In addition there are several modes of reactor operation—batch, fed batch and continuous—each posing different operational problems in terms of control system structure. The progress made

Abbreviations: AA, atomic absorption; AI, artificial intelligence; ATP, adenosine 5'-triphosphate; CARIMA, controlled autoregressive integrated moving average; CARMA, controlled autoregressive moving average; CER, carbon dioxide evolution rate; DO_2, dissolved oxygen; DOT, dissolved oxygen tension; EKF, extended Kalman filter; GC, gas chromatography; GCMS, combined gas chromatography and mass spectrometry; GFR, glucose feed rate; GMV, generalized minimum variance; GMVC, generalized minimum variance control; GPC, generalized predictive control; HPLC, high performance liquid chromatography; LQ, linear quadratic; LQG, linear quadratic Gaussian; MIMO, multi-input multi-output; MRAC, model reference adaptive control; NAD(P)H, reduced nicotinamide adenine dinucleotide (phosphate); OUR, oxygen uptake rate; PI, proportional-integral; PID, proportional-integral-derivative; RQ, respiratory quotient; SIMO, single-input multi-output.

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over the past six years in measurement technology and biosensors, automation and computer control, and the development and application of biotechnological process modelling together with advanced control methodologies is reflected by the contributions to a series of important International Federation of Automatic Control (IFAC) meetings devoted to biotechnological processes (Halme, 1982; Johnson, 1985; Fish and Fox, 1988). This chapter will concentrate on the modelling and control of fermentation processes. It should be recognized, however, that the techniques developed for, and used in, fermentation are readily applicable, for example, to wastewater treatment and other biotechnological processes.

Control of the normal physical and chemical states of a fermentation (environmental control) is relatively straightforward. Software-based methods, such as state estimation and adaptive and inferential control methodologies, are becoming of special interest because they usually only require conventional instrumentation to support them. These methods, however, are still some way from being applied routinely in industrial situations because of a number of problems related to the biological models required for their implementation. Difficulties concerning numerical robustness and user friendliness remain to be solved.

Measurements for fermentation supervision and control

In industrial fermentations the availability of measurements will be significantly influenced by reliability, ease of use and robustness. Modern micro-electronic instrumentation provides powerful, sophisticated and reliable measurement of electrical signals at relatively low cost. Fermentations provide a demanding environment for reliable, stable and noise-free measurement from high-output impedance sensors and transducers (Clarke *et al.*, 1982). In such circumstances it is largely sensor and transducer characteristics that determine reliability and robustness. The measurements must provide demonstrable benefits without compromising the process. In particular, for most fermentation processes this must be achieved without increased risk of contamination. These considerations often lead to well-instrumented research and development fermenters, while production fermenters, where control methodologies could provide considerable benefits, are the least well instrumented.

Present on-line fermentation process measurement, and as a result most on-line control, is based upon a few, robust, commercial devices (Flynn, 1982; Carleysmith and Fox, 1984). The instrumentation utilized is largely similar to that found in chemical plants, although the probes are in a more demanding environment in that they must withstand sterilization, and are subject to fouling by surface coatings of organisms, cellular macromolecules, such as proteins, and other media components. The most important sensors for fermentation control (e.g. pH and DO_2 probes) are the least reliable of the sensors widely used in chemical process control (Flynn, 1982; Lees, 1976). These on-line sensors are largely *in situ*, making simple physico-chemical measurements. The control action taken to maintain constancy of the measured variable (e.g. by acid/alkali addition, heating/cooling, antifoam

addition, etc.) is often related to growth and/or product formation and so may be subject to the effects of process disturbances, shifts in metabolism and also, indirectly, by other control actions. As a consequence, these controls could be, but usually are not, employed to afford useful information about the fermentation operation. For example, feedback control of pH if, as is usual, pH is under feedback control, the control action taken in regulation provides an indication of metabolic rate (Cooney, Wang and Wang, 1977).

The best, general, on-line growth-related measurements from amongst many available (Meyer, Kappeli and Fiechter, 1985) are probably those based upon gas analysis, enabling CO_2 production and O_2 uptake to be calculated from the fermenter exhaust and inlet gases. These are sometimes available continuously, e.g. with infra-red, paramagnetic and Zirconia analysers. In practice these instruments are often multiplexed, leading to discrete analyses. More popular, and becoming more cost effective, is mass spectrometry. This is inherently a fast, discrete analysis, which is also usually multiplexed (Buckland *et al.*, 1985; Coppella and Dhurjati, 1987). Although all these measurements lead to discrete data, they are relatively fast for control purposes. Flynn (1982) has commented upon the accuracy and precision of calculations (giving derived variables) based on gas analysis (Spriet *et al.*, 1982), indicating that care must be taken in their use.

Discrete-sample analysis using auto-analysers and a variety of other specific analytical measurement techniques (spectrophotometry, HPLC, GC, GCMS, AA) is widely used, giving infrequent and delayed off-line data. These techniques can give quite rapid results if samples are taken frequently, but in practice it is usually the case that samples are taken relatively infrequently, with 'returned' results taking one or two hours. These results may be used manually to adjust fermentation parameters and monitor the fermentation. The use of infrequent off-line data in estimation control schemes is discussed later.

Solutions to the problems induced by reliance upon off-line analyses are being found through new *in situ* sensors. The specificity and sensitivity required indicates significant potential for on-line biosensors (Cleland and Enfors, 1983, 1984; Karube, 1984; Brooks and Turner, 1987). There are problems with sterilization, stability and robustness, but developments in continuous flow-line sampling (Clarke *et al.* 1982; Mandenius, Danielsson and Mattiasson, 1984; Omstead and Greasham, 1988), and in off-line discrete sample measurements (*see* references in Meyer, Kappeli and Fiechter, 1985; and the clinical laboratory techniques described by Truchaud *et al.*, 1980), promise to overcome these problems.

However, many other options are being investigated, for example in the field of biomass determination alone a wide range of measurement techniques are being applied (Harris and Kell, 1985; Ramsay *et al.*, 1985). They are based upon such diverse principles as: acoustics (Clarke *et al.*, 1982); piezo-electric membranes (Ishimori, Karube and Suzuki, 1981); bioelectrochemistry (Ramsay *et al.*, 1985); laser light scattering (Latimer, 1982; Carr *et al.*, 1987); electrical admittance spectroscopy (Kell, 1987; Harris and Kell, 1985); fluorescence (Zabriskie and Humphrey, 1978; Srinivas and Mutharasan, 1987); calorimetry (Birou, Marison and Von Stockar, 1987); and viscosity (Picque and Corrieu, 1986).

In general, the measurements supplied by sensors are not simple linear correlations to the fermentation process variable of interest. Significant correlations can be made between these measurements and the state variables required for control, e.g. ATP or NAD(P)H for biomass. However, analysis of the measurement and the state variable usually indicates that the measured variable is a complex function of many factors. Under calibration conditions most of these factors vary little and/or cancel each other out to leave the required correlation. In practice, however, these factors can vary significantly and, as a result, the calibrated correlation may not be valid. This may well be significant for any new sensors introduced (e.g. Clarke *et al.*, 1982; Srinivas and Mutharasan, 1987). For example, new techniques for biomass determination such as admittance spectroscopy, infra-red optical fibre light scattering detection, on-line fluorescence probes for NAD(P)H, may all exhibit good direct correlations under suitable calibration conditions but are complex functions of biological and physico-chemical effects. Similarly, the development of a whole range of ion-selective electrodes allows the direct measurement of a wide range of important medium constituents, but the measured values are activities and correction is required for a whole range of interfering ions, ionic effects and chelation (Clarke *et al.*, 1982).

The converse of this is that these measurements contain a great deal of information about the environmental conditions and the physiological state of the organisms, but more effort will be required to use this information for control purposes. To this end developments in sensors and in numerical and model-based estimation techniques are essential.

Environmental and derived variable feedback control

The on-line measurement of process variables not only provides a means by which to monitor the progress of a fermentation but also allows the regulation of the environment to which an organism is exposed. Since the environment that exists inside a fermenter is a major influence on the performance of a fermentation, regulation of environmental conditions can be utilized to influence the organism state. It can be seen from the previous section that some variables in a fermentation are capable of measurement on a continuous basis with some degree of reliability. It is their feedback, to enable the maintenance of desirable environmental conditions, together with variables derived from these measurements, which form the basis of the majority of present fermentation control schemes.

On-line regulation is usually restricted to the maintenance of a small number of environmental conditions, such as broth temperature, pH and dissolved oxygen levels. This is achieved through the manipulation of fermenter heating and cooling, acid/alkali addition and aeration rate (stirrer speed), respectively. Feedback controller tuning (either on–off time period settings or proportional–integral–derivative settings) to obtain acceptable performance in the presence of system disturbances is not always straightforward. This is due to non-linearities of the bioprocess itself and the control variable actuators, as well as any non-linear characteristics in the process sensors. In addition, perturbation of process variables as an aid to on-line tuning of the controllers

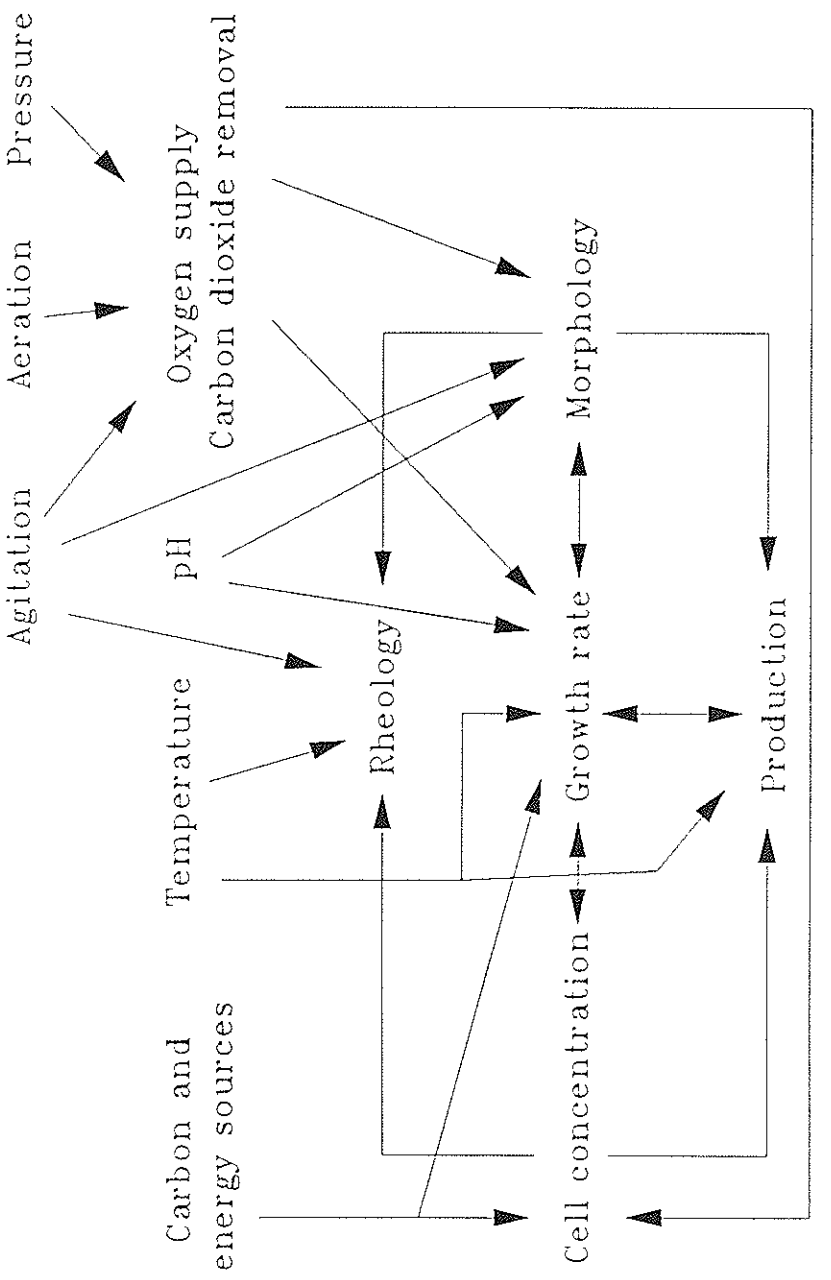

Figure 1. Typical bioprocess interactions

requires care, in order not to force the fermentation into significantly different operating regimes. For example, excessive growth or lysis conditions may result from perturbations in feed rates.

Fermentation processes can also exhibit the classic problem of multivariable system interactions which serve to complicate the controller tuning. *Figure 1* serves to demonstrate the complexity of the major interactions which can take place between variables within a fermentation. The interactions shown are by no means an exhaustive set, as several other interdependencies could also be postulated.

Controllers are usually tuned on a loop-by-loop basis, neglecting the effects of any process interactions. When all the control loops are closed the effect of the process interactions is to propagate disturbances between the loops, which can result in prolonged unsatisfactory behaviour of the individual environmental control loops. All the above problems can be overcome to a greater or lesser extent by using established control loop design and tuning techniques (e.g. Miller *et al.*, 1967).

In practice it is usually possible to maintain conditions reasonably close to desired values (e.g. ±0·1°C, ±0·1 pH) with well-tuned control loops. However, the determination of 'desirable operating conditions' (i.e. the controller set-points) poses the major problem. The environmental variable set-points are often chosen based upon past experience and trial and error approaches in order to 'optimize' the performance. This usually means regulation at a constant set-point, which in many cases is not ideal due to the constantly varying dynamics experienced in a fermentation. A set-point trajectory that would optimize the fermentation is difficult to specify and a more considered approach must be developed for its specification. These techniques will be outlined later.

While the feedback of established environmental measurements is common place, the consequences of fermenter engineering design can affect the quality and applicability of the measurements made. For example, by the very nature of the operation of an air-lift fermenter, an organism is subjected to cyclic variations in dissolved oxygen levels. In this case a number of dissolved oxygen probes may well be more appropriate in order to gain some insight to the cyclic nature of the variation. Even a relatively high frequency of exposure, compared with overall organism growth dynamics, can have serious consequences in terms of fermentation performance. Reuss and Brammer (1985) demonstrated that although control techniques may work well on the laboratory scale, the engineering implications of scale-up can significantly degrade the quality of performance obtainable from the fermentation. Cyclic variation of substrate in a large bioreactor due to inefficient mixing was demonstrated to markedly reduce the yield of biomass.

The previous section outlined some of the developments in sensor technology that are currently taking place. It is apparent, however, that at the present time there are many other measurements of environmental conditions (e.g. precursor, substrate concentrations) that are not routinely available on-line for direct feedback control. The usual method adopted for environmental fermentation regulation is therefore based upon the use of a

combination of off-line and on-line measurements for single-loop feedback control. Resorting to the use of off-line measurements in the feedback loop has important consequences on the quality of control that can be obtained. Montague, Morris and Bush (1988) discuss in detail the problems resulting and the considerations necessary before reasonable control can be achieved. In summary they conclude that it is the ability to obtain off-line measurements, with the minimum process/measurement delay and at a rate suitable for feedback control, which usually proves to be a problem. This said, the use of off-line measurement still forms a major component of many fermentation control schemes. More advanced techniques, aimed at overcoming the problems of off-line measurement and the resulting process delay, have been developed which utilize both on- and off-line measurements in on-line-measurement-based feedback control schemes. These have been shown to be successful, as will be discussed later.

The successful maintenance of fermentation environment using feedback control, however, does not necessarily imply that the fermentation is being operated under optimal conditions. In order to improve the performance of a fermentation system, it is necessary to consider variables (or states) that give some indication of the way in which an organism is behaving and not just the environment to which it is exposed. It is worthwhile at this stage considering what the aims of a fermentation control scheme should be. Ideally, it is desirable to be able to specify the condition (i.e. all the states) of the fermenter at any time. It is the present states coupled with the system inputs that completely specify the future fermenter condition.*

The important consequences are that knowledge of all the critical variables (states) and inputs that affect the bioprocess are required in order to specify future fermentation conditions. In this context, environmental regulation is the control of only a few of the many system states and therefore will not achieve the quality of control often demanded for improved operation. To move towards this improved operation requires the control of variables more closely related to the states of the system, while bearing in mind the problems of process measurement. In particular, the feedback control of the major carbon source in a fermentation is desirable, since it usually represents a major component of the production cost.

Off-gas (effluent gas) measurements have proved to be a popular method of gaining insight into the performance of an organism, since they are available on-line, without significant delay and give some indication of growth. Three common derived variables based upon off-gas measurements can be identified: OUR (oxygen uptake rate); CER (carbon dioxide evolution rate) and RQ (respiratory quotient), which is defined as the ratio of the amount of carbon dioxide produced to the amount of oxygen consumed, CER/OUR. RQ, CER and OUR thus represent 'control variables' that can be regulated throughout the fermentation period.

The behaviour of OUR, CER and RQ varies with the type of fermentation under investigation and the particular operating conditions which prevail.

* 'We ought to regard the state of the universe as the effect of its antecedent state and the cause of the state that is to follow' (Laplace, eighteenth century).

Johnson (1987), with reference to the baker's yeast fermentation, discussed the behaviour of RQ, OUR and CER during various operating regimes. In a fed-batch baker's yeast fermentation, two major causes for ethanol production can be identified. If the substrate concentration in the broth is too high then ethanol is produced (negative Pasteur effect). In addition, if insufficient oxygen is supplied to combust the substrate, ethanol will again be produced. In this case the production of large quantities of ethanol is undesirable since it represents wasted sugar. When ethanol is produced the CER is observed to rise while the OUR remains constant. Hence an increase in RQ is a good indication that ethanol is being produced.

The use of off-gas analysis-based control schemes has been adopted by many authors. Aiba, Nagai and Nishizavo (1976) and Wang, Cooney and Wang (1977), for example, applied the method to the regulation of a fed-batch baker's yeast fermentation and demonstrated good performance. Williams, Yousefpour and Swanick (1984) went further in the combination of RQ and dissolved oxygen control in an adaptive multivariable control scheme for regulation of a yeast fermentation. They showed that a high biomass and good conversion from nutrient to yeast could be obtained with their control strategy (*see* later).

The more straightforward control of OUR has also been widely studied. An early reference to OUR control can be found in Humphrey and Jeffreys (1973), who investigated the control of organism growth through variation of substrate addition rate. Squires (1972) utilized the dissolved oxygen, as an indication of OUR, in an attempt to control the substrate addition to a fed-batch penicillin fermentation.

In addition to the off-gas derived-variable techniques, other derived variables that are based upon environmental measurements have been investigated for use in feedback control. For example, an organism growing tends to result in changes of broth pH; in a weakly buffered broth, addition of sugar tends to lower the pH whereas insufficient substrate tends to cause a rise in pH. If, as is usual, pH is under feedback control, the control action taken in regulation provides an indication of metabolic rate (Cooney, Wang and Wang, 1977).

Heat production, from energy balances of the fermentation, has also been used in an attempt to define some measure of the metabolic activity. Mou and Cooney (1976) utilized heat evolution from a novobiocin fermentation to regulate the specific growth rate through variation of the substrate feed.

An alternative to energy balancing is the use of mass balancing techniques for on-line estimation. The conservation approach (whether it be mass or energy) avoids the necessity to specify yields and rate constants, although physiological models are still required. The technique has been demonstrated in both fed-batch and continuous yeast fermentations (Cooney, Wang and Wang, 1977; Cooney and Swartz, 1982). Mou and Cooney (1983) extended the mass balancing technique to cover secondary metabolite fermentation. The balancing technique is more suited to fermentations which utilize defined/semi-defined media, although even in these cases a considerable proportion of carbon can be unaccounted for.

Bioprocess modelling for control

From a control engineering point of view, an essential prerequisite for good supervision and operation of bioreactors is an understanding of the process (fermentation) behaviour (for example in the application of mass balancing techniques discussed previously). To progress beyond the purely physical and environmental control of the fermenter into the area of biological control requires a process model that is sufficiently comprehensive to relate all important process inputs (strains, medium, feeds, environmental conditions) and outputs (biomass, product, pH, temperature, dissolved oxygen, off-gases, etc.). The availability of a sufficiently descriptive mathematical model would also provide insight into the behaviour of the fermentation state variables which could be used for improved control. In general, there are four different forms of process model that might be considered for control purposes.

1. The first is a *physiological model* where a knowledge of the physiology of the growth process can be expressed in causal/consequent, and usually non-mathematical, terminology. These models are useful in initial control strategy synthesis and may play an even more important role as knowledge-based systems methods (expert systems) are developed—*see* later.
2. The second form is a *structured model* where partial and algebraic equations are used to describe the dynamic behaviour of the growth process. The basic idea here is that the biomass is structured, or classified, by some proper intracellular characteristic which describes growth, activity, metabolism, etc. of the biomass or cells. The classification basis might be any chemical species content of the cell (DNA, RNA, etc.), the cell's mass, volume, or chronological age (i.e. age distribution of the cell population or biomass). Sometimes the partial differential equations are simplified to ordinary differential form to provide a lumped parameter model. Such a simplification, however, will not explain the effects of the intracellular state on the process operation and dynamic behaviour.
3. The third form represents an *unstructured model* of the process where the fermentation behaviour is assumed to be represented by a single, homogeneously growing, organism. In spite of its limitations, this type of model has been most frequently used for the development of fermentation control strategies.
4. Finally, some of the newer methods being developed for fermentation control, especially of the adaptive type, utilize 'black-box' or 'input–output' models of the process. Here the *primary model variable (output variable) is specified in terms of a function of the relevant process inputs (control or manipulated variables)*. The parameters of such a model do not necessarily have any biological relevance. Johnson (1987) gives a very good survey of fed-batch fermentation modelling and control studies.

Once a mechanistic understanding of the process has been obtained, the next step is to try efficiently to represent such mechanisms in a model that is

appropriate for the particular problem being studied. The resulting model should be balanced with respect to its mathematical complexity and its ability to capture all the essential features for control purposes. It should also be simple enough to permit direct determination of its key parameters through feasible experimental procedures. Presently available structured models and lumped parameter models are not generally applicable—due to the large numbers of parameters involved in the case of structured representations, and, in contrast, the inability of the lumped parameter models to accurately predict dynamic behaviour.

The most commonly adopted model in the design of control strategies for fermentation processes is the homogeneous single organism form. Additionally, it is also commonly assumed that there is a single growth-limiting substrate. A typical state–space representation of bacterial growth systems is given by the following mass balance relationships:

$$\frac{dX}{dt} = \mu(X,S,t)\,X - DX - k_d X$$

$$\frac{dS}{dt} = -k_1\mu(X,S,t)\,X + D(S_F - S)$$

$$Y = k_2\mu(X,S,t)\,X$$

where X is the biomass concentration (kg m^{-3}), S the substrate concentration (kg m^{-3}), D the dilution rate (feed rate/volume of culture) (h^{-1}), S_F the feed substrate concentration (kg m^{-3}), Y the product production rate (kg m^{-3} h^{-1}), $\mu(X,S,t)$ the specific growth rate (h^{-1}), k_1, k_2 the yield coefficients, k_d the biomass death rate (h^{-1}) and t the time (h).

A number of analytical expressions for $\mu(X,S,t)$ have been developed and Spriet (1982) suggests nine possibilities, although many more exist. The most widely used representation is due to Monod (1950):

$$\mu(X, S) = \frac{\mu_m S}{K_m + S}$$

where μ_m is the maximum specific growth rate and K_m the Monod constant for growth on substrate.

The selection of an appropriate representation for $\mu(X,S,t)$ is not straightforward. In addition, the determination of the important parameters μ_m and K_m from actual plant data is not easy (e.g. Holmberg and Ranta, 1982; Dochain and Bastin, 1984). Indeed, Dochain and Bastin (1984) avoid some of these problems by identifying the time-varying growth rate $\mu(X,S,t)$ on-line as part of their adaptive control strategy. Other work by Bastin and Dochain (1986), shows how specific growth rates can be estimated on-line using continuous time-estimation algorithms. Such algorithms do not require an analytical description of the specific growth rate but consider it to be a function of unknown, time-varying identifiable parameters.

The above basic relationships have been extended and used by many workers to develop new on-line control strategies for a range of fermentations. Two particular fermentation models will be summarized here—baker's yeast and

penicillin. These have been selected for the purposes of demonstration since they are representative of a wide range of industrially useful fermentations, are quite well understood and have been used quite widely for both theoretical and experimental modelling, state estimation, optimization and control studies.

A dynamic model of yeast fermentation based on material balance and fermentation kinetics results in the following set of differential equations (Williams, Yousefpour and Swanick, 1984):

$$\frac{dx_1}{dt} = \left[\frac{\mu_{mS} x_5}{(K_s + x_5)} + \frac{\mu_{mA} x_4}{(K_A + x_4)} \right] * \left[\frac{x_2}{(K_c + x_2)} + K_{LA}(x_2^* - x_2) \right] x_1 \qquad \text{Eq. (1)}$$

$$\frac{dx_2}{dt} + K_{LA}(x_2^* - x_2) - K_1 \frac{dx_1}{dt} \qquad \text{Eq. (2)}$$

$$\frac{dx_3}{dt} = K_2 \frac{dx_1}{dt} + K_3(x_5 - x_5^*) \qquad \text{Eq. (3)}$$

$$\frac{dx_4}{dt} = K_3(x_5 - x_5^*) - \frac{x_1 x_4}{K_3} \left[\frac{x_2}{(K_c + x_2)} + K_{LA}(x_2^* - x_2) \right] \qquad \text{Eq. (4)}$$

$$\frac{dx_5}{dt} = F - \frac{1}{Y} \frac{dx_1}{dt} - K_3(x_5 - x_5^*) \qquad \text{Eq. (5)}$$

$$\frac{dx_6}{dt} = F \qquad \text{Eq. (6)}$$

where x_1 is the total quantity of yeast cells, x_2 the percentage of dissolved oxygen in fermenter broth, x_3 the percentage of carbon dioxide in exit gas, x_4 the total quantity of alcohol in the fermenter, x_5 the sugar available for yeast growth in the fermenter, x_6 the total sugar added, K_{LA} the oxygen transfer coefficient, F the sugar feed rate and * indicates the equilibrium value of the variable.

Yeast and bacterial fermentations have also been studied by, for example, Aiba, Nagai and Nishizavo (1976); Takamatsu *et al.* (1979); Wang, Cooney and Wang (1979); Dekkers (1982, 1983, 1984); Williams, Yousefpour and Swanick (1984); Dekkers and Voetter (1985); Shioya *et al.* (1985); and Williams, Yousefpour and Wellington (1986).

Attention has also been focused on mycelial fungal systems, in particular the penicillin fermentation. A number of modelling approaches have been adopted for this fermentation. Models closely related to that of Monod are typified by the work of Bajpai and Reuss (1980). Although the Monod model has been found to perform well at low cell densities, a structurally similar model developed by Contois (1959) has the additional ability to account for diffusional limitations experienced at high cell densities. This can be particularly important in high cell density fermentations such as industrial

penicillin fermentation. The model set out below (Bajpai and Reuss, 1980; Montague *et al.*, 1986a, b) has been found to give good agreement with the practical results of Pirt and Righelato (1967).

Growth of biomass is related to substrate concentration by the relationship:

$$\frac{dX}{dt} = \frac{\mu_x SX}{K_x X + S} - \frac{X}{V}\frac{dV}{dt} \qquad \text{Eq. (7)}$$

where S represents substrate concentration, μ_x the maximum specific growth rate, X the biomass, V the fermenter volume and K_x the Contois saturation constant for substrate limitation of biomass production.

Substrate inhibition kinetics, which other workers have used to good effect with the inclusion of a term to account for hydrolysis, has been used to model penicillin production. Penicillin production is related to substrate concentration and biomass by:

$$\frac{dP}{dt} = \frac{\mu_p S X}{K_p + S(1 + S/K_I)} - KP - \frac{P}{V}\frac{dV}{dt} \qquad \text{Eq. (8)}$$

where μ_p represents the maximum specific rate of product formation, K_p is the Monod saturation constant for one substrate limitation of product formation, K_I is the substrate inhibition constant for product formation, K is the first-order rate constant for penicillin hydrolysis, and P is the penicillin concentration.

Substrate concentration is modelled by assuming constant yields and maintenance requirements:

$$\frac{dS}{dt} = \frac{-1}{Y_{x/s}} \cdot \frac{dX}{dt} - \frac{1}{Y_{p/s}} \cdot \frac{dP}{dt} - m_x X + F - \frac{S}{V}\frac{dV}{dt} \qquad \text{Eq. (9)}$$

where $Y_{x/s}$ represents the yield of biomass on substrate, $Y_{p/s}$ the yield of product on substrate, m_x the maintenance requirement and F the term that accounts for the fermentation feed rate.

A term for the production of carbon dioxide, which the original Bajpai and Reuss model lacked, was adopted from the work of Calam and Ismail (1980). The carbon dioxide production relationships assumes that evolution is due to three processes—growth, maintenance and penicillin biosynthesis:

$$\frac{d\mathrm{CO}_2}{dt} = k_4 \frac{dX}{dt} + m_c X + k_5 \qquad \text{Eq. (10)}$$

where k_4 relates CO_2 production to growth, m_c relates CO_2 production to maintenance and k_5 relates CO_2 production to penicillin formation.

An alternative approach by Nestaas and Wang (1983) considered the various stages through which penicillin mould develops. They constructed a segregated model for which good agreement was again obtained with experimental data. However, the segregated nature of this model (biomass is considered to consist of a mixture of three different states) causes some difficulty in the measurement of kinetic parameters. This highlights the importance of selecting, or developing, a model the complexity of which balances parameter measurement

difficulties with its ability to represent dynamic behaviour.

A number of investigators have used the penicillin fermentation for their modelling, state estimation, optimization and control studies. For example, Fishman and Biryukov (1974); Mou (1979); Biryukov (1982); Kishimoto *et al.* (1982); Nakamura and Calam (1983); Thompson (1984); Montague *et al.* (1986a, b).

Estimation techniques in fermentation

It will have been seen from the earlier discussions that the sensors available today for bioreactor measurements do not cover all the necessary and important variables. The important 'internal' variables, such as biomass, substrate and secondary product concentrations, that characterize the state and progress of a fermentation, are very difficult to measure reliably and fast enough for fermentation supervision and control purposes. What few sensors/instruments there are available for such purposes are not robust enough, or are too expensive, to be used in routine industrial control applications. At present, though, quite rapid off-line analysis is a common technique in the fermentation industry and assay results can be returned within two hours on samples taken, say, every 1–8 hours. However, even off-line, biomass is difficult to measure due to the complex nature of the fermentation broths, which can contain mixtures of natural materials, including immiscible oils, and a wide range of soluble, and insoluble, biological and inorganic materials.

As has already been mentioned, for unrestricted growth, biomass can be estimated in proportion to the amount of oxygen consumed or carbon dioxide produced during the fermentation. Considering that biomass can multiply by a thousandfold or more during a fermentation, the method relies heavily on an accurate biomass measurement to start the integration of the off-gas analysis. Substrate-limited growth cannot easily be correlated to carbon dioxide evolution and, in this case, elemental mass balancing methods have been tried. It is important to stress here that the use of an inaccurate estimate of biomass, for control purposes, can result in the fermentation being driven away from its a priori defined optimum trajectory, thus defeating one of the main purposes of the control strategy. Periodic correction of estimated biomass by some form of direct measurement or assay is therefore essential. In spite of these difficulties, it has become common practice to use measurements of related variables (secondary variables), such as gaseous oxygen and carbon dioxide, to estimate or infer unobtainable, or difficult to measure, (primary) variables, such as biomass, products, etc.

The easiest, but potentially least accurate, way of obtaining an estimate of a primary variable with measurement difficulties is to establish, by experiment, a correlation between the primary and secondary related variables while neglecting all measurement errors, noise, etc. A more realistic approach is to take account of those errors and adopt a numerical estimation technique. Estimation methods can be used primarily for two different purposes—for estimating the parameters of a pre-defined model structure (parameter estimation or identification), or for estimating the actual process variables (state

estimation). In comprehensive fermentation process models, the model parameters are usually physical or biochemical in nature and the model structure is non-linear. The corresponding identification problems are therefore also non-linear. If these are carried out by off-line computation, then many well-known numerical optimization algorithms can be used. On-line recursive estimation can also be carried out using any of the many existing recursive algorithms. Most of the methods used, to a greater or lesser extent, employ least squares based ideas. When linearity and additivity assumptions on the process model are justified, then the optimal estimator is the Kalman filter. When they are not, then recourse must be made to a suboptimal estimator. Unfortunately, the most useful and reliable algorithms have been developed for linear systems, whereas the biotechnical estimation problem is inherently non-linear.

Standard non-linear estimators, such as the extended Kalman filter (EKF), Anderson and Moore (1979), can suffer from some numerical problems and convergence difficulties, especially when the process noise characteristics are not well known (Ljung, 1979). In spite of these problems, the EKF has proved to be the usual means of tackling bioreactor identification and estimation, and many successful application studies have been reported (e.g. Svrcek, Elliot and Zajic, 1974; Stephanopoulos and San, 1981, 1984; Nihtila, Harmo and Perttula, 1984; San and Stephanopoulos, 1984; Montague *et al.*, 1986a, b). The use of an adaptive filter based upon the techniques developed by Jazwinski (1970) has been recommended to overcome some of the problems encountered due to filter robustness in the presence of process and measurement uncertainties and growth model inaccuracy. A more recently developed non-linear filter that has some useful innovations is that due to Halme and Selkainaho (1982). This filter basically provides a Bayesian maximum likelihood estimate for the state variables. The most noticeable difference when compared with the EKF is in the estimator gain calculation. A further problem commonly faced in practice is the variation of the model parameters, e.g. yield coefficients, maintenance, etc., either with time, point in the fermentation cycle or environmental conditions. In such cases estimation of the model parameters and process state variables simultaneously is often necessary in order to obtain reliable results. This can also be achieved using well-known filtering methods, or modifications of them, as well as, in principle, standard non-linear filtering procedures (e.g. Holmberg and Ranta, 1982; Halme, Kuismin and Korteniemi, 1985; Holmberg and Olsson, 1985).

When estimating parameters, either for the purposes of model identification or in adaptive state estimation, choosing the number of parameters that should be estimated can present potential difficulties (Holmberg and Ranta, 1982). There are no standard ways of determining a priori the number of parameters that can be successfully estimated, and probably the best way is to rely on experience. It almost goes without saying, however, that the maximum number of parameters that might be successfully estimated depends upon the extent and quality of information made available to the estimator. For example, the more extensive the information pattern, the more reliable the estimation and the larger the number of parameters that might be estimated. It is therefore

extremely important to utilize all the relevant information that is available for estimation. In biotechnological processes some of this information comes from laboratory assays and off-line analyses. The incorporation of this data into the estimation algorithms requires account to be taken of their irregular sample intervals and associated delay times. This problem has been tackled successfully by Halme, Kuismin and Korteniemi (1985).

Applications of state estimation methods

There have been a number of successful developments and applications of state estimation, and advanced fermentation control, reported by several authors (e.g. Stephanopoulos and San, 1981, 1984; Dekkers, 1982; Halme and Selkainaho, 1982; Swiniarski *et al.*, 1982; Nihtila, Harmo and Perttula, 1984; San and Stephanopoulos, 1984; Halme, Kuismin and Korteniemi, 1985; Shioya *et al.*, 1985; Tarbuck *et al.*, 1985; Montague *et al.*, 1986a, b; Lakroi and Cheruy, 1988). It is interesting to observe that most work has used a mechanistic model-based approach and extended Kalman filtering. This requires the existence, or development, of a sufficiently accurate biochemical model of the fermentation being controlled.

Swiniarski *et al.* (1982), in their study of Kalman filtering methods for biomass estimation, used the bacterial degradation of cellulose by *Sporocytophaga myxococcoides*, which provides a convenient general model for bacterial growth and the conversion of an extracellular non-soluble growth substrate to soluble form. As such, the model is applicable to a wide range of fermentation processes. The stationary extended adaptive Kalman filter and adaptive EKF were derived and shown to give satisfactory results for biomass estimation. The algorithms, however, were demonstrated to be sensitive to errors in initial estimate of substrate and very dependent upon an initial estimate of the noise covariances.

Leigh and Ng (1984) reported significant batch to batch variations in what were initially expected to be nominally identical fermentations. Such unmodelled variations were shown to seriously degrade the biomass estimates when using a fixed parameter model for extended Kalman filter derivation. With this problem in mind, Leigh and co-workers suggested a combination of approaches based on adaptive state estimation, improved modelling and rigorous quality control to overcome possible batch to batch variations.

The adaptation and extension of the above work to represent a secondary metabolite antibiotic production system, *Streptomyces clavuligerus*, was later presented by Tarbuck *et al.* (1985). The authors showed that the application of an EKF was able to provide reliable estimates of biomass.

Shioya, Takamatsu and Dairaku (1982) and Shioya *et al.* (1985) have investigated the application of an EKF to the estimation of specific growth rate for control purposes. Modifications to the filter were proposed in order to use moving averages and dynamic mass balancing, as well as adaptively changing the noise covariance matrix according to the prediction error. An improvement in estimator performance was demonstrated. The estimator was then used in a 'profile control scheme' in a fed-batch baker's yeast fermentation.

An adaptive non-linear observer for the estimation of cell concentration and specific growth rate has been proposed by Dochain and Bastin (1985). Stability and convergence proofs were given for three of the four different systems studied, and simulation studies demonstrated the performance of the simplified algorithms. However, for systems where the number of fermentation measurements might be more limited than those studied, a more rigorous mathematical model might well be required and the use of an EKF approach might be at least as appropriate.

Adaptive control techniques in fermentation

Practical bioprocesses operating under industrial conditions exhibit non-linear and time-varying properties and will always be subject to unexplained disturbances. As discussed earlier, this can make it difficult to set 'optimum' values for standard proportional–integral–derivative (PID) controllers. In addition, fermentations are by nature multivariable and conventional control strategies may not provide a totally satisfactory performance. Adaptive control schemes—controllers whose parameters can be found (identified) and varied on-line as the fermentation proceeds—have therefore been considered by a number of researchers. *Figure 2* illustrates the underlying philosophy of an adaptive control strategy. Such control schemes will cope with, and to a greater or lesser degree overcome, the complexities and control difficulties of biotechnological processes.

Over the past decade many adaptive algorithms have appeared in the literature. However, there are no clear guidelines available as to which algorithm is the most suitable for any particular type of process of interest. Categorization of the adaptive algorithms into a small number of classes, and investigation of the important performance characteristics of each class, should help ease the choice of algorithm, and this has been carried out for those algorithms of practical interest (Montague, 1987). Three categorization principles have been proposed:

1. The control action can be calculated either to minimize a cost function, i.e. a generalized minimum variance (GMV) type of algorithm (e.g. Clarke and Gawthrop, 1979; Astrom, 1983), or according to a pre-specified closed loop response based upon frequency domain considerations, for example a pole placement type of algorithm (e.g. Wellstead and Sanoff, 1981).
2. The parameter estimation routine can be applied either to estimate the parameters of the system from which the controller parameters are calculated—an explicit algorithm [e.g. generalized predictive control (GPC), Clarke and Mohtadi, 1985], or to estimate the controller parameters directly—an implicit algorithm (generalized minimum variance control, GMVC).
3. The system model used to calculate the control action may be either a time series model (GMVC, GPC) or a state space model (pole placement control—of the type discussed by Warwick, 1981).

A common basis for the majority of adaptive algorithms is the assumed

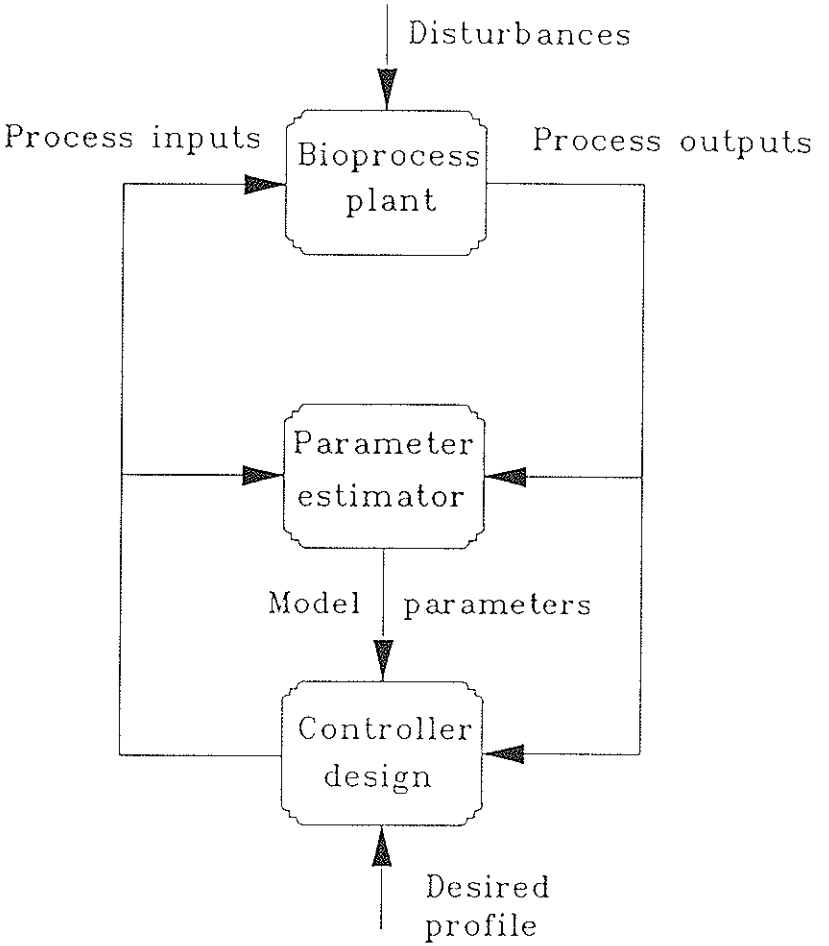

Figure 2. A typical adaptive control scheme.

general linear process model. For derivation of adaptive control laws, it is usually assumed to be of the controlled autoregressive moving average (CARMA) form. However, the choice of a controlled autoregressive integrated moving average (CARIMA) form provides additional benefits for the control algorithm.

Consider a process which is described by the following difference equation:

$$A(z^{-1})y(t) = z^{-k}B(z^{-1})u(t) + z^{-l}L(z^{-1})v(t) + C(z^{-1})e(t)/\Delta \quad \text{Eq. (11)}$$

where A, B and L polynomials in the backward shift operator z^{-1} [the backshift operator implies, for example, that $z^{-1}y(t) = y(t-1)$]. $y(t)$, $u(t)$ and $v(t)$ are the system's output, manipulative input and measurable load disturbance respectively. k and l are the process time delays (expressed as integer multiples of the sample time) exhibited by the output to manipulative control input and load disturbance input, respectively. In explicit adaptive control schemes these delays, or variations in them, can be accommodated by including leading zero

elements in the $B(z^{-1})$ and $L(z^{-1})$ polynomials, respectively. $e(t)$ is a random zero mean disturbance with finite variance and, for simplicity, $C(z^{-1})$ is often considered to be unity. Δ is the differencing operator $(1-z^{-1})$. The disturbance term, $e(t)/\Delta$, can be thought of as being Brownian motion and, for controller design purposes, realistically represents the form of disturbances affecting the process. The design of control strategies (adaptive or otherwise) based on a CARIMA representation of the bioprocess leads to controllers with inherent integral action.

To develop a self-tuning controller, a regressor (data-vector) $\boldsymbol{x}$ and parameter-vector ϕ are defined in terms of the process data (y, u, l) and 'unknown' process parameters (A,B,L). The process model parameters (A,B,L) are identified 'on-line' and are passed to an appropriate control algorithm and the control increment calculated to update the controller output setting at every control interval. This is shown schematically in *Figure 2*. The theory and techniques of on-line recursive parameter identification, and the data filtering requirements, have been discussed at length by many authors, e.g. Ljung and Soderstrom (1983).

Although, as discussed earlier, a number of authors have investigated the application of modern control techniques to bioreactor control, the recent work of, for example, Takamatsu *et al.* (1979); Dochain and Bastin (1984, 1985); Dekkers and Voetter (1985); Frueh *et al.* (1985); Montgomery, Williams and Swanick, (1985); Poulisse and van Helden (1985); Shioya *et al.* (1985); Verrbruggen, Eelderink and van den Broecke (1985); Dochain (1986); Dochain, De Buyl and Bastin (1988) are most relevant to the adaptive control studies described here. Almost all the studies are concerned with yeast fermentations, with only Frueh *et al.* (1985) investigating penicillin fermentation. Dochain and Bastin (1984) initially tried to use a minimum variance control policy before adopting a self-tuning GMV control law of the Clarke and Gawthrop (1979) type; whereas Poulisse and van Helden (1985), Dekkers and Voetter (1985) and Montgomery, Williams and Swanick (1985) utilize LQ and LQG methods of adaptive control. Frueh *et al.* (1985) used a minimum variance type of self-tuning control law with a cascaded PID compensator, whereas Verrbruggen, Eelderink and van den Broecke (1985) applied a pole placement self-tuning controller with an outer loop integrator. Almost without exception, the models used for control law derivation have been of the controlled autoregressive moving average (CARMA) form, leading to the problem of including integral action in the resulting control law. In the work of Montague *et al.* (1986a, b) integral action is inherently included in the controller by use of a CARIMA representation of the process with a Brownian motion type disturbance model. This method has the added advantage that the adaptive control law parameter estimator uses incremental data in the data vector (Δy, Δu, Δl), rather than positional data as in many other self-tuning algorithms.

Of interest in bioreactor control are the profile–model reference adaptive control (MRAC) studies of Shioya *et al.* (1985). They suggest a programmed controller to follow the desired (biomass concentration or specific growth rate) profile, with a feedback compensator to regulate the system against

disturbances. It is interesting to note that the GPC algorithm used by Montague *et al.* (1986a, b) could also be used in model-following form by including P weighting in the control law, where the transfer function P can be interpreted as the inverse of the desired closed-loop system response (e.g. Clarke and Gawthrop, 1979).

Two particular areas of application of state estimation and adaptive control techniques will be briefly reviewed. The baker's yeast fermentation and the penicillin fermentation have been selected since they are the most widely studied and the techniques developed on these fermentations serve to demonstrate the wide applicability of the algorithms.

Estimation and adaptive control of yeast fermentation

In order to maximize the cell numbers and productivity in a yeast fermentation it is necessary to control all the states of the system. This was discussed previously. In a yeast fermentation direct measurement of cell mass is not usually available on-line. Williams, Yousefpour and Swanick (1984) and Williams, Yousefpour and Wellington (1986) recognized this problem and chose to control RQ and dissolved oxygen in order to regulate cell mass. They adopted an adaptive control strategy that used measurements of dissolved oxygen concentration, CER, OUR, alcohol production rate, pH, and temperature to control the fermentation by manipulation of nutrient feed rate and stirrer speed. Temperature and pH were regulated by individual single-loop on–off controllers. The structure of the estimator and control algorithms were assessed using a mathematical model of yeast fermentation. Both single-input multi-output, SIMO, (nutrient feed control only) and multi-input multi-output, MIMO, (both nutrient feed and stirrer speed controlled) were studied. The SIMO control strategy was found not to achieve the target of maximum cell growth and yield. The MIMO control strategy satisfied the desired performance criteria as well as meeting existing plant operator levels of performance.

Figure 3 shows the responses of RQ and dissolved oxygen, with *Figure 4* showing the corresponding control manipulations of glucose addition and agitation rate. The suggested technique utilizes a linearized model of the fermentation and was therefore anticipated to be of wide applicability. In practice, careful choice of controller parameters (e.g. discrete time sample period, cost function weightings, model order, etc.) was found to be essential to ensure satisfactory performance. Dekkers and Voetter (1985) also investigated the adaptive control of a fed-batch baker's yeast fermentation. Experiments on three different batches were discussed. RQ was identified as the major control parameter and two different controllers were studied. These were a feed-forward adaptive controller based upon a gain scheduling approach, and a self-tuning controller based upon the work of Astrom (1983). *Figure 5* shows the responses obtained in RQ through the controlled variation of glucose feed rate (GFR) with the feed-forward adaptive controller. OUR/M, where M represents the total amount of biomass, is also plotted since it provides additional important information concerning fermentation performance.

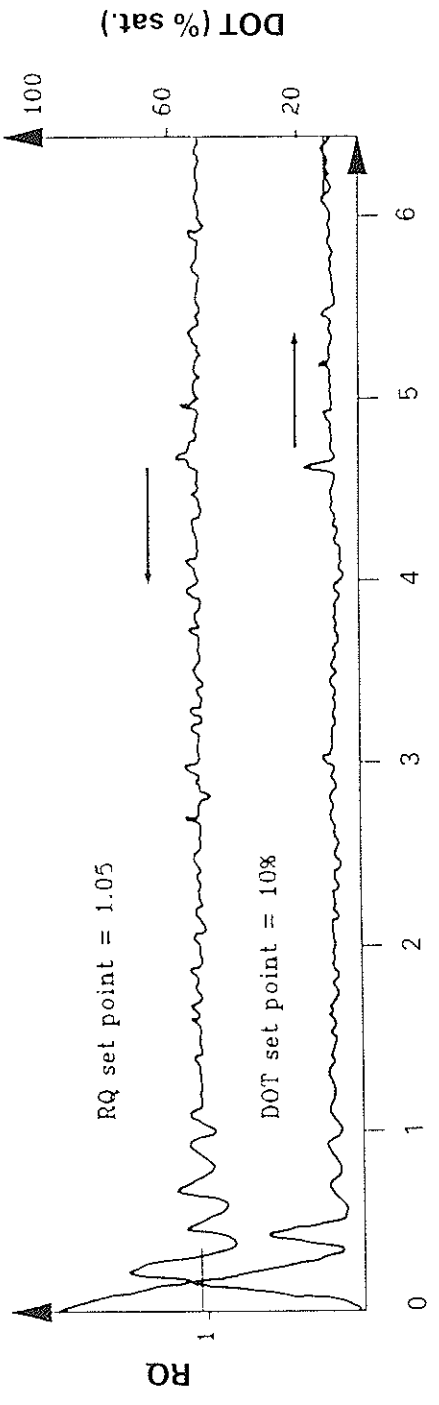

Figure 3. Adaptive control of a fed-batch yeast fermentation using RQ and dissolved oxygen tension (DOT) (reproduced from Williams, Yousefpour and Wellington, 1986, by permission of John Wiley and Sons).

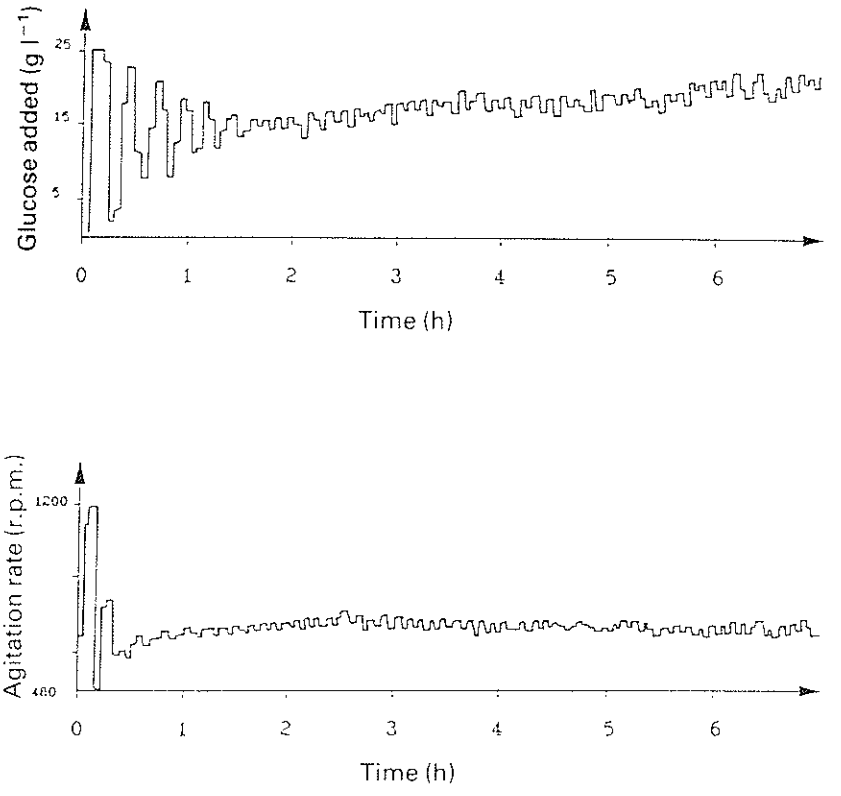

Figure 4. Controller-manipulated variables, nutrient feed rate and stirrer speed, corresponding to *Figure 3* (reproduced from Williams, Yousefpour and Wellington, 1986, by permission of John Wiley and Sons).

Figure 6 shows similar responses, but this time the fermenter was under self-tuning control. In this case RQ control appears satisfactory, except during the period 8·5–9·5 hours when CO_2 measurement drift caused some problems. At time 9·5 hours the analyser was recalibrated, causing a drop of 0·02 in the measured RQ. Stirrer speed (N) and dissolved oxygen (DO) are also plotted. *Figure 7* again shows similar controlled responses under self-tuning control. However, the effects of controller parameter estimation problems result in the poor performance observed for several hours starting at 9·7 hours. At times 10·1 and 10·5 hours RQ again deviates from the set point due to controller instabilities. These investigators also comment on the importance of careful choice of the controller parameters that influence the overall performance of the algorithm.

Further examples of many studies that have been undertaken to investigate the improved control of yeast fermentation can be found in the work of Peringer and Blachere (1978), Dairaku *et al.* (1982) and Wu, Chen and Chiou (1985).

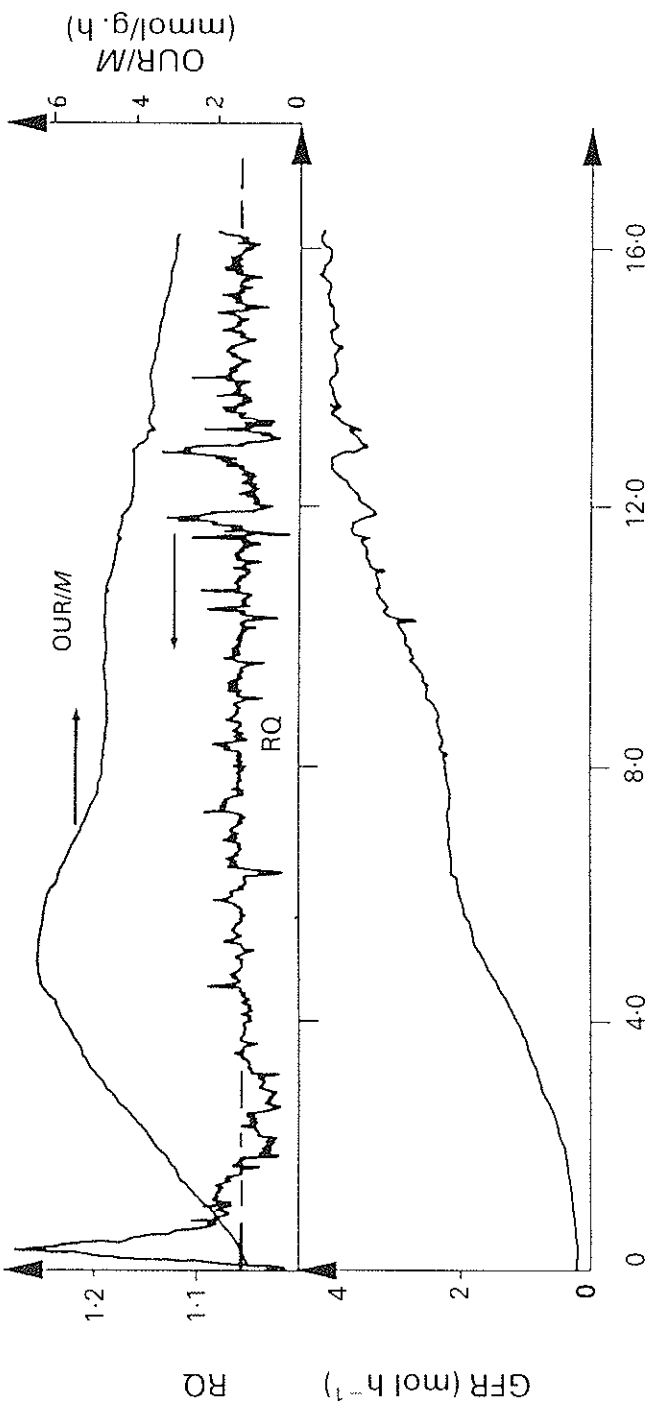

Figure 5. Feedforward control of RQ in fed-batch yeast fermentation—fed batch 1 (reproduced from Dekkers and Voetter, 1985, by permission of IFAC Publications).

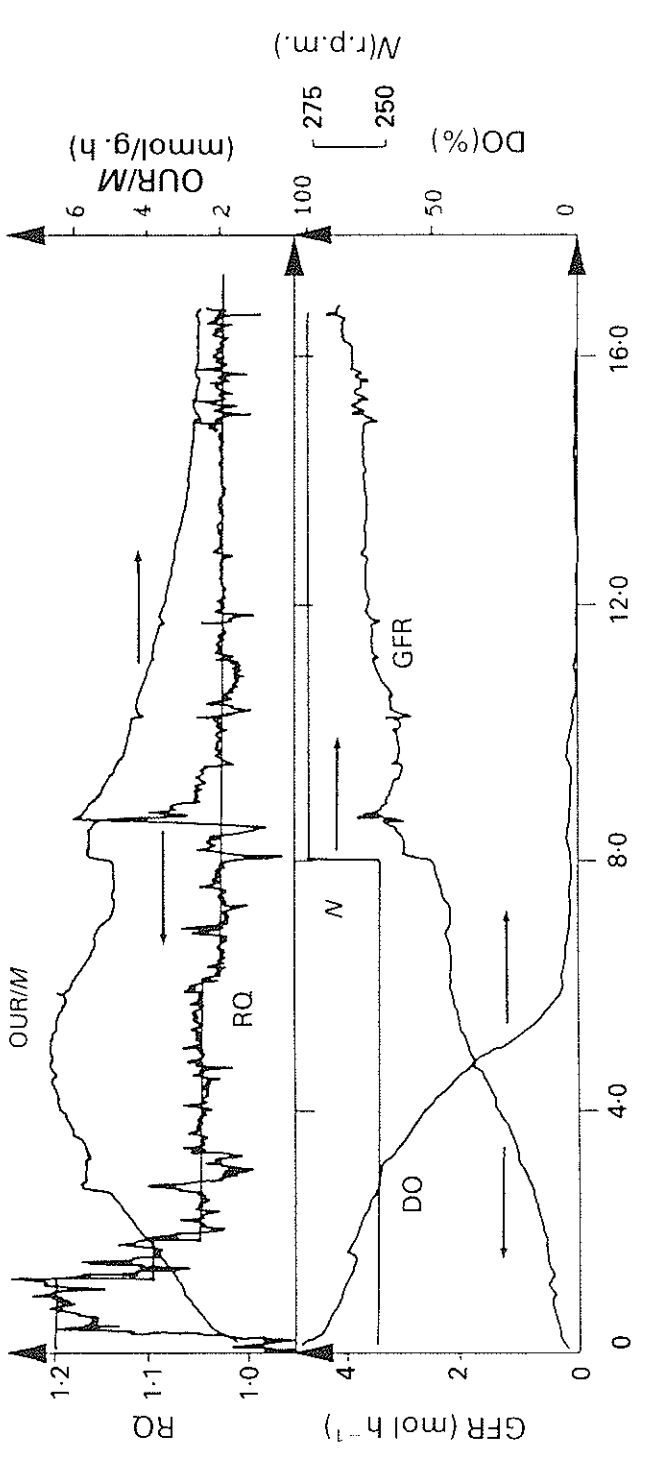

Figure 6. Self-tuning control of RQ in fed-batch yeast fermentation—fed batch 2 (reproduced from Dekkers and Voetter, 1985, by permission of IFAC Publications).

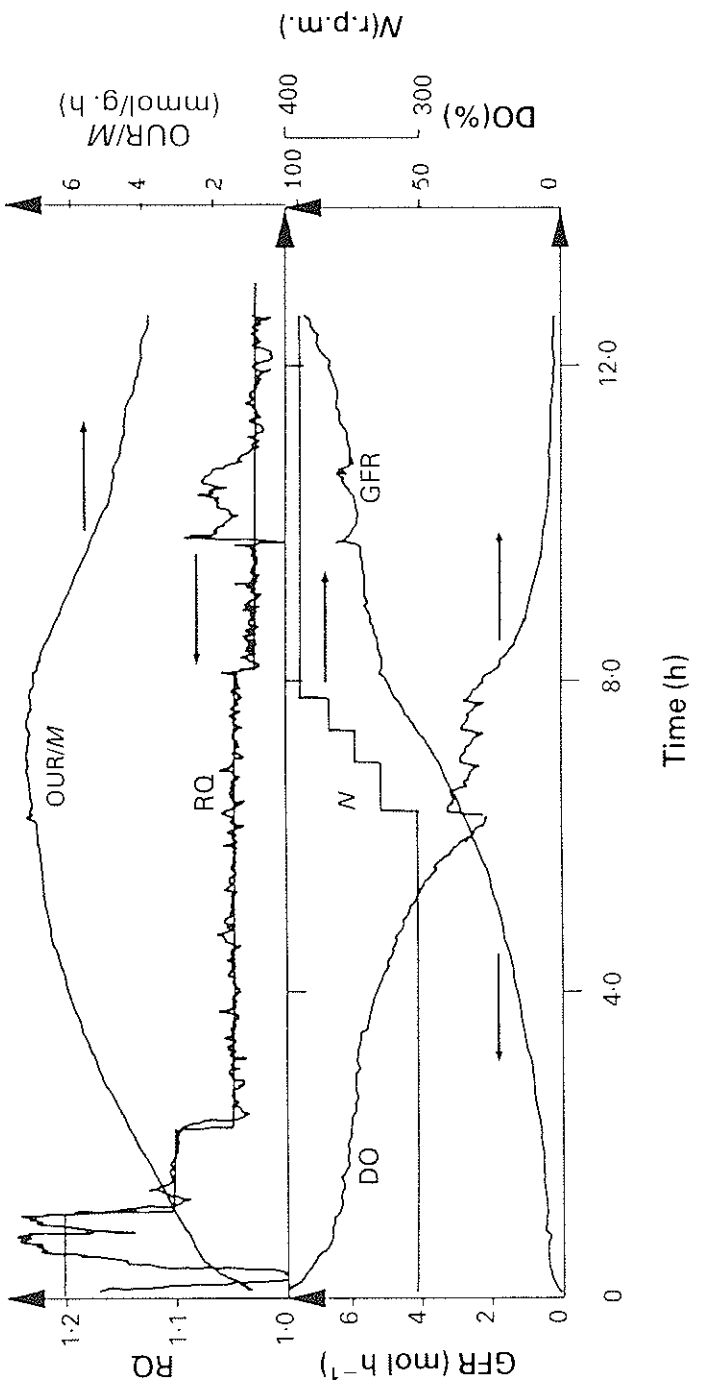

Figure 7. Self-tuning control of RQ in fed-batch yeast fermentation—fed batch 3 (reproduced from Dekkers and Voetter, 1985, by permission of IFAC Publications).

Estimation and adaptive control of penicillin fermentation

The studies of Montague *et al.* (1986a, b) on the control of biomass in a fed-batch penicillin fermentation serve to demonstrate the importance of the correct choice of control algorithm. Here a generalized predictive controller (GPC), a long-range receding-horizon predictive-type algorithm developed by Clarke and Mohtadi (1985), was used to control biomass estimated by an EKF. This controller was shown to overcome many of the problems experienced with previous types of adaptive algorithms. With reference to fermentation, the most important controller characteristics are its robustness, the ease of tuning and low process overshoot. In addition, the GPC algorithm allows for the inclusion of pre-programmed set-point profiles (future set points) in the calculation of control action increments. It is anticipated that this facility could make a significant contribution to bioreactor production control. The controller is of the explicit type; that is, identification is applied to estimate the parameters of a linear system model.

Figure 8 shows responses of fermenter biomass (superimposed upon desired biomass profile—broken line), substrate addition (control-manipulated variable) and penicillin concentration (secondary metabolite product). These demonstrate the quality of control that is achievable through the application of GPC. Following the initial rapid growth phase, which is operated open-loop, the control loop is closed and the GPC algorithm is used to regulate growth rate during the penicillin production phase. It is in the early penicillin production phase where biomass profile overshoot is particularly undesirable as it would lead to a lowering of penicillin production. Then, if normal growth rate were maintained, oxygen transfer problems would arise towards the end of the fermentation.

Although a number of successful simulation and pilot-plant studies have been reported, as referenced above, it is fair to observe that in general the algorithms still need to be 'engineered' to the point where robust consistent control can be achieved without operator intervention or supervision.

Adaptive estimation for inferential control

In practice, laboratory analyses/assays are required to support fermentation supervision and control. This imposes financial costs associated with laboratory support as well as operational restrictions. Delayed and infrequent measurement of some process outputs, determined by sampling limitations, prevents the early detection of process disturbances. One approach to deal with the problem of controlling infrequently sampled process outputs is to use the information provided by other more easily measurable variables. For example, this information can be used to provide an estimate of the controlled output. The estimated values of the output can then be used for overall control of the plant. Control schemes based on the feedback of estimated outputs are often termed 'inferential control schemes'. An ideal situation arises when the process states are completely observable from the secondary outputs. Under such circumstances, Kalman filtering techniques can be employed to estimate plant

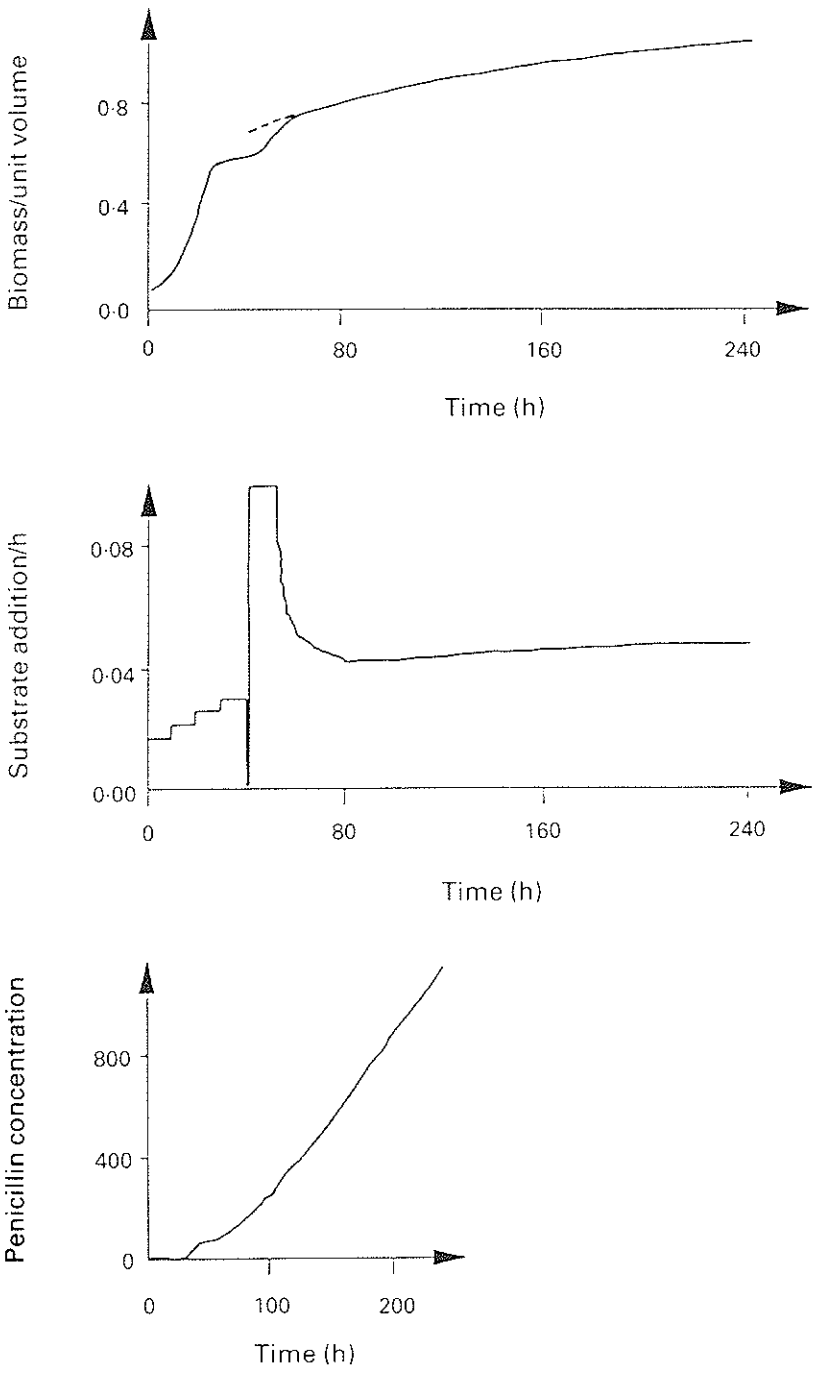

Figure 8. Generalized predictive control of estimated biomass in penicillin fermentation.

states using the secondary output measurements. Estimates of the controlled output can then be computed and control of the plant achieved by feedback of either the state estimates or the output estimates to appropriate controllers. Published literature on the above methods is extensive. As mentioned earlier, however, the use of Kalman filters is confined to situations where the plant is completely observable from the secondary outputs and an adequately accurate process model is available. Recently, other techniques have been developed that allow the estimation of a 'primary' controlled output (for example, biomass) from measurements of other 'secondary' outputs (for example, fermenter outlet gas CO_2, fermenter feed, etc.). Of particular interest are the adaptive estimation and control methodologies being researched by Guilandoust, Morris and Tham (1987, 1988), Montague, Morris and Tham (1988), and Dochain and Bastin (1984), Dochain, De Buyl and Bastin (1988).

An interesting approach adopted by Dochain and Bastin (1984) is to exploit the known non-linear structure of the bioprocess, rather than consider it to be approximated by time-varying linear 'black-box' models. In their approach the parameter estimation and process control are performed simultaneously. In addition, the specific growth rate is considered as a time-varying function of the unknown parameters being estimated rather than being modelled by an analytical function.

The experimental validation of an observer for the on-line state estimation of fermentation performance (biomass and product) has also recently been presented by Dochain, De Buyl and Bastin (1988). Here poly-3-hydroxybutyrate production, in a 60 litre pilot-scale fermenter, is used to demonstrate the performance of the observer. The algorithm uses on-line measurements of reactor volume, substrate feed rate, dissolved oxygen concentration and the gaseous oxygen balance to estimate biomass ($\hat{X}$), product concentration ($\hat{P}$) and ammonia ($\hat{N}$). *Figures 9, 10* and *11* compare estimated and off-line measured data, showing good agreement. An important aspect of this observer is that prior knowledge of specific growth rate and

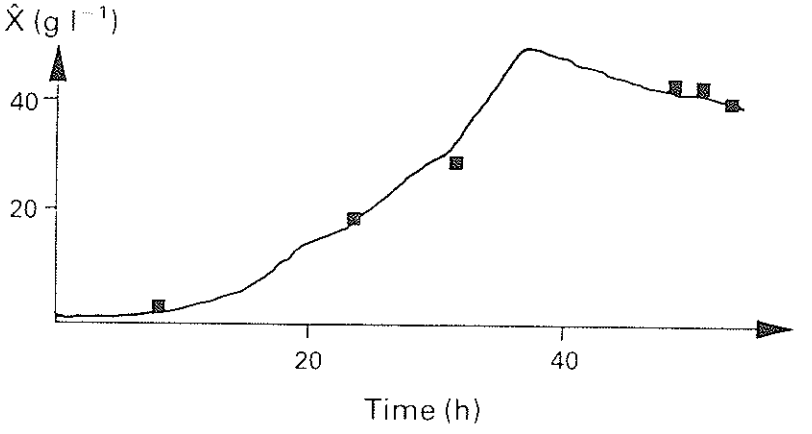

Figure 9. On-line state estimation—biomass concentration: ■ denotes off-line data used for validation only (reproduced from Dochain, De Buyl and Bastin, 1988, by permission of the Society of Chemical Industry).

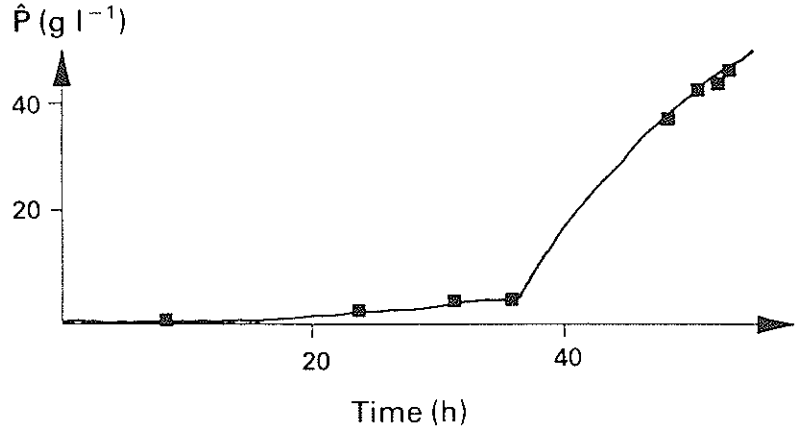

Figure 10. On-line state estimation—product concentration; ■ denotes off-line data used for validation only (reproduced from Dochain, De Buyl and Bastin, 1988, by permission of the Society of Chemical Industry).

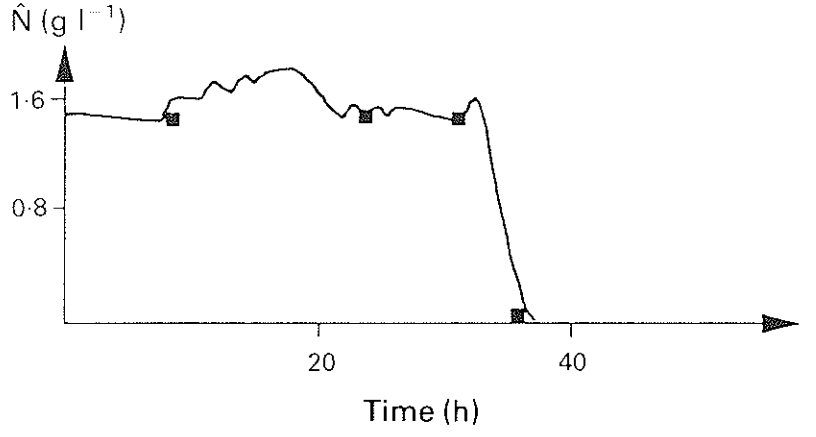

Figure 11. On-line state estimation—ammonia concentration; ■ denotes off-line data used for validation only (reproduced from Dochain, De Buyl and Bastin, 1988, by permission of the Society of Chemical Industry).

specific production rate is not required. These rates can, however, be computed on-line (Bastin and Dochain, 1986).

An alternative starting point for the development of the adaptive estimation (soft-sensor) algorithms adopts the philosophy that the bioprocess dynamics can be represented in either of two quite general forms (an observer canonical form and an input–output form). The approach using a general input–output model of the process is similar to that adopted for the development of well-known adaptive control laws, briefly discussed previously, except that an additional term representing the CO_2 measurement is included. The techniques adopted in both of these approaches reflect the experiences of

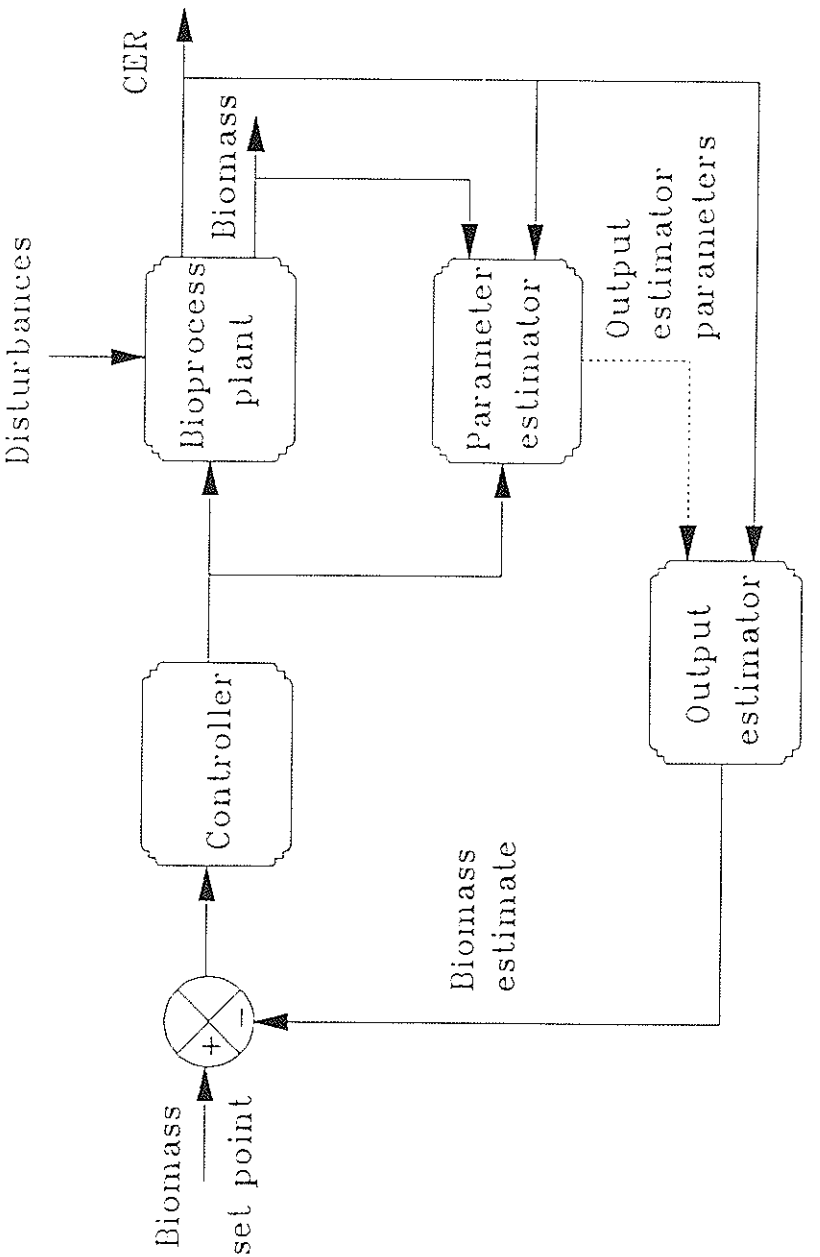

Figure12. Adaptive estimation of biomass.

previous work on adaptive and self-tuning control (e.g. Morris, Nazer and Wood, 1982; Montague *et al.*, 1986a,b).

An interesting option that becomes available with the availability of a 'fast' estimate of controlled output (e.g. fermenter biomass concentration) is closed-loop 'inferential' control of that variable—adaptive inferential control. The values of estimated primary output can be used as a feedback signal for almost any constant parameter or adaptive control algorithm. *Figure 12* shows the underlying philosophy. Here 'fast' measurements of fermenter feed and CER, coupled with 'slow' laboratory assays of biomass, allow a 'fast' estimate of biomass to be inferred which could be used for closed-loop control.

Industrial fermentation verification of the inferential estimation scheme has been undertaken on a continuous fermentation, operated in stirred-tank and air-lift-fermenter configurations, producing a fungal mycelium. Present industrial biomass regulation is based upon the analysis of four-hourly samples and the dilution rate adjusted upon the return of the assay information. Adaptive estimators (Montague, Morris and Bush, 1988), provide a means by which frequent estimates of biomass can be obtained. The estimator is supplied with hourly CO_2 and dilution rate measurements and four- or eight-hourly biomass concentration assays, which enable the prediction of dry weight at hourly intervals. Industrial-scale trials have been promising.

Figure 13 shows the CO_2 and dilution rate measurements over a 470-hour period of continuous operation. For reasons of confidentiality, the ordinate scales have been removed in this and following figures. (It is noted, however, that the ordinate scale does not start at zero.) *Figure 14* shows the biomass assay results (dry weight analyses—step-like response) over the fermentation period and how they compare with estimated biomass concentration. Here the estimator is supplied with filtered dilution rate and off-gas CO_2 measurements every hour, and dry weight assays every eight hours. It can be seen that the ability of the estimator to predict biomass transient behaviour (at the measurement rate of off-gas CO_2) is very acceptable. Due to the adaptive nature of the algorithm, it is, of course, capable of dealing with the slow drift in calibration sometimes experienced with on-line CO_2 measurements.

Optimization of fermentations

The large variety of fermentation products, the complexity of fermentation processes and the multitude of control variables offer a wide range of possibilities for optimization schemes. A fermentation such as that of yeast can be optimized in order to maximize a yield of biomass, whereas secondary metabolite fermentation can be controlled in order to maximize antibiotic production, for instance. Optimization is not only concerned with product maximization; other process considerations are essential, for example, production costs and fermentation time span. Optimization of present industrial fermentations has been carried out predominantly on an empirical basis. Although this has resulted in some considerable improvements in operability, the application of modern mathematical optimization theory offers the potential of even greater benefits.

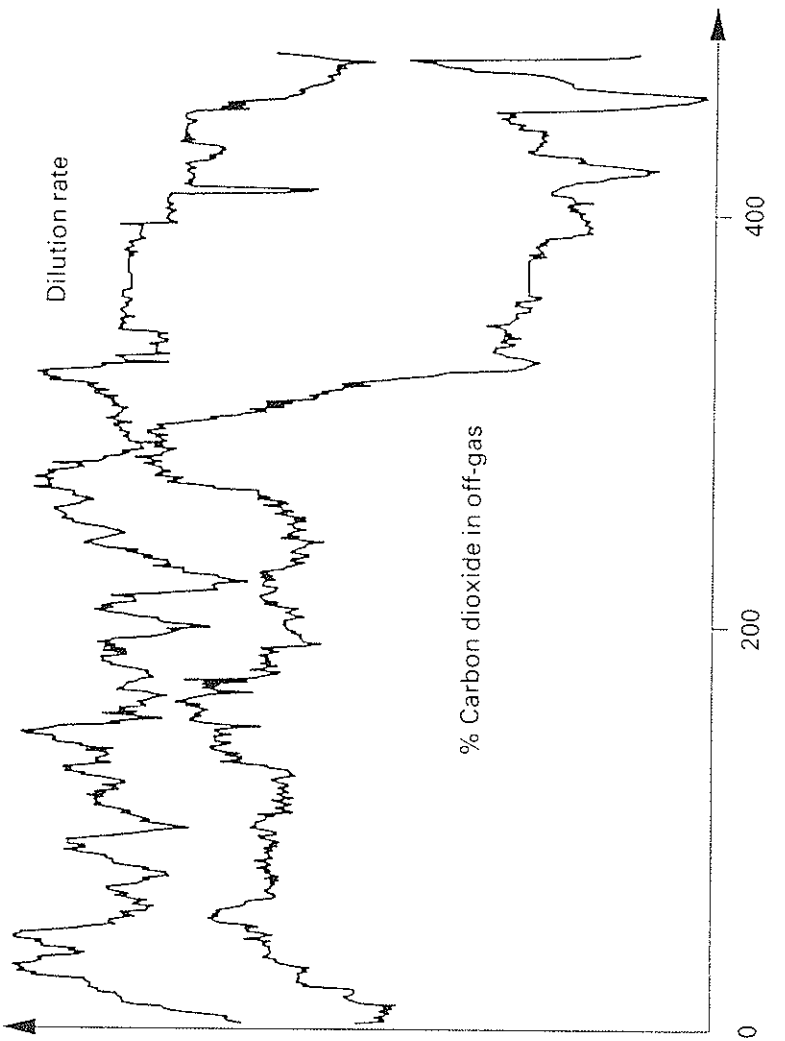

Figure 13. On-line fermenter dilution rate and CO_2 evolution rate.

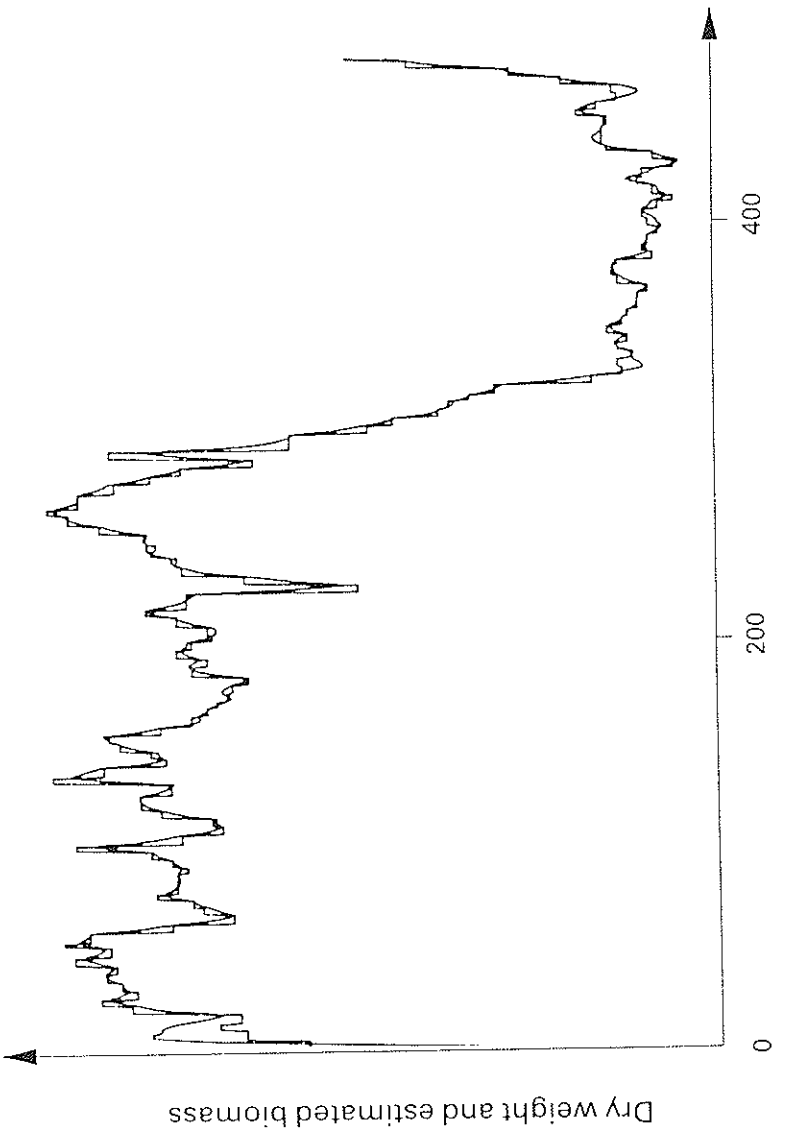

Figure 14. Comparison of laboratory-assayed dry weight with estimated biomass.

At the heart of any optimization scheme is the development of a mathematical model of the system which is capable of adequately describing behaviour. The models discussed previously for estimation and control purposes can, and have been, applied in fermentation optimization studies. Numerous references can be found and many are set out in Constantinidies (1979). For example, Biryukov (1982) who applied a continuous maximum principle approach to the optimization of a fed-batch penicillin fermentation. In this case the substrate addition was varied in order to maximize penicillin production. Takamatsu *et al.* (1975) applied the continuous maximum principle and Green's theorem to a continuous fermentation for amino-acid production. Here the optimization enabled the maximization of amino-acid production and minimization of transient time by the variation of the feed flow rate. The use of a process model can be highlighted in the work of Constantinidies and Rai (1974) in their attempt to predict the optimum temperature profile for the maximization of pencillin production.

Modelling the rate of cell growth by the well-known logistic law:

$$\frac{dX}{dt} = b_1 X(1 - X/b_2) \qquad \text{Eq.(12)}$$

where b_1 is a parameter closely related to the specific growth rate in the early period of the fermentation and b_2 is approximately equivalent to the maximum concentration of cells that can be achieved. The rate of penicillin biosynthesis increased proportionately to the concentration of mature cells and decreased in proportion to the concentration of penicillin (accounting for hydrolysis), hence:

$$\frac{dP}{dt} = b_3 X_{t-tm} - b_4 P \qquad \text{Eq.(13)}$$

where tm is the maturation time and P is the penicillin concentration.

The parameters b_i were determined experimentally for a range of operating temperatures. The maturation time was assumed to be 20 hours, that is cells that were 20 hours old or older were capable of producing penicillin.

Applying Pontryagin's Maximum Principle to the above equations, they obtained a continuous profile for the control variable, temperature, which enabled the optimization of penicillin production. This profile, shown in *Figure 15*, suggests a lowering of operating temperature during the penicillin production phase, contradicting the standard industrial operating procedure of maintaining a constant temperature throughout the fermentation.

An alternative technique, closely linked with the adaptive inferential ideas discussed previously, is the use of a generally structured linear model, which adapts as changes in the process occur. This adaptation enables the tracking of process dynamics and hence provides a means by which on-line optimization can be achieved. Golden and Ydstie (1987) demonstrate the methodology in studies aimed at optimizing the yield of yeast in a continuous fermentation.

The optimization of fermentation processes offers the possibility of high returns, since the raw materials costs are high while yields can be low. Even a

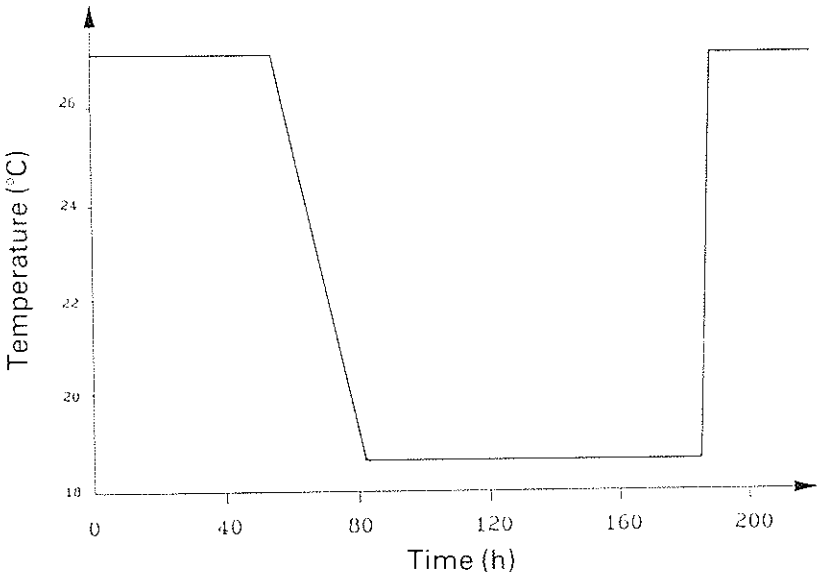

Figure 15. Optimal temperature profile in penicillin fermentation (reproduced from Constantinidies and Rai, 1974, by permission of John Wiley and Sons).

small increase in yield could result in considerable savings. The area of fermentation optimization is therefore one of growing interest. An excellent review and background to the principles involved can be found in Constantinidies (1979).

Knowledge-based systems in fermentation supervision and control

It should be clear from the discussions above that biological systems are extremely complex and in many cases not well understood. In addition the environment that exists within a fermenter is often uncertain, subjecting the biological system to unknown localized disturbances. Indeed, the fermentation process itself is only a small part of an equally complex industrial–sociological system. Although the algorithmic approaches described above can offer much insight and provide for improved process operability, the comprehensive management of a fermentation process requires more than simply an algorithmic approach at every level. 'Expert' and 'knowledged-based' systems, as replacement experts, may not be the complete answer, but the artificial intelligence (AI) techniques developed in the past 20 years do offer a number of solution strategies (e.g. Stephanopoulos and Stephanopoulos, 1986). These may be appropriate for supervisory environmental control and optimization (e.g. Karim and Halme, 1988; Stephanopoulos and Tsiveriotis, 1988), fault diagnosis (Halme, 1988), integration of lower-level control algorithms and utilization of qualitative and quantitative biological data. AI may also have a wider role in the design and control of the biological processes actually taking place in the fermentation (Seressiotis and Bailey, 1988).

Real-time expert systems software environments are now starting to become available, an excellent example being Gensym's G2 (Moore and Kramer, 1986), and are being applied to fermentation processes (Aynsley *et al.*, 1989). Data input from new sensors, improved signal analysis and statistics, advanced control algorithms, state and parameter estimation leading to predictions of future state and output behaviour, improved data handling and learning algorithms, and AI techniques should result in both a deeper understanding of fermentation behaviour and better process supervision and control of bioreactors.

Discussion

As the importance of biotechnology-based industries increases in the market place, there is a growing need to operate bioprocesses in a cost-effective manner. Whereas the original route to process improvement was through strain development, an alternative complementary approach discussed above is that of improved process supervision and control. As outlined, these techniques provide quite significant potential for improvements in process operability and hence increased profitability. Although the techniques and methodologies have been discussed with respect to their application to fermentation processes, the underlying fundamental theories, and to a greater or lesser extent their practice, are directly applicable to a wider spectrum of bioprocess systems—for example, wastewater treatment. The discussions on fermentation system control have made wide reference to three important IFAC meetings (Halme, 1982; Johnson, 1985; Fish and Fox, 1988). These conference proceedings provide a valuable source of reference for modelling and control studies on the whole range of biotechnological processes.

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