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Substrate Concentration Control in Bioreactors

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Introduction

Manufacturing economics are becoming increasingly dependent upon the care with which processes are managed. To compete on an international scale, manufacturing companies are turning to improved methods of management and process control in an unending effort to reduce the unit costs of production. There are many aspects to this concerted effort on the part of financial, personnel and production managers in manufacturing companies of every type. Regardless of the characteristics of the product or the manufacturing process, several common techniques can be applied to all manufacturing industries. Manufacturing resource planning is a technique which greatly improves planning of the flow of materials through a manufacturing process, reducing delays and inventories while increasing throughput and productivity. Computer-integrated manufacturing is another technique which allows for the control of complex assembly operations through the integration of production machinery with design and reference databases, reducing labour costs and increasing the reliability and reproducibility of the manufacturing process.

In both of these generalizable techniques, the ultimate control of the manufacturing process must rest with highly trained individuals. Virtually all attempts to develop completely independent, computer-controlled processes have met with either operational or product failure.

Fermentation processes are a subset of manufacturing processes and are in many respects similar to complex assembly operations. Within the range of

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improved manufacturing techniques available to managers, fermentation processes are as susceptible to improvement by the careful application of manufacturing resource planning as are any other manufacturing process. These techniques can be, and have been, adopted by the fermentation industry with relative ease and without extensive modification of the methods and procedures used in other manufacturing industries.

Computer-integrated manufacturing is much the same for all industries in concept, but systems and techniques must be designed in detail for each class of industry. A computer-integrated manufacturing system designed for automobile assembly could not be modified to be of any use to the fermentation industry. Central to computer-integrated manufacturing systems is the computer control of the generalized critical assembly process.

In the fermentation industry, there are usually two critical process steps: fermentation and purification. Techniques are now available to implement computer-based process control in some form to each of these major components of the overall manufacturing process. Some parameters, such as pressures and temperatures, are easily controlled with well-established technologies. Other parameters, such as substrate or product concentration control, present more difficult challenges to process development scientists and engineers.

In the development of detailed computer control systems and strategies for use in the fermentation industry, it is important to recognize that the computer and associated control equipment will not perform usefully unless guided by experienced and knowledgeable individuals working within a well-managed production environment. When applied rationally and designed to utilize fully the invaluable experience gained through experimentation, computer-controlled fermentation techniques can give a competitive edge to manufacturers.

There is thus a hierarchy of techniques available to the fermentation production manager. First, the production process must be well planned to eliminate scheduling problems and uneven equipment utilization patterns. Once this is accomplished it becomes worth considering the improvements in productivity which could result from increased information flow, most probably in the context of well-instrumented equipment reporting to a distributed computing system. Nested in this hierarchy, controlled fermentation processes can make a significant contribution to increasing the actual production capacity of a fermentation plant.

In the ensuing discussion we present a summary of both the theoretical background and the experimental basis for the development of computer-controlled fermentation processes. There is no easy way to develop a new fermentation process based on computer control techniques, and the control of substrate concentrations is currently one of the most difficult aspects. However, knowledge of the basics of control theory, of the techniques for simulating the effects of various control stragegies, of the basic aspects of process optimization, and of the experimental results that others have obtained, can provide a solid basis from which to start.

Fermentation system dynamics

CATEGORIES OF FERMENTATION PROCESSES

Production processes based on cell cultivation fall into three categories: batch, fed-batch and continuous. This review discusses techniques for the development and optimization of useful fed-batch fermentation processes involving substrate concentration control. The fed-batch approach offers the opportunity of using the best aspects of both batch and continuous processes. Process control can improve titres and yields, as in continuous cultivation; genetic drift can be minimized, as in batch cultivation, and cell metabolism can be stabilized in a particular mode in ways uncommon to both alternate techniques.

Batch fermentation

Batch fermentation processes are characterized by continually diminishing substrate concentrations, and continually increasing biomass and product concentrations. By the time that biomass levels are greatest, and thus the capacity of the system for product production is greatest, the conditions for product production are often at their worst.

Continuous fermentation

Continuous fermentation processes are characterized by constant substrate, biomass and product concentrations, but usually one substrate is in growth-limiting concentrations. This results in a culture which has a controlled growth rate, but does not necessarily result in optimal productivity or titre. In essence, continuous cultivation allows the cell population to control medium composition to the point at which diminished substrate concentrations are limiting cell density to the level that is imposed by the dilution rate. This is a good way to control growth rate, but may not be the best way to control other aspects of metabolism. Development of large-scale processes using continuous culture techniques has often been limited by the genetic drift of the cell population, since there is continuous selection for faster-growing rather than more productive genotypes. This may be advantageous in the production of biomass from a toxic substrate such as methanol.

Fed-batch fermentation

Fed-batch fermentation processes are intermediate in character, and many industrial fermentation processes are varieties of fed-batch. In fed-batch culture, certain substrates are added as required to maximize productivity, efficiency or final titre. This approach often results in the maintenance of the optimal substrate concentrations for the maximization of the rate of product formation. Fed-batch processes are not continuous and thus in any given batch the likelihood of difficulties resulting from genetic drift are minimized.

As feedback control becomes a more common feature of fermentation process development, it is likely that the distinctions between these categories will fade. Thus, continuous processes may be developed in which specific substrates will be individually sensed and controlled at optimal levels to control metabolic activity directly in preference to growth rate. Such developments will combine the capital and operating cost advantages of continuous cultivation with the productivity advantages of fed-batch cultivation.

SCALE-UP OF FED-BATCH PROCESSES

Many microbial processes are affected by sub-optimal behaviour at excessive substrate concentrations. In batch fermentation the substrate concentration might initially be supra-optimal, eventually pass through the optimal concentration range and then become sub-optimal. To overcome this type of problem, early yeast manufacturers supplied a feed of malt throughout the culture period. One of the reasons for this was oxygen limitation resulting from high oxygen demand during rapid growth at high substrate concentrations. Oxygen limitation is the most common limiting factor in the scale-up of microbiological processes (Einsele, 1978; Whitaker, 1980).

Table 1. Fermentation processes using fed-batch techniques

Product	Substrate added	Reference
Yeast	Sugar, nitrogen, phosphate	Reed and Peppler, 1973
Glycerol	Sugar, carbonate	Eoff, Linder and Beyer, 1919
Acetone/butanol	Sugar	British Patent 176284
Riboflavin	Carbohydrate	British Patent 615847
Penicillin	Sugar, ammonia	Hosler and Johnson, 1953
Glutamate	Ammonia	Kinoshita *et al.*, 1958
Vitamin B_{12}	Sugar	US Patent 2970220

Today, many industrial fermentation processes use fed-batch techniques to optimize product formation. *Table 1* lists a selection of these processes by product, and it is clear that fed-batch techniques either have been, or are still being, used in many of the most economically important fermentation processes. Further details of some of these are given in later sections.

Fed-batch techniques offer the opportunity to overcome the limitations of large-scale equipment by the adjustment of substrate concentrations to give optimal performance under those limitations. For example, substrate feed can be used to match the rate of oxygen uptake to the rate of oxygen supply, thus preventing anaerobic conditions or controlling dissolved oxygen concentration at a desired level. As another example, substrate feeds can be at a high rate to promote growth during the early phases of the process and then can be adjusted to lower levels to promote product formation.

Generally, substrates are added either at a continuous rate or at punctuated rates which are either timed or keyed to biomass or other detectable levels useful for process control. Little use has been made thus far of continuous process control in fed-batch fermentations. There are three main reasons for this: (1) it has been difficult to obtain sensors for the substrates and products in

question; (2) it has been difficult to relate substrate concentrations to product production rates; and (3) it has been difficult to develop control systems capable of reliable operation as the concentration of biomass increases by as many as 2–6 orders of magnitude, with concomitant decreases in the response times of the system. With the recent developments in microelectronics and sensor technology, these limitations are beginning to disappear: enzyme sensors can detect substances such as glucose or penicillin; computer models of metabolic pathways can be constructed which consume only modest computer time and can be used to guide control systems; and adaptive control methods based on system models can provide real-time control in systems with time constants that change progressively by many orders of magnitude.

Once developed, fed-batch control strategies which depend on measurements of substrate or product concentrations will be much less dependent on scale and should provide acceptable product formation rates with less scale-up research. This should reduce the time required to go from the pilot plant to production. This approach to the scale-up of biotechnological processes can be of significant economic benefit to the introduction of new biotechnological products.

BATCH FERMENTATION DYNAMICS

The dynamics of microbial growth follow a basic outline upon which is superimposed the vagaries of the metabolism of particular microbial species and strains. Examination of the basic equations for growth under various conditions provides the only significant background for understanding the results which are obtained in a given fermentation process, and for planning future strategies for process optimization. The dynamics of batch and fed-batch fermentation processes are outlined below to summarize these two cultivation technologies. The kinetic equations will then be combined with some realistic characteristics of fermentation processes in a simulation model, to provide a basis for the expected process strategies.

In batch growth, the rate of biomass production is given by Equation 1, which describes simple exponential growth if one assumes that the specific growth rate is constant.

$$\frac{dX}{dt} = \mu X \tag{1}$$

where:

X = biomass concentration
μ = specific growth rate
t = time

Many factors combine to limit exponential growth, and their effect is seen through functions which determine the value of the specific growth rate, μ. In general, μ depends on substrate concentrations, and its value depends on the product of μ specific for each substrate (Eq. 2).

$$\mu = (\mu_{sugar})(\mu_{nitrogen})(\mu_a) \ldots\ldots (\mu_z) \tag{2}$$

There are alternative approaches to the assignment of the effects of multiple substrates on μ; however, this factorial approach is the most generally useful in the context of the development of a useful simulation model of fermentation processes.

This factorial approach has the advantages that growth ceases, as it should, if the concentration of any essential nutrient becomes unacceptably low, and that interactive effects between nutrients can be approximately modelled.

Each factor in Eq. 2 that relates to a major substrate depends on substrate concentration according to the Monod equation (Eq. 3).

$$\mu = (\mu max_s)\,(S) / (S+K_s) \tag{3}$$

where:

S = substrate concentration
K_s = Monod constant for the substrate under the given conditions (concentration of half-maximal growth rate)
μmax_s = maximum specific growth rate on the substrate under otherwise optimum conditions

Substrate utilization rate, however, depends on more than just biomass production since substrate is also consumed for product formation and for cellular maintenance. The rate of substrate consumption is also affected by the yields of biomass or product which are obtained under the prevailing conditions. Equation 4 gives an overall balance for the substrate utilization rate.

$$\frac{dS}{dt} = \frac{-\mu X}{Y_{x/s}} - \frac{q_p X}{Y_{p/s}} - mX \tag{4}$$

where:

$Y_{x/s}$ = yield of biomass from substrate
$Y_{p/s}$ = yield of product from substrate
q_p = specific product formation rate at the given S
m = maintenance coefficient

The combination of equations 1–4 with empirical data for the dependence of yields and product formation rates on substrate concentrations can provide a fairly accurate model of microbial growth in batch fermentation. It is clear, however, that if there are optimal substrate concentrations for $Y_{x/s}$, $Y_{p/s}$ or q_p, they will be achieved only for short periods of time under batch conditions.

Product formation rate is given approximately by Equation 5.

$$\frac{dP}{dt} = q_p X = \frac{Y_{p/s}\mu X}{Y_{x/s}} \tag{5}$$

where:

P = product concentration

It is important to realize that all of these equations are coupled and non-linear.

In real situations the yield factors, specific growth rate factors, maintenance coefficients and specific productivities are all functions of substrate concentrations and physical environmental factors such as pH and temperature. It is thus quite difficult to estimate the outcome of fermentations without the assistance of computer simulations which faithfully account for changing conditions over the time-course of the process.

FED-BATCH FERMENTATION DYNAMICS

Fed-batch fermentations can be conducted under conditions of variable volume or constant volume. Maintenance of constant volume can be achieved either by the removal of whole fermentation broth or by selective removal of medium with cell recycle. These possibilities increase the complexity of the model and we will only deal with the variable (increasing) volume case here.

The total biomass balance under conditions of substrate feed and continual volume increase is given by Equation 6.

$$\frac{d(XV)}{dt} = \mu XV \tag{6}$$

where:

V = volume of culture

The volumetric feed rate of substrate is $F = dV/dt$, and thus the dilution rate, D, of culture is given by F/V. The rate of change of biomass concentration is given by Equation 7.

$$\frac{dX}{dt} = (\mu - D)X \tag{7}$$

where:

$$D = \text{dilution rate} = \frac{1}{V}\frac{dV}{dt} = \frac{F}{V}$$

Equation 6 generally describes fed-batch fermentation processes when the effect of the substrate feed on μ is taken into account. Fed-batch processes are distinguished from continuous processes in that V varies in fed-batch process but is constant in continuous processes, and in that there is no outflow of biomass or product in fed-batch processes. Generally, if the rate of substrate feed, F, is constant, μ will decrease with time.

The substrate balance in fed-batch is analogous to the batch situation and is given by Equation 8.

$$\frac{dS}{dt} = D(Sr-S) - \frac{\mu X}{Y_{x/s}} - \frac{q_p X}{Y_{p/s}} - mX \tag{8}$$

where:

Sr = substrate concentration in feed reservoir
q_p = volumetric specific product formation rate
m = maintenance coefficient

Under conditions in which $Sr >> S$, it is possible to approach conditions of constant substrate concentration. The level at which S is constant is determined approximately from the Monod equation (Eq. 3) with $D = \mu$.

The rate of product production is usually complicated by non-linear empirical relations between product formation rate, biomass concentration, substrate concentration and various physical factors. Nevertheless, the general situation is given by Equation 9, in which q_pX is the volumetric product formation rate and D_p is the dilution of product caused by the feed.

$$\frac{dP}{dt} = q_pX - D_p \tag{9}$$

where:

P = product concentration
q_p = volumetric specific product formation rate
D_p = product dilution rate

Under conditions of constant substrate feed, one would expect dilution to predominate (lowering *P*) until the biomass concentration increases sufficiently to result in a net increase in product concentration. This situation applies, of course, to a secreted, soluble product. In situations in which product formation is strictly intracellular, product formation may parallel biomass level. More often, product formation rate appears to be a function of biomass production.

SUMMARY

The dynamics of batch, fed-batch and continuous fermentation systems may be compared both qualitatively and quantitatively. While technically the least difficult, batch systems are almost always sub-optimal. Continuous systems allow control of the growth rate in preference to product formation rate, and this technique is best suited for physiological or genetic studies. Fed-batch systems, especially with feed-back control, offer the greatest opportunity for the control of substrate concentrations and thus for the optimization of productivities and yields.

Fermentation system simulation

INTRODUCTION

There are many models of fermentation systems. The basic structure of the fermentation system has been described above and is discussed in detail by Pirt

(1975). The basic structure is intended to appear to be linear, simplistic and stripped of the peculiar attributes of real fermentation systems.

In process development, fermentation systems have generally been treated as though the outcome is a natural and unavoidable outcome of the initial conditions, and this is indeed the case for batch fermentations. However, interactions during the fermentation between environmental conditions and the growth rate of the organisms make the outcome very difficult to determine from the initial conditions by an intuitive process. More recently, the trend has been increasingly towards the use of simulation models and control systems to optimize the fermentation for biomass or product production.

In many of the cases reviewed in later sections, control of one system variable or substrate has improved the final biomass concentration, productivity rate or other desirable aspects of the fermentation. In practice, however, many attempts at parameter or substrate concentration control are either ineffective or go awry: only successes are published.

In the development of new fermentation processes for industry, there are usually significant constraints on manpower, equipment and financial resources. It is relatively expensive to investigate a series of possibly useful control schemes, while it is relatively inexpensive to develop a simulation model of a process, however unknown, and use it to guide experimental programmes.

This section sets forth a dynamic simulation model of an hypothetical example of a fermentation system. It is characterized by an approach balanced between the simplistic, apparently linear basic structure described above, and the esoteric, highly detailed type of model which attempts to describe precisely each mechanistic detail of cellular biochemistry and physiology in the context of the chemical engineering of fermentation systems.

This model combines aspects of cell physiology and biochemical reactor engineering into useful categories within the context of the development of new fermentation processes for industry. It is intended to display realistically the characteristics of an unremarkable fermentation system. It is not intended to be a perfect model dealing with all hierarchical levels operating in real fermentation systems, but is the type of model that can be very helpful during new process development programmes. This approach offers an extension to the basic fermentation system structure that is not only useful and realistic, but is also within the grasp and range of interests of most microbiologists and biochemical engineers involved in fermentation process development.

The fermentation system

The fermentation system is composed of relatively few classes of components: cells, major substrates, and products. Equipment only provides a location, a separation from potentially contaminating organisms, and a means for enhanced mass and heat transfer, with possibilities for control.

The ultimate limit to cell population growth could be simplistically considered to be the availability of major substrates. Substrate availability is the primary constraint on growth, but other environmental conditions can

decrease growth before substrate depletion. Toxic or inhibitory products or by-products, ethanol or acidity for example, frequently terminate growth even though substrate concentrations may be substantial. In the absence of these limitations and in the presence of adequate, non-inhibitory substrate concentrations, growth is essentially exponential. However, exponential growth is eventually slowed by limitations in the system.

The nature of exponential growth is worthy of reconsideration. An exponential growth law is a relatively simple statement: the number of organisms doubles in a particular time. The consequences to a cell population in a fermentation system are quite remarkable: for many doubling periods, growth is unaffected by the seemingly ideal environment; then suddenly in one doubling time, the last, substrates are depleted to starvation levels and products accumulate to toxic concentrations. When one critical limit is reached, growth stops abruptly. Considering the economics of production, this is wasteful since at the very time the culture has its greatest cell density and its greatest potential for product formation, it is allowed to cease all useful activity. This sharp termination of exponential growth is characteristic of uncontrolled batch fermentation.

Growth to equilibrium

Substrate concentration control, and parameter control in general, begins to address the transition in process development from the use of abruptly terminated exponential systems to the use of gradually slowed systems carefully brought into a productive steady state. Without substrate addition, cells experience substrate concentrations well in excess of K_s for many doublings, often until the last doubling. Suddenly, the large population depletes substrate concentrations to within a small fraction of K_s. Growth rate slows, but high cell concentrations, maintenance requirements and product formation consume the relatively small remaining quantities of substrate in a very short time. Growth rate then approaches zero, product formation halts and death rates increase.

The investment in the culture to the time of the last doubling is usually considerable if a full accounting is made of raw materials, equipment, time and manpower. To obtain greatest benefit from that investment, it would be desirable to use every effort to bring the culture into a sustainable and productive metabolic state. It is, however, probably too late to begin control measures only as substrate concentrations reach critical levels: cellular metabolism will either have adapted to the earlier conditions of substrate surplus or will be adapting to incipient starvation, and be slow to respond to novel imposed conditions.

To achieve this desirable, controlled steady state it may be necessary to control growth from the start, to minimize toxic or undesirable by-products, and to allow the induction and development of the enzyme systems responsible for the desired product. The greatest current challenge in fermentation technology is to develop methods to guide the transition from exponential growth to a productive steady state. To do this may require counterintuitive

measures: growth rates may have to be suppressed by the accurate control of combinations of parameters or substrate concentrations at nearly inhibitory or starvation levels. There are many options for limiting growth while maintaining metabolic functions required for product formation, and it is quite probable that combinations of several options will be more useful than the potentially brutal effects of exercising one option at the extreme.

The economic opportunities of fermentation technology will not be fully realized until we develop the understanding and the techniques, for controlling cell cultures, that are required to achieve stable, productive steady state cell populations. To do this, a much more analytical approach to fermentation process development than is prevalent today will need to be achieved.

BATCH CULTIVATION MODEL

This section describes a batch cultivation model of an hypothetical fermentation system. This model contains those aspects of the system which are independent of the mode of cultivation, and is thus a core model that can be adapted to model fed-batch or continuous cultivations of the same hypothetical organism.

All dynamic models consist of three categories or elements of structure: levels, rates and information flows. Levels are, in this case, quantities; information flows generally tell us how the level of one quantity affects the rate of change of another. For example, a level might be the concentration of biomass (X, grams/litre); the relevant rate is the growth rate (dX/dt, grams/litre-hour). Information relevant to the growth rate includes the concentrations of all major substrates, temperatures, pH, and so on. Rates of flow or of change are affected by concentrations or physical conditions, but cannot be affected by other rates of change directly: for example, the cell population density is affected by the rates of cell division and cell death and the difference between these is the net rate of population change; as food is exhausted, population levels only change as a result of the relative effects of, say, glucose concentration on growth and death rates—the rate of glucose consumption does not directly affect the rate of cell growth.

The Dynamo Simulation Language (Richardson and Pugh, 1981) is a computer simulation language designed to allow the relatively uncomplicated development of computer simulations without requiring the modeller to be concerned with the mechanics of the integration process. Dynamo is a discrete time-step simulator, which further increases its ease of use and decreases the likelihood of logical errors. Dynamo divides time into three steps: J is 'last time', K is 'now', and L is 'next time'. Thus, the current value of a level variable (X.K) is the last value of the variable (X.J) plus the change that has occurred over the time period between the 'last time' and 'now'. From the modeller's point of view, the software takes care of the numerical details, allowing full attention to be placed on the structure of the model itself. Dynamo has been used in the model which follows.

Cell growth

Cell growth involves three components: growth rate, cell concentration and death rate. The growth rate has been taken as the highest observable specific growth rate, under constant and ideal conditions, times the cell concentration. The death rate has been estimated at a constant fraction of the population. Using the Dynamo Simulation Language, the growth in cell concentration is expressed as follows:

$$
\begin{aligned}
X.K &= X.J + DT * (XRATE.JK) \\
X &= IX \\
IX &= 0{\cdot}01 \\
XRATE.KL &= X.K * (U.KL - DTH)
\end{aligned}
$$

where:

X is cell density (grams/litre)
IX is initial cell density (grams/litre)
XRATE is net cell growth rate (grams/litre-hour)
U is growth rate coefficient (per hour)
DTH is death rate coefficient (per hour)
DT is the length of the time interval (hour)
J, K, L are time subscripts (*see* text)

The Dynamo Simulation Language will accumulate the rates of growth and death in each time increment to give an accumulated cell density at any given time. As in an actual laboratory situation, there must be an initial cell density to initiate the growth process. The value of IX chosen here represents a 1% inoculum, as could be used in a practical situation. In subsequent time intervals, XRATE will increase by an amount U*X and decrease by an amount DTH*X. Both U and DTH could change as conditions in the culture medium change as a consequence of growth. In this simulation, emphasis is placed on the changes in U which result from changes in substrate concentrations.

Cell growth rate

Cell growth rate (XRATE) depends on the growth rate coefficient (U) and the death rate coefficient (DTH). In this model, it has been assumed that the death rate coefficient is constant, and DTH was set at 0·00075 regardless of conditions. This is probably unrealistic, but alternative relationships could be built into the model if data suggested they were relevant. The growth rate coefficient, U, depends on the concentrations of all major nutrients in the growth medium. A characteristic of the hypothetical system modelled here is the ability to grow under either aerobic or anaerobic conditions. The assumption used is that the only factor in the determination of the growth rate coefficient affected by the difference between these two conditions is the factor relating to oxygen. The growth rate coefficient is described by the following equation:

$$U.KL = UANA.KL + DO.K * (UAER.KL - UANA.KL)/CSTAR$$

where:

U is the growth rate coefficient (h^{-1})
UANA is the anaerobic growth rate coefficient (h^{-1})
UAER is the aerobic growth rate coefficient (h^{-1})
DO is the dissolved oxygen concentration (g/l)
CSTAR is the saturation concentration of dissolved oxygen (g/l)

The equation describes a shift of growth rate between UAER and UANA as dissolved oxygen concentration decreases from CSTAR to zero. In practical situations, many factors affecting the growth rate coefficient could change as growth conditions bring anaerobic metabolism into play, and the shift could display other responses to dissolved oxygen concentration; however, for the purposes of a hypothetical model, this provides a functional relation.

The effects of substrate concentrations on cell growth rate are generally considered to be successfully modelled by the Monod equation described above. The effects of multiple substrates are best modelled by a series of multiplicative factors, since growth will not be sustained in the absence of any major nutrient class. Aerobic growth rate coefficient is given by the following equation:

UAER.KL = UP * UO.KL * UN.KL * UG.KL * UPH.KL

Anaerobic growth rate coefficient is given by the following equation:

UANA.KL = UP * UN.KL * UG.KL * UPH.KL

where:

UAER is the aerobic growth rate coefficient (h^{-1})
UANA is the anaerobic growth rate coefficient (h^{-1})
UN is the nitrogen growth rate factor (h^{-1})
UP is the phosphorus growth rate factor (h^{-1})
UG is the glucose growth rate factor (h^{-1})
UPH is the pH growth rate factor (h^{-1})
UO is the oxygen growth rate factor (h^{-1})

Substrate concentration factors

In general, the effect of concentration on the various substrate growth rate factors is given by the Monod equation with an appropriate value for the μ and K_s of each. In addition, it is necessary to define the level and rate equations explicitly for the concentration of each substrate included in the model. The equations used for each substrate are as follows.

For phosphorus, a substrate generally supplied in excess, and for which sufficient data were not available to develop a generalized picture of the effects of phosphate concentration on growth rates, it was decided to assume that the phosphorus concentration was at growth rate saturating levels. Thus, the phosphorus growth rate factor, UP, was set as a constant.

UP = 0·7025

Oxygen concentration also follows Monod kinetics and the oxygen growth rate factor is as follows:

$$\begin{aligned} UO &= UOMAX * DO.K/(KSO + DO.K) \\ UOMAX &= 1{\cdot}01 \\ KSO &= 1{\cdot}3 \times 10^{-5} \end{aligned}$$

where:

UO is the oxygen growth rate factor (h^{-1})
UOMAX is the μmax attributable to oxygen (h^{-1})
KSO is the half saturation constant for oxygen (g/l)

Selection of appropriate values for UOMAX and KSO would require some careful examination of the literature, or experimentation designed to determine the effects of oxygen concentration in the presence of ample concentrations of other nutrients. The values chosen here are similar to values appropriate to *Escherichia coli*. Use of this equation requires knowledge of DO, the dissolved oxygen concentration, and this is dealt with below.

Glucose and nitrogen follow a similar pattern, although in practice it is possible for both substrates to cause substrate inhibition at high substrate concentrations.

$$\begin{aligned} UG.KL &= UGMAX * GCON.K/(KSG + GCON.K) \\ UGMAX &= 2{\cdot}1 \\ KSG &= 2{\cdot}5 \times 10^{-2} \\ UN.KL &= UNMAX * NCON.K/(KSN + NCON.K) \\ UNMAX &= 0{\cdot}8 \\ KSN &= 1{\cdot}4 \times 10^{-2} \end{aligned}$$

where:

UG is the glucose growth rate factor (h^{-1})
UGMAX is the μmax attributable to glucose (h^{-1})
KSG is the half saturation constant for glucose (g/l)
GCON is the glucose concentration (g/l)
UN is the nitrogen growth rate factor (h^{-1})
UNMAX is the μmax attributable to nitrogen (h^{-1})
KSN is the half saturation constant for nitrogen (g/l)
NCON is the nitrogen concentration (g/l)

The pH of the growth medium reflects the hydrogen ion concentration, but hydrogen ions are not normally considered to be substrates for growth. Growth rate is strongly affected by pH through a variety of mechanisms and these effects must be considered in the construction of a realistic model of a fermentation process. To model the effects of pH, a number of experimental results relating μ to pH in various bacteria were examined. Generally there is an optimal pH and a symmetrical decrease in growth rate on either side of the optimum, with growth rate declining to very low values by about 3 pH units above or below the optimum. For the purpose of the model, pH values outside this range brought growth rate to zero. The effects of pH on growth rate were modelled as follows:

UPH.KL = 1 − 0.1*(PH.K − PHOPT)**2
PHOPT = 7·0

where:

UPH is the pH growth rate factor (h^{-1})
PH is the pH of the growth medium
PHOPT is the optimal pH for growth

Substrate concentrations

Glucose concentration is the accumulated difference between the initial glucose concentration, plus any additions (nil in batch mode) less all utilizations of glucose for growth or product formation. Glucose is consumed for several purposes in the cell: growth or biomass production, metabolic maintenance, and product production. The glucose uptake rate, glucose maintenance rate and glucose consumption rate for product combine to give the overall glucose consumption rate:

GRATE.KL = −GLUR.K − GLMR.K − GCRPR1.K
GLUR.K = X.K * (U.KL/YLDXG)
GLMR.K = X.K * MGX
GCRPR1.K = YPR1G * PR1PR.KL
YLDXG = 0·27
MGX = 0·005
YPR1G = 0·3

where:

GRATE is the glucose consumption rate (g/l-h)
GLUR is the glucose uptake rate for biomass production (g/l-h)
GLMR is the glucose maintenance rate (g/l-h)
GCRPR1 is the glucose consumption rate for product (g/l-h)
MGX is the maintenance coefficient for glucose (h^{-1})
YLDXG is the yield of X on glucose (g/g)
YPR1G is the yield of PR1 on glucose (g/g)
PR1PR is the product production rate (g/l-h)

Glucose concentration in the growth medium is given by the accumulation of these rates over time with respect to the initial glucose concentration:

GLC.K = GLC.J + DT * GRATE.JK
GLC = IGLC
IGLC = 10·0

where:

GLC is the glucose concentration in the growth medium (g/l)
IGLC is the initial glucose concentration (g/l)

The sub-model for nitrogen concentration and utilization rate is of the same form as that for glucose. The nitrogen consumption rate and associated terms are as follows:

```
NRATE.KL = -NUR.K - NMR.K
   NUR.K = X.K * (U.KL/YLDXN)
   NMR.K = 0·0005 * X.K
  YLDXN = 20·0
```

where:

NRATE is the nitrogen consumption rate (g/l-h)
NUR is the nitrogen uptake rate for biomass production (g/l-h)
NMR is the nitrogen maintenance rate (g/l-h)
YLDXN is the yield of X on nitrogen (g/g)

Nitrogen concentration in the growth medium is depleted in each time increment by the nitrogen consumption rate:

```
NIT.K = NIT.J + DT * NRATE.JK
  NIT = INIT
 INIT = 2·0
```

where:

NIT is the nitrogen concentration (g/l)
INIT is the initial nitrogen concentration (g/l)

Oxygen balance depends not only on the initial concentration of oxygen in the growth medium, but also on the rate of supply of oxygen to the liquid by the mixing and sparging systems. Oxygen consumption follows a model similar to that of other substrates, but includes a term for the rate of oxygen supply to the system.

```
DO.K = DO.J + DT * (OTR.JK - OUR.JK)
  DO = IDO
 IDO = 0·0058
```

where:

DO is the oxygen concentration in the growth medium (g/l)
OTR is the oxygen transfer rate (g/l-h)
OUR is the oxygen uptake rate (g/l-h)
IDO is the initial dissolved oxygen concentration (g/l)

In consideration of the dissolved oxygen concentration, solubility limits become a recognizable problem, and accessory statements must be built into the model to limit dissolved oxygen to less than or equal to CSTAR, the saturation concentration for oxygen in water under the conditions of growth. Oxygen transfer rate is given by the standard relation:

```
OTR.KL = KLA.K * (CSTAR - DO.K)
 KLA.K = (0·0014 / H) * ( (P**0·53) * (V**0·67))
 CSTAR = 0·006
     H = 0·00117
```

where:

OTR is the oxygen transfer rate (g/l-h)
KLA is the oxygen mass transfer coefficient (h^{-1})

H is the Henry's constant appropriate to the operating conditions (kg mole/ATM-m^3)
P is the power per volume ratio (HP/l)
V is the superficial air velocity (l/m^2h)
CSTAR is the saturation concentration of oxygen under the operating conditions (g/l)

Relations converting mixing rate, in rev/min, to power per volume and sparging rate to superficial air velocity allow the model to be adjusted as one would adjust the operation of an actual fermentation system, and allow realistic simulations of oxygen concentration control systems.

Oxygen uptake rate, OUR, depends on the yield of biomass on oxygen, the maintenance requirements for oxygen and the rate of growth:

OUR.K = OURX.K + OMR.K
OURX.K = X.K * (UAER.KL/YLDXO)
OMR.K = 0·005 * X.K
YLDXO = 3·25

where:

OUR is the oxygen uptake rate (g/l-h)
OURX is the oxygen uptake rate for X production (g/l-h)
OMR is the oxygen maintenance rate (g/l-h)
YLDXO is the yield of biomass on oxygen (grams/gram)

Product production

To complete a reasonably realistic model of the hypothetical fermentation process, consideration must be given to the production of three products: carbon dioxide, hydrogen ions and an extracellular product (PR1, assumed to consist of C, H and O only). The model of product production follows a similar pattern:

PR1.K = PR1.J + DT * PR1PR.JK
PR1 = IPR1
IPR1 = 0·0
PR1PR.KL = QPR1 * X.K
QPR1.K = YLDPX.K * UAER.KL
YLDPX.K = YPR1G/YLDXG

where:

PR1 is the concentration of product (g/l)
IPR1 is the Initial concentration of Product (g/l)
QPR1 is the specific Product Production Rate (h^{-1})
PR1PR is the Product Production Rate (g/l-h)
YLDPX is the Yield of Product in relation to biomass (g/g)

Assumptions built into this sub-model are that the product is extracellular, growth associated and produced only under aerobic conditions.

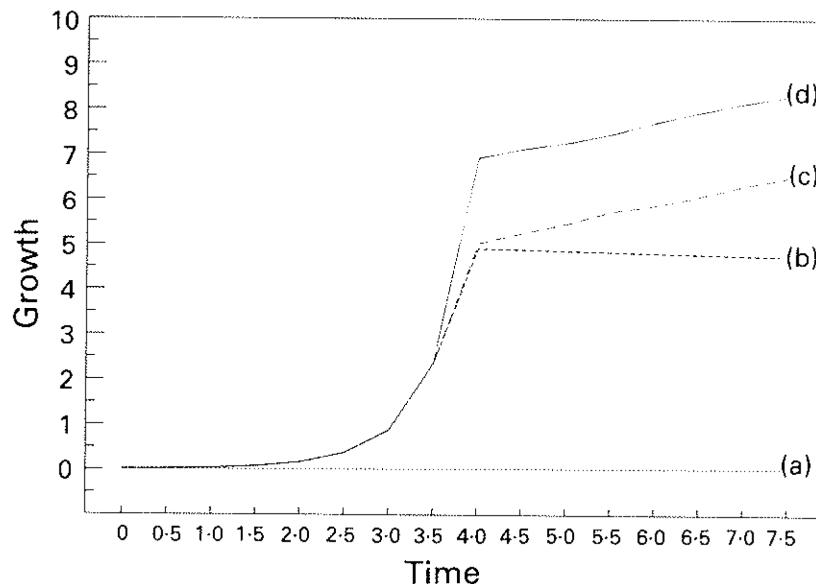

Figure 1. Simulated fermentations of a hypothetical organism under various process conditions: (a) batch, (b) pH controlled batch, (c) fed-batch with constant feed rate, and (d) fed-batch with oxygen-controlled substrate feed.

The production of hydrogen ions and CO_2 can be modelled in a similar manner, although adjustments must be made for the equilibria between the various ionic species involved. The production of hydrogen ions is assumed to be primarily from the deprotonation of ammonium so that, on a molar basis, one hydrogen ion is expelled into the medium for each ammonium ion consumed by the cell. Similarly, carbon dioxide comes from the oxidation of glucose.

Batch cultivation dynamics

Figure 1a gives the output of this model under conditions of simple batch cultivation. Growth begins, the growth rate is quite high, but in a very short time the pH falls, abbreviating growth and by 8 hours, essentially no biomass has accumulated. In practice, this is a common initial result with the early runs of a new fermentation. Many industrial fermentations run without pH control only as a result of alterations in the medium composition or extensive mutation and selection programmes which minimize acid or base production. In the development of new production processes, it is preferable to control pH with the usual pH electrode–controller–pump set-up, and to optimize the medium on the basis of a constant pH. While the technical difficulties can be considerable with large equipment, pH is the variable most easily controlled and of the greatest pleiotropic effects.

Figure 1b gives the results obtained with the model modified to contain a pH controller set at pH 7·0, the optimum pH for growth. As would be expected,

the growth is dramatically improved. In this case, the pH controller is designed to act like a typical on/off controller with constant feed rate, which induces some minor variations in growth rate as pH varies within the dead-band of the controller; acid and base additions slowly dilute the broth, reducing cell concentration (growth).

SIMULATED FERMENTATIONS WITH SUBSTRATE CONCENTRATION CONTROL

The most common attempt to control substrate concentration is to supply a steady feed of a single substrate. This is the classic 'fed-batch' strategy, although now the term is more widely applied. This usually promotes growth in comparison to a batch fermentation, but at some point, the feed rate is only sufficient for the maintenance requirements, and growth ceases. A sample run of the model developed above with constant glucose feed is given in *Figure 1c.*

To actually control substrate concentration at a prescribed level, it is necessary to develop either a closed-loop or open-loop control system capable of supplying the desired substrate to the cultivation on demand. There are many possible loops which could be considered. A closed-loop controller readily available on many fermentation systems is dissolved oxygen control: the dissolved oxygen electrode output is compared to a set-point value for dissolved oxygen concentration and the error signal is applied through an algorithm to control either air flow rate, agitator speed or both. This is useful under a variety of circumstances: (1) when there is a definite optimum dissolved oxygen concentration for either growth or product formation or, (2) when it is desirable to minimize air flow, while maintaining a minimum dissolved oxygen concentration, in order to prevent undesirable effects such as volatilization of by-products or formation of foam. The model developed above does not have either of these characteristics, so closed-loop dissolved oxygen control has little effect—growth ceases when the glucose concentration becomes zero.

An open-loop controller that is often useful in practical situations is based on the following control system: the dissolved oxygen concentration is compared with a set-point and a control signal is applied to control the rate of glucose addition. The case of an on-off controller, with a set-point of KSO is given in *Figure 1d*: the relatively low set-point slows growth through reduction of the oxygen factor in the growth rate equation, but the glucose concentration remains high for a relatively long period. Adjustments in the set-point and feed rate can give a fairly wide variety of results, but the qualitative result remains the same. Early in the fermentation, oxygen demand increases until the dissolved oxygen concentration falls below the set-point, turning off the glucose feed. Glucose remains at relatively high levels until the biomass concentration is such that glucose concentration approaches the KSG value. When this occurs, growth rate declines, reducing oxygen demand and, thus, increasing the dissolved oxygen concentration. Eventually the dissolved oxygen concentration exceeds the set-point, turning on the glucose feed. This, in turn, increases oxygen demand until the dissolved oxygen concentration falls below the set-point, turning off the glucose feed. These cycles continue at an

increasing frequency until the maximum oxygen transfer rate of the fermentor is sufficient only to supply oxygen for the maintenance requirements of the culture and growth rate slows to zero.

Even with the feed of a single substrate, the dynamics of this open-loop system are fairly complicated. It is easier and generally more productive to plan experiments of this type by iterations between computer simulations and experimental runs. In this way, it is possible to understand better the results of changing experimentally variable parameters such as the dissolved oxygen concentration set-point or the glucose feed rate.

With some experience, it is also possible to extend the use of such a model to situations in which a complex mixture is used as the feed, as has been successfully done by one of the authors using a yeast extract/glucose/minerals feed solution with PID (Proportional-Integral-Derivative) control of pump rate. This system allowed biomass concentration to reach more than 35 g/l before the dissolved oxygen concentration deviated by more than 5% of the set-point (D.F. Gerson, unpublished observations).

Experimental approaches to substrate concentration control

INTRODUCTION

Fermentation media

Fermentation media used in the cultivation of micro-organisms must contain all elements in a form suitable for the synthesis of biomass and metabolic products. Micro-organisms can obtain energy from their environment in a variety of ways. Only autotrophs require carbon as CO_2. Heterotrophs require carbon in a more complicated molecular form. The micro-organisms of greatest commercial importance are the heterotrophs (Frobisher, 1968).

The microbial environment is largely determined by the composition of the growth medium. Different media are used in the identification process and study of microbial metabolism (Burnett, Pelezar and Conn, 1957). Synthetic, or defined, media are prepared using pure compounds in precisely defined proportions. This type of medium is suitable for laboratory research with micro-organisms, and increases the ease of product recovery and purification. However, in many cases, the low product yield and poor economy of synthetic media make complex, natural or semi-synthetic media the preferred choice in industrial fermentations (Casida, 1968).

Carbon sources

The constituents of fermentation media can be classified as sources of carbon, nitrogen, inorganics or vitamins, according to their principal function in the medium. Carbohydrates are an excellent source of carbon, oxygen, hydrogen

and metabolic energy for many micro-organisms. They are available as simple sugars such as glucose, molasses, sucrose; or sugar polymers such as starch, dextrin, cellulose and hemicellose. As biomass is typically 50% carbon on a dry weight basis, carbohydrates are frequently the main medium components. Whey, a liquid by-product of cheese manufacturing, contains lactose, protein and lactic acid and can be used commercially as a source of fermentable carbohydrate and nitrogen (Peppler, 1979). Sulphite waste liquor from the paper-pulping industry has been used in the commercial production of single-cell protein (SCP) from *Candida utilis* (Reed and Peppler, 1973; Biochem Technology, Inc., 1980), *Paccilomyces variotii* (Romantschuk, 1976) and torula yeast (Litchfield, 1977).

Cellulosic materials have always had considerable commercial interest as a source of carbon. A number of processes have been developed to hydrolyse cellulosic materials to fermentable sugars. Cellulase from *Trichoderma reesei* is particularly effective for this purpose (Ladish, Ladish and Tsao, 1978).

The widespread availability of inexpensive hydrocarbons and a world shortage of protein has resulted in the development of fermentation processes for the production of SCP (Biochem Technology Inc., 1980). Liquid alkanes are used by a variety of micro-organisms including *Candida*, *Acinetobacter cerficans*, *Achromobacter delaevate* and *Pseudomonas* sp. (Litchfield, 1979). In most cases, hydrocarbons are used to produce SCP, although vitamins, amino acids, nucleotides, lipids, surfactants, and enzymes have also been reproduced from hydrocarbons (Ratledge, 1977; Gerson and Zajic, 1979).

Alcohols are another group of compounds used as carbon sources for fermentations. Methanol can be used for the production of biomass, glutamic acid, valine, lysine, threonine, α-ketoglutaric acid, citric acid, fumaric acid, hypoxanthine, serine and vitamin B_{12}. Like methanol, ethanol may be used as a carbon source for the production orotic acid, orotidine, ergosterol and cephalosporin (Ratledge, 1977).

Acetic acid and other organic acids and oils may also be used as sole carbon sources in fermentation media. Acetic acid is used for the production of glutamic acid and citric acid. Oils (liquid fats) have been reported to be used in the production of penicillin, tetracycline, streptomycin, celphalosporins, noenomycin, riboflavin, citric acid and β-carotene (Ratledge, 1977).

Tables 2 and *3* summarize the use of fed-batch cultivation techniques with carbon and nitrogen source additions for the production of enzymes, amino acids, antibiotics and alcohol.

Nitrogen sources

A large number of microbial strains are able to use ammonium or nitrates as their sole source of nitrogen. Ammonia gas or ammonium hydroxide are used for controlling the pH while supplying nitrogen to the culture medium. Many species are able to assimilate ammonium salts, ammonium sulphate being the least expensive and most commonly used (Solomons, 1969). Nitrates, such as sodium nitrate and ammonium nitrate, are common nitrogen sources used by

Table 2. Several processes in which fed-batch cultivation was used for addition nitrogen sources

Product	Additions	Reference
Cellulase	Ammonia	Sternberg and Dorvai, 1979
Cellulase	Ammonia Cellulose	Gottvaldova, Kucera and Padrazy, 1982
Protease	Ammonia Glucose	Kole, Draper and Gerson, 1988
Amino Acid	Urea	Su and Yamada, 1960 Miescher, 1962 Oki *et al.*, 1968
Amino Acid	Ammonium Salt	Kinoshita *et al.*, 1958 Miescher, 1962 Yamamoto *et al.*, 1972
Glutamic Acid	Ammonium Benzoate	Yamamoto *et al.*, 1972
Isoleucine	Urea	Sugisaki, Yamamoto and Koyama, 1970
Glutamic Acid	Nitrate	Kole *et al.*, 1986
Penicillin	Ammonia	Hosler and Johnson, 1933 Hockenhull and Mackenzie, 1968 Wright and Calam, 1968 McCann and Calam, 1972
Streptomycin	Glucose Ammonium Sulphate	Singh, Bruzelius and Heding, 1976
Oxytetracycline	Glucose Ammonium Salt	Orlova and Pushkina, 1972
Novobiocin	Glucose Sucrose Fructose Ammonium Salt Urea	Smith, 1956

Table 3. Alcohol production by fed-batch fermentation

Additive	Ethanol	Reference
Cellulose	8·5 g/l	Spinnler, 1986
Cellulose	0·40 g/g sugar hydrolysate	Beck, 1986
Xylose	—	Beck and Strickland, 1984
Cellulose	—	Wang *et al.*, 1978
Cellulose	0·3–0·9%	Zertuche and Zall, 1982
Starch	—	del Rosario and Wong, 1984; Gibson and Westby, 1983
Glucose	15% (v/v)	Ohta, Supanwong and Hayaskida, 1981
Glucose	11·3% (w/v)	Silman, 1984
Glucose	8–12 g/l	Jones *et al.*, 1984
Glucose	—	Verduyn *et al.*, 1984
Glucose	—	Tyagi and Ghose, 1980
Glucose	—	Toda, Tabe and Yamagata, 1980
Glucose and magnesium	12% (v/v)	Dombek and Ingram, 1986
Lactose	13·6 g/l/h	Terrell, Bernard and Bailey, 1984
Glucose	90–92 kg/m^3	Luong and Tseng, 1984
Glucose	25 g/l/h	Lee and Wang, 1982
Glucose	5–11 g/10 g glucose	McGhee, Julian and Detroy, 1982
Glucose	50 g/l	Rainina *et al.*, 1986
Glucose	124 g/l	Mota *et al.*, 1987

many algae and fungi but less extensively by bacteria and yeasts (Rose, 1968). Various species of bacteria, for example *Klebsiella*, *Azotobacter*, *Clostridium*, *Rhizobium* and most blue-green algae, have the ability to fix molecular nitrogen into organic molecules (Frobisher, 1968; Rose, 1968; Wallace and Stokes, 1979).

Specific amino acids, purines, pyrimidines and urea may be used as organic sources of nitrogen in synthetic media. Many commercial fermentations use complex organic nitrogen sources which are by-products of the food processing and agricultural industries. These media components are less expensive and often more effective than salts, and can contain various growth factors and vitamins. For example, a fivefold increase in antibiotic yield was obtained when corn steep liquor (CSL) was added to the fermentation medium for *Penicillium chrysogenum*. The CSL contained phenylalanine and phenylethylamine which are precursors of penicillin G (Perlman, 1979). Peptone, tryptone, soybean meal, cotton seed extracts and yeast extracts are also used as nitrogen sources in fermentation media.

Inorganic components

Micro-organisms require many inorganic nutrients. Phosphorus and sulphur are required in large amounts. Smaller amounts of potassium, magnesium, sodium, calcium, iron, copper, manganese, molybdenum, cobalt, zinc, and chloride are also required (Riviere, 1977). In the cell, phosphorus occurs principally in the form of sugar phosphates such as nucleotides which compose DNA, RNA and ATP; sulphur is present in the amino acids cysteine and methionine. Intracellular potassium is often in the 100 mM range; iron is associated with the cytochromes; zinc is essential to the function of alcohol dehydrogenase; magnesium activates many enzymes, such as hexokinase, and regulates the degree of association of ribosomes (Rose, 1968).

GLUCOSE CONCENTRATION CONTROL

General aspects and ethanol production

Glucose is one of the simplest carbon sources used in the fermentation industry for the production of various industrial chemicals and other supplies. It has been reported that substantial initial glucose concentrations in batch culture result in growth inhibition leading to longer fermentation times, poor substrate utilization (King and Houssain, 1982) or product inhibition by products such as ethanol and CO_2 (Worden, Donaldson and Shumate, 1983; Nipkow, Sonnleitner and Fiechter, 1985).

Yeast has been conventionally exploited in the manufacture of alcoholic beverages and pure alcohol. A promising alternative to yeast as an industrial alcohol producer is the bacterium *Zymomonas mobilis* which utilizes the Entner–Doudoroff pathway for ethanol formation (Gibbs and DeMoss, 1954; Moat, 1979). In comparison to other microbial candidates for ethanol

production such as *S. uvarum* and *S. cerevisiae*, it offers improved ethanol yields with high ethanol tolerance (Rogers *et al.*, 1982). Several researchers (Lee, Tribe and Rogers, 1979; Rogers, Lee and Tribe, 1979; King and Houssain, 1982) have examined the effect of pH, temperature and initial glucose concentration on yields and kinetics of biomass production and ethanol formation in batch cultures of different strains of *Zymomonas mobilis*. Ohta, Supanwong and Hayaskida (1981) studied *Zymomonas mobilis* with regard to ethanol tolerance. Fermentation tests were carried out in batch cultures with stepwise addition of glucose to complex or synthetic medium. *Z. mobilis* produced 15·1% and 15% (v/v) ethanol respectively in complex synthetic media at 30°C.

A fed-batch culture of *Z. mobilis* was grown at 38°C for ethanol accumulation, the medium was recycled to the fermentor from the still and the culture was fed continuously with concentrated glucose syrup (Mescle and Pesche, 1986). In this study, the fermentation was 15 h long with a total consumption of 171 g/l of glucose. The maximum uptake rate was 20 g/l/h. These results are related to the high growth of the strain and the suppression of ethanol toxicity. Jain and Nath (1987) found that *Z. mobilis* NRR2 B-806 cultures with an initial glucose concentration of 100 g/l have a specific ethanol productivity (q_p) of 6·4 g/g-h, with a final ethanol concentration of 42·5 g/l. The effects of CO_2 and ethanol concentration on the *Z. mobilis* fermentation were investigated using continuous and fed-batch cultivation systems (Burrill, Doelle and Greenfield, 1983). It was observed that CO_2 affects the glucose uptake rate, and ethanol inhibition is responsible for the final ethanol concentration obtainable during *Z. mobilis* fermentations. Silman (1984) studied the effect of feed rate and total feed consumption in fed-batch fermentations of *Z. mobilis* for ethanol production. By optimizing the feed rate and feed volume, the final ethanol concentration was 11·3% (w/v) compared with 10·0% (w/v) by batch fermentation.

Several continuous ethanol fermentation designs with high ethanol productivities have recently been proposed. These include continuous stirred tank fermentors in series (Tyagi and Ghose, 1980), recycle fermentations (del Rosario, Lee and Roger, 1979), immobilized cell reactors (Tyagi and Ghose, 1982) and tower fermentors (Prince and Barford, 1982). A major advantage of the tower fermentor is its ability to maintain stable continuous operation for periods exceeding 6 months (Ault *et al.*, 1969). Jones *et al.* (1984) studied a mutant of *Saccharomyces cerevisiae* which forms large, multicellular flocs in liquid culture. This strain rapidly fermented media containing high concentrations of glucose (100–180 g/l) in a continuous non-aerated tower fermentor at 30°C. Ethanol productivities of 8–12 g/l were achieved, giving conversion efficiencies exceeding 90% of the theoretical yield.

Of all the factors which influence alcoholic fermentation in yeast cultures, the concentrations of glucose and oxygen are probably the most important. An enzyme electrode was used, in combination with a reference oxygen electrode, to study the short-term kinetics of alcohol fermentation by aerobic yeast suspensions after pulsing with glucose (Verduyn *et al.*, 1984). Samples from aerobic, glucose-limited chemostat cultures of *S. cerevisiae* not producing

ethanol, exhibited aerobic alcoholic fermentation within 2 min of being pulsed with excess glucose. Ethanol formation occurred only at initial glucose concentrations exceeding 150 mg/l, and the rate of alcoholic fermentation increased with increasing glucose concentrations up to 1000 mg/l glucose.

Batch and single-flow, four-stage continuous ethanol fermentations of bagasse hydrolysate have been investigated at pH 4·0 and 30°C with a strain of the yeast *Saccharomyces cerevisiae* (Tyagi and Ghose, 1980). The studies were carried out in laboratory-sized four-stage cascade continuous-stirred tank fermentors at varying feed glucose concentrations (10%, 14%, 18% and 22%). The dilution rate was varied from 0·05 to 0·2/h. The hydrolysate was supplemented with other nitrogen and mineral sources. The conditions of continuous culture in a multistage system were predicted by a graphical method based on batch culture data. The experimental data were as expected, within the limits of experimental error.

Toda, Tabe and Yamagata (1980) developed a model to analyse the biphasic growth of yeast on both the glucose remaining and the ethanol produced in continuous and fed-batch cultures of *Saccharomyces carlsbergensis*. Ciftci, Constantinides and Wang (1983) studied alcohol fermentation with an emphasis on the separation of the cell growth and alcohol production stages. It was found that glucose concentrations should be kept under 10% (w/v) and the temperature should be between 40 and 42·5°C for maximum specific alcohol productivity by *S. cerevisiae*. Dombek and Ingram (1986) observed that supplementing fermentations with 0·5 mM magnesium prolonged exponential growth, resulting in increased cell mass. The addition of magnesium also reduced the decline in fermentation activity. These two effects reduced the time required for ethanol conversion, with no measurable loss in ethanol yield (98% of theoretical maximum yield). Sreenath, Chapman and Jeffries (1986) observed, during xylose fermentation by *Candida shehatae*, that young inocula exhibit a higher ethanol production rate than older inocula. Aerobic conditions limited the uptake of sugar, and continuous feeding of glucose increased the xylose utilization rate. During continuous fermentation with a catabolite repression-resistant mutant of *Saccharomyces cerevisiae*, a complete conversion of cheese whey lactose to ethanol has been demonstrated. The first step in this process was a computer-controlled fed-batch operation based on the carbon dioxide evolution rate of the culture (Terrell, Bernard and Bailey, 1984).

The continuous ethanol processes currently under investigation can be divided into two basic categories: continuous stirred tank reactors (CSTR) and immobilized cell reactors (ICR). Continuous stirred tank reactors have definite productivity limitations due to the fact that the dilution rate cannot be greater than the specific growth rate of the organism. Immobilized cell systems, however, can be operated at feed rates exceeding the growth rates of the micro-organisms. Dense cell populations and high productivities are two distinct advantages that immobilized cell systems have over continuous stirred tank systems.

Luong and Tseng (1984) developed a two-stage fermentation process for continuous ethanol production by immobilized cells of *Zymomonas mobilis*.

About 90–92 g/l of ethanol was produced with a 4-hour residence time. Entrapped cells of *Zymomonas mobilis* have a capability to convert glucose to ethanol at 93% of the theoretical yield. The application of a repeated fed-batch ethanol fermentation employing immobilized yeast cells accompanied by an activated carbon extraction system has been investigated to determine the effect of product removal on product yield (Lee and Wang, 1982). The maximum ethanol productivity achieved in this system was 25 g/l-h. Glucose was converted to ethanol by calcium alginate-entrapped *Saccharomyces cerevisiae* NRRL Y 2034 cells in continuous and static repeated batch fermentations (McGhee, Julian and Detroy, 1982). The continuous fermentations were better than the static ones in producing maximum ethanol yields (5·11 g/10 g of glucose) over extended time periods. Immobilized cells of *Zymomonas mobilis* were used for the conversion of glucose to ethanol in batch and continuous fermentations. The specific ethanol productivity increased fivefold in the batch and tenfold in continuous mode (Rainina *et al.*, 1986).

A strong correlation between CO_2 evolution rate and ethanol production rate at the stationary stage was observed in continuous alcohol fermentations. Mota, Strehaiano and Gomma (1985) have shown that productivity and yield could be increased if substrate inhibition is removed. The fed-batch approach, therefore, seems to be a good technique to reach high productivity. Mota *et al.* (1987) devised a fed-batch control system to keep the glucose level at 20 g/l during alcohol fermentation. This increased the production of alcohol to 124 g/l, with a product yield of 0·47 (92% of the theoretical possible yield), and a biomass yield of 0·019.

Glucose to acetone and other solvents

The production of acetone and butanol (solvents) by *Clostridium acetobutylicum* during its fermentation of carbohydrates has been studied by numerous authors (BuLock, 1975; Spivey, 1978). Most studies have been concerned with establishing the metabolic pathways involved in the production of acetone and butanol or with the more practical problem of defining the most favourable combination of substrates and environmental conditions that support good solvent production. Gottschal and Morris (1981) observed that there was no solvent production when glucose or nitrogen was limited in a continuous culture of *C. acetobutylicum*, but a nominal amount of solvent was produced during fermentations of glucose in a defined minimal medium in a pH controlled batch culture.

C. acetobutylicum was grown in fed-batch cultures at different glucose feeding rates. The proportion of sugar converted to butanol and acetone increased with increasing the glucose feed rates. On the contrary, the conversion to butyric acid was highest at a slow glucose feed rate (Fond *et al.*, 1984). Taya, Ishii and Kobayashi (1985) described microbial butanol production by extraction fermentation in which the product is removed from broth during the fermentation by liquid extraction *in situ*. The same author also developed a fed-batch culture system in *C. acetobutylicum* to control the butanol concentration below the toxic level, using the volume of gas evolved

during the fermentation as a control parameter. Fond *et al.* (1986) studied the kinetics of acetone–butanol fermentations with *C. acetobutylicum*, in fed-batch culture mode, using glucose, xylose and a mixture of both sugars as the feed. Within the range studied, the final conversion yield of sugars into solvents always increased with the sugar feed rate.

Glucose to antibiotics

There have been many studies of fed-batch fermentation techniques to improve penicillin yields (Whitaker, 1980). Daily addition of nutrients improves penicillin yield; glucose feeds at 6·0 g/l/day were almost as effective as the use of other carbon sources (Moyer and Coghill, 1946). After these initial studies, a variety of techniques of batch, fed-batch and continuous feed have been tested, with the most attention being given to the carbon source in the medium. Glucose has most often been the best carbon source for the production of penicillin. A computer-aided methodology was described for on-line monitoring and control of cell growth in fed-batch penicillin fermentations using a semi-defined medium containing a corn steep liquor (CSL) concentration of 5–7 g/l (Mou and Cooney, 1983a). The authors used an experimental correlation between CO_2 production and growth in combination with a carbon-balancing equation. The computer programme calculated and controlled cell growth on a real-time basis throughout the fermentation. In this experiment, the glucose concentration in the broth was kept below 1 g/l. Mou and Cooney (1983b) broadened the practicality of on-line growth monitoring and control by application of the technique to fed-batch penicillin fermentations using high concentrations of CSL. By employing a calculation method together with a feedback control strategy, it was possible to control glucose feed rate and maintain a preselected growth rate in each production phase.

Bajpai and Reuss (1981) developed and used a substrate inhibition model to evaluate the effect of different glucose feeding policies upon penicillin productivity. Using a substrate inhibition model, Tayeb and Lim (1984) presented a new method for computing optimal feeding strategies to obtain various performance indices. They developed a computer simulation and optimization programme for the penicillin fermentation. The optimal glucose feed rate profiles produced 80 g penicillin in 131 h compared with a constant feed rate producing 78 g penicillin in 150 h.

During batch fermentations for the production of the macrolide antibiotic tylosin by *Streptomyces fradiae*, the maximum production occurred when the organism was in a growth phase. Vu-Trong and Gray (1982) used a microprocessor to control the feeding of monosodium glutamate and glucose in a cyclic on–off mode. The feed rate was adjusted to maintain a linear volumetric aeration rate for the tylosin fermentation and resulted in a 114% increase in tylosin compared with batch fermentations. During the production of tylosin, Gray and Vu-Trong (1987) found that a continuous, constant feed rate of glutamate, glucose and methyloleate, initiated during the period of high tylosin productivity, increased total tylosin production. Tylosin fed-batch

fermentations with glutamate and glucose being fed in a cyclic on–off mode with excess methyloleate increased final tylosin titres several-fold. By varying the amplitude and period of the feed cycles, it was found that maximum tylosin productivity occurred when the glutamate cycle amplitude was 600 mg/l and the glucose cycle amplitude reached 42·5 mg/l each 24 h.

Vu-Trong and Gray (1986) showed that cyclic feeding of the key nutrients allowed RNA and tylosin synthesis to continue during a period when such activities had ceased in control, batch cultures.

Suzuki, Yamane and Shimizu (1987) developed a pH-stat, multi-substrate feeding strategy for the production of the antibiotic thiostrepton. Production of the antibiotic at high concentrations was achieved by fed-batch culture of *Streptomyces laurentii*. During the fermentation, a mixture containing several substrates was fed in response to pH changes. When glucose had been exhaustively consumed, the pH increased immediately, and the multi-substrate solution, containing glucose, CSL, nitrogen and salts, was automatically supplied by an on–off controller. During fed-batch culture, glucose concentration was kept below 5 g/l by regulating the feed rate. By this method, 10·5 g/l of thiostrepton was produced by a total biomass concentration of 157 g/l.

Glucose to glycerol and sorbitol

Glycerol is non-toxic to the culture and does not significantly affect fermentation rates. High glycerol concentrations in the final broth of batch fermentations could be obtained by using high initial concentrations of glucose in the medium; however, this may decrease the fermentation rate. Glycerol fermentations require a high aeration rate. Improved results could be obtained by using the fed-batch method to add glucose progressively during the course of the fermentation. Button, Garver and Hajny (1966) obtained a 17% glycerol concentration in the final broth using the fed-batch method with *Torulopsis magnoliae* at pH 4·0. In this process the glucose was added for conversion to glycerol by a stationary cell population.

The commercial feasibility of the fermentation process, depends on the concentration of glycerol in the final broth. Vijaikishore and Karanth (1987) attempted a fed-batch mode of addition of carbon source and nutrients in a culture of the osmophilic yeast, *Pichia farinosa*. The nutrients were added in a powder form and the fermentation achieved a high concentration of glycerol (30%). The production of glycerol from molasses could be improved by adding 5% sodium carbonate, making the pH alkaline. Successive additions of sodium sulphite and sodium bisulphite as aqueous solutions or solids have also been proposed (Carhampton and Lilly, 1919; Cocking and Lilly, 1922).

Glycerol productivity in a batch fermentation was improved significantly in the conventional sulphite process by operating it under vacuum on continuous sparging with carbon dioxide (Kalle, Naik and Lashkari, 1985). In such a system, the competence of the yeast cell could be conserved for a long period, allowing the accumulation of glycerol up to 230 g/l in the medium, as long as glucose was fed. A continuous fed-batch vacuum fermentation system has been described for glycerol production from cane molasses by a conventional

sulphite process. A glycerol concentration of 80 g/l was achieved, which is twice that from vacuum batch process and four times that obtained without vacuum (Kalle and Naik, 1985).

Glucose to yeast biomass

The production of bakers' yeast from simple sugar has been studied mainly in aerobic batch and continuous culture experiments. Recently, a few investigations (Aiba, Nagai and Nishizawa, 1976; Wang, Cooney and Wang, 1977) have been made on fed-batch processes which are generally employed in the industrial production of bakers' yeast. In fed-batch cultures, molasses is added incrementally to the bioreactor. If the sugar is overfed, the formation of ethanol will decrease the cell yield, and this cannot be avoided even in the presence of excess oxygen. Low sugar feed rates, however, decrease biomass production. In order to optimize the productivity and cell yield of aerobic, fed-batch cultures of glucose-sensitive yeasts, it is important to analyse the growth behaviour when metabolism switches between total respiration and aerobic fermentation. The shift between respiration and aerobic fermentation has been studied in detail by Meyenburg (1969) and Leuenberger (1972) in continuous culture. Woehrer and Roehr (1981) used a highly instrumented computer-coupled bioreactor to investigate metabolic changes of *Saccharomyces cerevisiae* in the aerobic fed-batch systems which are applied to baker's yeast production.

During an experiment on fed-batch yeast fermentation, Ghoul, Ronat and Engasser (1986) observed that the growth of *Candida utilis* is optimal at a glucose level near 0·5 g/l, that maximizes the intracellular production of ethanol while maintaining a relatively fast growth rate. During fermentation, the glucose level was maintained constant at 0·5 g/l by an automatic on–off controller feeding a solution of 100 g/l glucose. In this system, the yeast concentration rises from 2 g/l to 14 g/l after 10 h and to 30 g/l after 18 h, without any appreciable extracellular or intracellular accumulation of ethanol.

Glucose to organic acids

Very little attention has been directed towards fed-batch or controlled feeding of glucose or other nutrients for the production of organic acids. In lactic acid production, repeated fed-batch has been proposed. During fermentations of *Clostridium barkeri* in glucose medium, Stadtman *et al.* (1972) and Haggstrom (1986) observed that the main end products were lactate and butyrate. In a glucose-limited chemostat culture, butyrate production was partly growth associated while acetate and lactate production were growth associated. Lactate was, however, only produced at a high dilution rate. By varying the glucose concentration in the inflowing medium, it was shown that lactate production was stimulated by a high feed rate of the carbon source (Haggstrom, 1986).

It has been reported that citric acid yields with *Aspergillus niger* improved with the addition of ammonia, ammonium hydroxide or ammonium carbonate

during a fermentation (Miles Laboratories Ltd, 1962). An increase of citric acid production was also found by extending the culture with sugar feed (Lockwood and Batti, 1965). An aerobic continuous stirred-tank bioreactor with cell recycle was used in preference to batch cultivation to produce citric acid from glucose with the yeast *Saccharomycopsis lipolytica*. The specific rate of total acid production was 0·045 h^{-1}, yield on glucose was 0·86 g/g and volumetric productivity was 1.16 g acid/l-h. The effluent acid concentration was 75 g/l (Enzminger and Asenjo, 1986). Chun, Sung and Park (1985) isolated a mutant strain of *Candida lipolytica* which increased production from 72 g/l to 85 g/l of citric acid. A semi-continuous cell recycle system was used to prolong the effective production phase, to minimize product inhibition and to shorten the lag phase. The productivity of semi-continuous cell recycle was 0·79 g/l-h while that of a comparable batch system was 0·53 g/l-h (Chun, Sung and Park, 1985).

Acetic acid is an important feedstock for many chemicals such as vinyl acetate monomer, cellulose acetate, acetic acid esters and acetic anhydride. Production of acetic acid via fermentation using renewable biomass feedstock has been studied by numerous researchers (Ljungdahl, 1983). When low-value commodity chemicals such as acetic acid are produced by fermentation, conversion yield is an important factor for successful commercialization. Schwartz and Keller (1982) conducted fed-batch fermentations at various pH values, using several strains of *Clostridium thermoaceticum*. Although continuous fermentations have reportedly been conducted, few data are available in the literature. Busche, Shimsick and Yates (1982) stated that acetate was produced in a continuous fermentor at a concentration of 15–18 g/l and at a yield greater than 85% over a period of 26 000 h, but no actual data were reported. During batch fermentations with *Clostridium thermoaceticum*, using automatic pH control, the maximum specific growth rate was 0·14 h^{-1} with a low yield of acetic acid. Continuous fermentations at high dilution rates indicate that the maximum specific growth rate was well above 0·17 h^{-1} when fresh feed medium was used (Sugaya, Tuse and Jones, 1986). Acetate yield in continuous fermentations was about 77% of theoretical yield, or 2–3 moles of acetic acid per mole of glucose.

One of the most important aspects in the control of aerobic fermentations of *Saccharomyces cerevisiae* is the Crabtree effect or catabolic repression due to an excess of glucose, with ethanol production. In many cases, the respiratory quotient, the ratio between CO_2 production and O_2 consumption, can be a good indicator of cellular metabolism. This led to control strategies for fed-batch production with control of the respiratory quotient (Aiba, Nagai and Nishizawa, 1976). This method cannot, however, be extended to all experiments as different kinetic behaviour can be observed with some strains during batch, continuous and fed-batch cultivation (Pons, 1984; Rajab, 1984). During production and assimilation of acetate from glucose and ethanol by *Saccharomyces cerevisiae*, Pons, Rajab and Engasser (1986) found that a respiratory-quotient-based policy for fed-batch control cannot be used for yeast strains with high acetate production because of the competition between O_2 over-consumption (due to acetate production) and CO_2 over-production (due to ethanol production).

Itaconic acid and methylene succinic acid are important intermediates in polymer production and are used to improve fibre properties such as dying characteristics. Itaconic acid is produced by submerged cultures of *Aspergillus terreus*, *A. itaconicus* and *Ustilago azea* (Miall, 1978), and by a number of *Candida* species (Kawamura, 1983). Recently, Horitsu *et al.* (1983) reported the production of itaconic acid by polyacrylamide-gel-entrapped *A. terreus* mycelium from 6% glucose at a yield of 60 mg/l employing 40 g of biocatalyst gel in a continuous bioreactor. Kautola *et al.* (1985) immobilized the spores of *Aspergillus terreus* in calcium alginate beads or alternatively, the fungal mycelium was immobilized either on Celite R-626 or in agar gel cubes. The biocatalyst was employed both in repeated batch and in continuous column reactors to produce itaconic acid from D-xylose and D-glucose. In a repeated batch fermentation with xylose at 60 g/l, mycelium immobilized in agar gel had a productivity of 0·12 g/l-h, and mycelium immobilized in calcium alginate gel had a productivity of 0·06 g/l-h. The best immobilized biocatalyst system used employed Celite R-626 as a carrier. The productivity was 1·2 g/l-h from glucose and 0·56 g/l-h from xylose (both at 60 g/l) in a continuous column operation.

Glucose to amino acids

In order to increase amino acid production, several authors have used the addition of a carbon source, nitrogen sources, or surface active agents, during the fermentation. Substrate addition was tried during the early stages of glutamic acid production in Japan. Molasses has been used by Miescher (1962) during glutamic acid fermentations. Glucose and anthranilate, or glucose, indole and anthranilate were used for the production of tryptophan in a fed-batch culture.

The use of genetically engineered organisms offers an interesting possibility to overcome the problem of low productivity and high cost of L-threonine obtained by traditional fermentation procedures. However, these strains are unstable and lose their plasmid during growth unless they are kept under selective pressure (Imanaka, Taunerkawa and Aiba, 1980; Dwivedi, Imanaka and Aiba, 1982). It is necessary to solve this problem before plasmid-bearing strains can be used satisfactorily in the commercial production of metabolites. Recently, Nudel *et al.* (1987) tried to produce threonine by a recombinant *Escherichia coli* which over-produced threonine in comparison to the original strain. Batch culture experiments were performed with 3%, 4% and 6% glucose added at the initial stage or in a discontinuous feed. During the fed-batch experiment it was observed that the plasmid were stable and there was an increase of the production of threonine (Nudel *et al.*, 1987).

In recent years, most glutamate has been produced by fed-batch fermentation processes (Kim and Ryu, 1982). The use of either immobilized whole cells or cell-free homogenate to reduce process cost appears to be very attractive. For the purpose of reducing the energy and raw materials required by glutamate fermentations, an immobilized whole-cell system was designed and its performance in a continuous reactor system was evaluated (Kim and Ryu, 1982). The continuous production of glutamate was carried out in an

air-stirred fermentor. The performance of the continuous whole-cell reactor system was evaluated by measuring glutamate productivity for a period of 30 days. It was found to be far superior to the performance of conventional batch reactors systems using free cells.

Glucose to polysaccharides

Polysaccharides of microbial origin are receiving increasing attention as possible replacements for natural gums for use as emulsifiers, stabilizers, thickeners and gelling agents. Phillips, Pik and Lawford (1983) studied biosynthesis of curdlan in batch and continuous cultures of *Alcaligenes faecalis* var. *myxogenes*. Curdlan is an unbranched polysaccharide composed of D-glucose linked in a β–(1–3) chain (Saito, Misaki and Hazada, 1968). Curdlan production is associated with the post-stationary phase of a nitrogen-depleted aerobic batch culture. The same author developed a two-stage continuous process for curdlan production. The specific rate of curdlan production was four times higher in post-stationary batch cultures than in single-stage continuous fermentations. During the production of extracellular polysaccharide by *Zoogloea ramigera*, Norberg and Enfors (1982) found that the yield coefficient of exopolysaccharides on the basis of consumed glucose was in the range of 55–60% for batch cultivation with an initial glucose concentration of 25 g/l. An extracellular polysaccharide concentration of up to 38 g/l could be attained if glucose, nitrogen and growth factors were added during fed-batch culture.

Glucose to monoclonal antibodies and growth factors

Reuveny *et al.* (1985, 1986) and Velez *et al.* (1986) described some factors which affect the growth and viability of hybridoma cells and the production of monoclonal antibodies (McAb) in batch fermentations. After several studies (Reuveny *et al.*, 1986), it became apparent that McAb yield increased considerably when cells were maintained in a viable condition for an extended period of time. This became a major strategy for optimizing antibody production in fermentors. Cultural conditions which prolonged cell viability and increased antibody production included optimal maintenance of the dissolved oxygen level, and feeding cultures a combination of glucose and glutamine on a daily basis. It was also observed that accumulation of ammonium ion, as a waste product from glutamine metabolism could decrease cell viability and antibody yield.

Several other investigators have studied McAb production using systems for continuous or perfusion feeding of hybridoma cells. Seaver *et al* (1984) compared production in batch culture with a system employing membrane-entrapped cells fed by perfusion. Fazekas de St Groth (1983) and Boraston, Thompson and Birch (1984) compared batch and continuous cultures. Tolbert and Feder (1983) investigated McAb production in stirred vessels in which cells were fed recycled medium in a perfusion system.

Velez *et al.* (1986) have previously shown that in one type of hybridoma, the

average production of McAb in batch culture was 15 mg/l of culture per day. Reuveny *et al.* (1986) increased the productivity to 27 mg/l per day in a fed-batch culture when increments of fresh medium were added daily. In a semi-continuous culturing system where medium was added twice daily and an equivalent amount of the culture was harvested, the productivity was raised to 34 mg/l per day. Harvesting the culture from the semi-continuous system and holding it for an additional 4 days in a second-stage vessel, where the cells were periodically fed with glucose and glutamine, increased the yield to 62 mg/l per day. The highest productivity of all was found using a perfusion mode of propagation in which cells were continuously fed on one side of a filter screen and cell-free supernatant containing the antibody and waste products was removed continuously from the other side. This resulted in a steady-state concentration of cells at $2{\cdot}2 \times 10^7$/ml and a daily productivity of 660 mg McAb/1.

During studies of the effect of organic nitrogen and glucose on the production of recombinant, human insulin-like growth factor (IGF-1) in high cell density *E. coli* fermentations, Tsai *et al.* (1987) used single and dual fed-batch fermentation processes in a 10 l fermentor. In the dual-feed system, organic nitrogen was delivered at a higher rate (50 g/h) than in single-feed systems (5 g/h). The dual-feed system resulted in a significant increase in IGF-1 yield, 30 mg IGF-1/g dry cell weight obtained in the single-feed system. An increase of glucose supply rate from 17 g/h to 80 g/h during the induction period did not enhance the IGF-1 gene expression in the dual-feed process, which was mainly dependent on a high level of organic nitrogen and an appropriate level of glucose in the medium.

In *E. coli* the multicopy plasmid pBR-322 is well known and has been used frequently. The stability of the plasmid in fed-batch (Mizutani *et al.*, 1986) and continuous (Mizutani *et al.*, 1985) cultures has been studied for the production of various gene products. In fed-batch cultivation of *E. coli* harbouring a runaway replication plasmid pCP3, copy number can be amplified 40-fold in the middle of the logarithmic growth phase by shifting the temperature and feeding intermittently with glucose, yeast extract, threonine, leucine and tryptophan.

SUCROSE

Glucose is a rather expensive raw material. To investigate the potential of *Zymomonas mobilis* for future usage in the conversion of sugar-cane juice to ethanol, the fermentation pattern of the sucrose to ethanol conversion was established. Lyness and Doelle (1981) observed the fermentation pattern of the sucrose-to-ethanol conversion process by *Zymomonas mobilis*. General patterns of sucrose fermentation by two strains of *Zymomonas mobilis*, designated Z7 and Z10, were established using sucrose concentrations from 50 to 200 g/l. High sucrose-hydrolysing activity in strain Z7 led to glucose accumulation in the medium at high sucrose concentrations. The high glucose concentrations at the termination of the fermentation were claimed to be due to the limitation of metabolic activity under these conditions (Cromie and Doelle, 1980). Ethanol production and fermentation time depends on the rate of

catabolism of the products of sucrose hydrolysis, glucose and fructose. Doelle and Greenfield (1985) studied the fermentation pattern of *Zymomonas mobilis* at high sucrose concentrations. At a high concentration of sucrose (200–400 g/l) during fermentation, the efficiency of sucrose hydrolysis dropped from 94% to 78% whereas the efficiency with which hydrolysed products were converted to ethanol decreased from 94% to 43%. The ethanol yield was relatively constant for final sucrose concentrations which lay between 80 and 132 g/l.

Suntinanalert, Pemberton and Doelle (1986) isolated a fructose-negative mutant of *Zymomonas mobilis* which was unable to use fructose as a sole carbon source. This strain was able to cleave sucrose to glucose and fructose and then ferment only glucose to ethanol while accumulating fructose at close to the theoretical yield. Under controlled fermentation conditions, sucrose was converted to ethanol plus high-purity fructose syrup in the fermentation process. Lee *et al.* (1981) studied the kinetics of ethanol production by *Zymomonas mobilis* on fructose and sucrose media. *Z. mobilis* strain ZM4 was grown on 250 g/l fructose and sucrose media in batch culture and on 100 and 150 g/l sucrose media in continuous culture. With fructose, a significant reduction of growth rate and cell yield was apparent although other kinetic parameters were similar to those previously reported for the fermentation of glucose. With sucrose, the major differences were a reduction in ethanol yield and a linear final increase in ethanol concentration. Ethanol inhibition of sucrose metabolism occurred at relatively low ethanol concentrations compared with those inhibiting glucose metabolism (Lee *et al.*, 1981). During the continuous fermentations of sucrose by *Z. mobilis*, Viikari and Linko (1986) observed that the rather low levels of monomeric sugars in the broth prevented the formation of sorbitol and oligomers, whereas the production of levan was increased compared with that in batch fermentations. The overall ethanol production rate was limited by the uptake rates of glucose and fructose.

Environmental manipulations affecting the growth rate, such as fed-batch or continuous culture, are often used to deregulate enzyme synthesis. Carbon-limited growth frequently stimulates the production of catabolite-repressed enzymes. Manipulations such as these would be expected to improve maltase (EC 3.2.1.20) production. The production of maltase, an inducible and repressible catabolic enzyme in *Saccharomyces italicus*, was studied and compared in batch, fed-batch and continuous fermentations (Schaeffer and Cooney, 1982). A mutant strain of *S. italicus* was isolated that can grow in sucrose, which is a non-inducing substrate that is hydrolysed by maltase. *S. italicus* does not possess invertase (EC 3.2.1.26) and will not normally grow in sucrose. Maltase production by this mutant was studied during growth on sucrose in batch and continuous culture, and a marked improvement in enzyme production was observed. The specific activity of maltase produced by this mutant was more than twice that of the wild type; 2210 and 1370 U/g of cells for the mutant versus 890 and 510 U/g of cells for the wild type in batch and continuous culture respectively.

During extracellular polysaccharide production by *Xanthomonas cucurbitae*, Baig, Qadeer and Shamsi (1985) used a sucrose, salts medium in batch

stirred fermentors. The authors observed that the fermentation patterns of microbial polysaccharides were greatly improved by the addition of cotton seed meal, as a source of amino acids, and other growth factors.

STARCH

Starch, one of the cheapest carbon sources, is found in corn and grains such as barley, wheat, and in many agricultural by-products. Potatoes and other roots such as cassava also contain large amounts of starch. α-Amylase, 1,4-α-D glucan glucanohydrolase (EC 3.2.1.1.), is used to dextrinize starch and glucoamylase, 1,4-α-D glucan glucohydrolase (EC 3.2.1.3), converts starch into glucose. Yeast, or a suitable bacterium such as *Zymomonas mobilis*, may then be employed to ferment glucose into ethanol (Prince and Barford, 1982). An alternative process does not employ added enzymes but uses an enzyme-producing micro-organism. A conversion of starch from potato, sweet potato or cassava into ethanol can be accomplished using a mixed culture of *Rhizopus niveus* and yeast (Sreekantiah and Satyanarayana Rao, 1980). *Aspergillus awamori* and *Saccharomyces cerevisiae* have been used to convert cassava root flour into ethanol. A continuous two-stage fermentation gave an improved production of ethanol (del Rosario and Wong, 1984). Gibson and Westby (1983) found that, during ethanol and feed production, continuous fermentation increased alcohol production at low dilution rates and thereby reduced the required fermentation volume.

The relative concentrations and nature of carbon and nitrogen sources are important factors in medium composition for amylase production (Coleman and Grant, 1966; Markkanen and Enari, 1972). Extracellular enzymes produced by *Bacillus subtilis* in fermentations are synthesized during the stationary growth phase. Certain nutrients may already be exhausted from the medium at this stage. Feeding of the carbohydrate nutrients at this stage enhances the production of enzymes (Johnson, 1971; Heineken and O'Connor, 1972). Increased carbohydrate concentrations at the beginning of the fermentation do not have the same effect because of the metabolic control system of the organism.

Markkhanen, Reinwall and Linko (1976) showed that starch feeding is a method to control the level of carbohydrates in the medium. In this study, slow feeding of starch after the exponential growth phase resulted in increased yield of β-1,4-glucanase (from 450 units/ml to 500 units/ml). Starch feeding considerably extended the active production phase, although the production continued at a lower rate. The higher yield of enzymes makes the feeding of starch profitable. Fed-batch cultures of *Bacillus megaterium* AJ3355 with two kinds of feeding mode, either constant or exponentially increasing feed rates, gave almost the same enzyme yield when operated optimally (Yamane and Tsukano, 1977). The yield of the enzyme was 3·6 times that in batch culture when a modified exponential fed-batch culture was used.

CELLULOSE

Cellulose is the most abundant renewable biopolymer produced in the world. The large amount of cellulosic products produced by modern societies, and subsequently generated as wastes, causes serious disposal problems. Microbial fermentation has been studied as a possible solution for disposal of these wastes as well as a means of generating valuable by-products such as food and food supplements. Miller and Srinivasan (1983) observed the production of SCP from cellulose by *Aspergillus terreus* in semicontinuous cultivation studies. They found that 90% of the biomass was withdrawn at the end of the growth cycle and that 84% of added cellulose was utilized, with the biomass containing 32% crude protein. In comparison with continuous cultivation of *A. terreus*, it was found that 78–84% of cellulose consumption occurred over growth temperatures ranging from 35 to 45°C.

Agricultural residues such as corn stover or wheat straw are attractive because they are cheap sources of polysaccharides and the materials are more abundant than the agricultural products from which they are obtained (Detroy and Hesseltine, 1978). Cellulose is the main polysaccharide which can be biodegraded into ethanol. The conventional techniques to achieve this bioconversion include the acid or enzymatic hydrolysis of cellulose followed by fermentation of soluble sugar into ethanol (Wilke and Blanch, 1979).

An alternative process, from an economical and technical point of view, may be the direct bioconversion of cellulose to ethanol by a bacterium such as *Clostridium thermocellum* (Wang *et al.*, 1978). The feasibility of producing ethanol in a continuous system from cellulose using *Clostridium thermocellum* was investigated (Zertuche and Zall, 1982). During continuous fermentation at 60°C and pH 7·0 using 1·5% and 3·0% pure cellulose as a limiting substrate, the maximum ethanol concentration was 0·3% and 0·9% respectively. It was therefore concluded that cellulose could be degraded continuously in a system with *C. thermocellum* for the production of ethanol. Eleven alternative fermentation schemes for ethanol production were compared (Maiorella, Blanch and Wilke, 1984) on an identical basis using a consistent model for yeast metabolism. Molasses and cellulose hydrolysate have also been considered as feeds.

Cultures of *Clostridium thermocellum* permit ethanol to be obtained from cellulose in one step (Wang *et al.*, 1978; Wiegel, 1980). However, it is very desirable to increase the conversion rate and the final ethanol conversion (Ng, Ben Bassat and Zeikus, 1981; Johnson, Reese and Demain, 1982; Guiliano, Asther and Khan, 1983). An increase in cellulose concentration can improve the cellulose hydrolysis rate as shown by Gordon (1981), Zertuche and Zall (1982) and Volfova *et al.* (1985). Wang *et al.* (1978) attempted to increase the final ethanol concentration using fed-batch fermentation in co-culture. Fed-batch fermentation of *Clostridium thermocellum* gave improved conversion rates and a high ethanol concentration in the broth; 8·5 g/l of ethanol was obtained in 60 h with a maximum ethanol productivity of 1·0 g/l-h (Spinnler, 1986).

The addition of ammonia during cultivation of *Trichoderma viride* in the

medium containing a high concentration of cellulose gave a useful enhancement of cellulase (EC 3.2.1.4) production in batch cultivation (Sternberg and Dorvai, 1979). High concentrations of cellulose are known to interfere with oxygen transfer rates (Gottvaldova, Kucera and Podrazy, 1982). The cultivation of *Trichoderma viride* was also derived in a fed-batch system using soluble sugars or cellulose as additional carbon sources (Kirk, 1975; Ghose and Sahai, 1979). In this method, the cellulase production was enhanced by 30% in comparison with the batch cultivation process. Gottvaldova, Kucera and Podrazy (1982) observed that production of cellulase by *Trichoderma viride* had been increased remarkably by means of a fed-batch fermentation with the addition of cellulose as the carbon source and ammonia as a nitrogen source during cultivation. The double carbon/nitrogen fed-batch procedure yielded 80% more cellulase than control (batch). In cellulose fed-batch without the addition of ammonia, the yield of cellulase was higher than control by 40%. Sahai and Ghose (1978) showed that pH regulation with NaOH increases cellulase production. During cellulase production by *Trichoderma* sp. the most important factor, apart from the carbon source supplementation, appears to be the nitrogen source, for which ammonia is superior to urea (Gottvaldova, Kucera and Podrazy, 1982). Substrate repression was observed in most cases when a high concentration of substrate was used for production. This could be avoided by adding the substrate stepwise when substrate quantity decreased from the optimal concentration. During cellulase production by *T. reesei* with Solka floc as a carbon source, an increase of cellulase production was observed (Hendy, Wilke and Blanch, 1982). The major benefit of fed-batch operation is the ability to achieve high enzyme titre and high productivity concurrently. An added advantage is reduced production of cell mass. The mycelium does not attain the high density found during high-substrate batch growth. Fed-batch operation also ensures adequate agitation and aeration with low power input.

The conversion of xylose to ethanol by yeast has received much attention recently. Processes such as the two-stage dilute acid hydrolysis of hardwood for ethanol production (Beck and Strickland, 1984) include fermentation of both xylose-rich as well as glucose-rich hydrolysate streams to ethanol. The recent work of Jeffries, Fady and Lightfoot (1985) illustrated increased rates of xylose utilization through periodic addition of glucose to a xylose fermentation using *Pachysolen tannophilus*. These experimental results suggest that the glucose additions repress respiration of ethanol by the yeast, a cause of reduced ethanol yields from xylose under the semi-aerobic conditions at which conversion of xylose to ethanol is optimum (Maleszka and Schneider, 1982). Beck (1986) observed that intermittent feeding of cellulose hydrolysate to hemicellulose hydrolysate of hardwood resulted in greater yield of ethanol using *Pachysolen tannophilus* than batch fermentations of either hydrolysate alone or as a mix. Conversion efficiencies as great as 0·40 g ethanol/g sugar were achieved in this fed-batch mode.

Yu, Chan and Saddler (1985) investigated the conversion of lignocellulosic substrate to butanediol and ethanol by a co-culture approach. Using *Clostridium thermocellum* and *Klebsiella pneumoniae*, as a sequential culture, resulted in the rapid accumulation of fermentative products, including

butanediol, ethanol and acetoin. Chung and Lee (1985) observed that continuous fermentation with cell recycle was superior to batch fermentations for ethanol, using acid hydrolysate of wood cellulose as a carbon source. During single-cell protein production by *Candida utilis*, Gonzalez-Valdes and Moo-Young (1981) observed that mild acid hydrolysis of corn stover is one of the most economically attractive possibilities to date. High efficiency of substrate utilization and protein productivity were obtained with yeast strain CM1–23311, studied in batch and continuous systems.

METHANOL

Microbial utilization of non-carbohydrate carbon-energy sources such as *n*-paraffins and methanol for the production of SCP and biochemicals has been gaining interest all over the world. As an industrial fermentation substrate, methanol has several advantages compared with other hydrocarbons. Methanol is available at a low cost, has high purity and is completely miscible with water. Methanol inhibits the growth of micro-organisms and prolongs the lag phase of growth even at relatively low concentrations in industrial batch fermentation processes. An exponential increase of growth lag with increased methanol concentration was found by Amano *et al.* (1983).

The practice adopted to overcome such difficulties is the fed-batch culture, in which a growth-limiting substrate is fed continuously or intermittently (Yokota, Sugimoto and Abe, 1974) into a batch fermentor. In fed-batch cultures with a constant feed rate, exponential growth was observed at an early period, but growth became linear later due to limitations of substrate supply (Yoshida, Yamane and Nakamoto, 1973). White (1954) suggested that during the production of baker's yeast, the feed rate of the growth-limiting substrate must increase in proportion to the exponential growth of the micro-organisms. It will then be possible to maintain a high rate of growth for a longer period of time while keeping the substrate concentration in the culture broth at a constant low optimal level. Although there have been no studies on this type of culture from an engineering standpoint, studies were performed by Yamane, Kishimoto and Yoshida (1976) on the exponential fed-batch culture of a strain of methanol-assimilating bacteria which were carried out with the use of a specially designed exponential feed-rate programmer. Experimental results agreed reasonably well with mathematical predictions. An attempt was made to produce vitamin B_{12} by a fed-batch process with an exponentially increasing feed rate. An increase in production compared with conventional batch culture was obtained. Yamane and Hirano (1977) determined a suitable feeding rate for a fed-batch culture of the methanol-utilizing micro-organism *Corynebacterium* sp x G, a vitamin B_{12} producer. Using the fed-batch system, the final biomass and vitamin B_{12} concentration reached 16·3 g/l and 880 mg/l respectively (Andriantsoa *et al.*, 1984).

Pseudonomas sp. K was cultivated to produce large amounts of poly-hydroxybutric acid (pHB) from methanol by means of microcomputer-aided, fully automatic fed-batch culture technique (Suzuki, Yamane and Shimizu, 1986 a,b). During this study, the dissolved oxygen concentration was

also controlled by means of a DO-stat (Yano, Kobayashi and Shimizu, 1980). A microporous teflon tubing sensor combined with a flame ionization detector and controller have been employed in fed-batch cultures for the control of the feeding of volatile carbon sources (Yamane, Matsuda and Sada, 1981a, b). The ammonium concentration may be controlled by pH-controlled ammonia feeding, with mineral ion supplementation based on stoichiometrical estimation (Suzuki *et al.*, 1985). A simple on-line computer control strategy based on dissolved oxygen level has been developed to control methanol addition during a fermentation of the methylotroph *Pseudomonas* AMI. This strategy has led to significant and reproducible improvements in the performance of the fermentation (Renard, Mansouri and Cooney, 1984).

Huang and Chu (1981) reported automatic control of substrate feeding by detecting either the ethanol vapour in the exhaust gas or measuring the dissolved O_2 concentration in the cultivation broth where ethanol was used as a sole carbon source. Controlled fermentations with substrate concentration control of substrate feeding were compared with those based on dissolved O_2 concentration control of substrate feeding. Direct substrate control yielded a biomass of 64 g/l, while open-loop, O_2-based control yielded only 55 g/l. Although the ethanol sensor used in this study did not exhibit a fast response, the performance of the automatic control system for fed-batch culture operated satisfactorily in this cultivation system.

Yoshida, Yamane and Nakamoto (1973) observed that during fed-batch, hydrocarbon fermentations of *Candida tropicalis* with colloidal emulsion feed, the cell yield during the linear growth phase, where growth was limited by the supply of the substrate, was much higher than the yield during the exponential growth phase, indicating a substrate concentration effect on cell yield from substrate.

Several studies were made in recent years on the optimization of fed-batch cultures (Fishman and Biryukov, 1974; Ohno, Nakanishi and Takamatsu, 1976; Yamane *et al.*, 1977) in which optimization problems were solved in terms of rate of addition of substrate throughout the time course of fed-batch cultures. In many cases of non-growth associated product formation, however, it is difficult to construct a model that adequately describes the complex nature of the problem. Kishimoto, Yoshida and Taguchi (1980) used regression analysis (Dunn and Clark, 1974) during the optimization of glutamic acid production by *Brevibacterium divaricatum* NRRL2311 with ethanol feeding. The results provided an effective solution to the optimization problem.

Yano, Kobayashi and Shimizu (1978) observed that high concentrations of biomass (85 g/l) were obtained in fed-batch cultures of *Protaminobacter ruber*. Similarly, Mori *et al.* (1979) obtained 125 g/l of *Escherichia coli* biomass in a fed-batch culture. A process control system with a microcomputer was constructed to maintain nutritious conditions, including dissolved oxygen concentration, at an optimal value (Yano, Kobayashi and Shimizu, 1985). In this system, the author obtained a very high concentration of biomass (268 g/l) of *Candida brassicae* after 28 h when the dissolved oxygen concentration was controlled at 0·12 ppm, simultaneously with the feeding with ethanol and ammonium.

To achieve high cell-mass concentrations in fed-batch culture, while maintaining optimal culture conditions, requires that all nutrients (carbon, nitrogen, oxygen and mineral ions) necessary for cell growth must be fed to the culture. Oxygen concentration control has been automated by the DO-stat technique with the aid of microcomputers for rapid date processing and effective feedback control (Yano, Kobayashi and Shimizu, 1980). To achieve a high concentration of cell mass, it is necessary to supply substantial amounts of mineral ions for growth. The requisite mineral salts may be added intermittently into the broth during cultivation according to estimates of deficits (Yano *et al.*, 1980). Sudden changes in the ion concentration may also change metabolic activities, and could inhibit microbial growth. To overcome these disadvantages, Suzuki *et al.* (1985) have developed an automatic continuous feeding schedule for minerals that is designed to maintain relatively constant mineral concentrations in a fed-batch culture up to high cell concentrations. Equations have been derived to calculate the required concentrations of each mineral ion in the mineral feed solution on the basis of mass balance principles. The mineral solution was supplied automatically, linked either with ethanol feed or ammonia water feed. The mineral supplementation gave better results coupled with ethanol feed than with ammonia feed, and resulted in relatively stable concentrations of K^+, Mg^{2+}, Na^+, Fe^{2+}, Zn^{2+}, CO^{2+}, Cu^{2+}, Mn^{2+}, NH_4^+, PO_4^{3-} and SO_4^{2-}.

NITROGEN SOURCES

Nitrogen sources to enzymes

Protease production by *Bacillus subtilis* was examined by Guntelberg (1953–1956). It was found that an excess of glucose and the control of the nitrogen level in the medium caused an increase in protease production. Recently, a number of publications have appeared on the synthesis of exoenzymes, many of which are reviewed by Schaeffer (1969). Coleman (1967) reported that amylase and protease of *Bacillus subtilis* are synthesized in the stationary phase of growth. Mandelstam (1967) postulated catabolite repression of only the enzymes directly or indirectly responsible for producing protease. It was observed by Heineken and O'Connor (1972), during continuous culture studies, that limiting growth with glucose was advantageous for α-amylase synthesis, while nitrogen-limited growth was advantageous for the synthesis of protease.

The production of protease depends on the availability of both nitrogen and carbon sources in the medium; either an excessive or an insufficient nitrogen concentration may cause an inhibition of the biosynthesis of protease by species of *Bacillus* (Cherdyntseva, Rozinkov and Egorov, 1982; Cohen, Morris and Drucker, 1975; Himelbloom and Hassan, 1986; Kole, Draper and Gerson, 1988). Jensen (1972) found enhanced protease production by the extension of stationary phase in a two-stage fermentation of *Bacillus*. During batch fermentations of *Bacillus subtilis* NC1B 8054, Kole, Draper and Gerson (1988) also observed maximum protease production during the stationary phase of

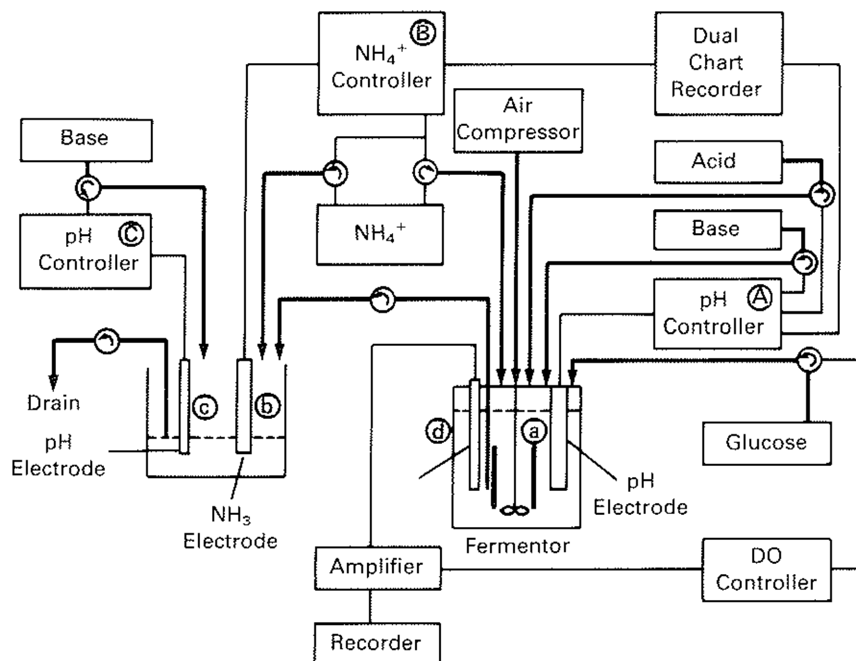

Figure 2. Ammonium and glucose controlled fermentation system. Controller A maintains the pH in the fermentation vessel. Controller B measures the ammonium concentration sensed by the electrode in the measurement vessel and controls ammonium concentration by pumping ammonium sulphate (25 mmol/dm^3) into the measurement vessel. Controller C maintains the pH in the measurement vessel at pH 11·0 by adding NaOH (4 mol/dm^3) to satisfy the requirements of the ammonia electrode. The dissolved oxygen electrode measures the DO level which activates the pump to add glucose to the fermentor. The concentration of the glucose feed solution was 250 g/dm^3.

growth. By controlling the ammonium level at 5 mM, a 1·5 times increase in the production of protease was obtained compared with uncontrolled, batch fermentations (Kole, Draper and Gerson, 1988). Simultaneous control of ammonium at 5 mM and glucose at 0·15 g/l, using a control based on an ammonium electrode for ammonium feed and an oxygen electrode for glucose feed (*Figure 2*), doubled protease production compared with fermentations having only an ammonium control system, and tripled protease production compared with uncontrolled batch fermentations. The protease yield on glucose and protease yield on ammonium were both increased in fermentations with simultaneous glucose and ammonium control (Kole, Draper and Gerson, 1988).

Stepwise addition of ammonia during fed-batch cultivation of *Trichoderma viride* in media containing a high concentration of cellulose (8%) gave an enhancement of cellulase production in batch cultivation (Sternberg and Dorvai, 1979). The production of cellulase by *Trichoderma viride* has been increased by 80% by means of a fed-batch fermentation, pH controlled with the

addition of cellulose as a carbon source and ammonia as a nitrogen source, during cultivation (Gottvaldova, Kucera and Podrazy, 1982).

Nitrogen source to amino acids

To increase the yield during amino acid fermentations, additions of both urea (Su and Yamada, 1960; Miescher, 1962; Oki *et al.*, 1968) and ammonium salts (Kinoshita *et al.*, 1958, 1959; Miescher, 1962; Yamamoto *et al.*, 1972) have been used a number of times both to maintain nitrogen concentration and to control pH. Slow feeding has made it possible to use substrates which would be inhibiting for glutamic acid production if used in high concentrations at the beginning of a batch culture. For instance, the feeding of ammonium benzoate increased the production of glutamic acid (Yamamoto *et al.*, 1972). The Kyawa Fermentation Company Ltd has used ethanol-urea feed and ethanol ammonium feed for lysine production. Continuous feeding of a 20% urea solution to control pH increases isoleucine production by *Arthrobacter globiformis* (Sugisaki, Yamamoto and Koyama, 1970).

A nitrate control system has been developed for the maintenance of stable nitrate concentrations throughout fed-batch fermentations of *Corynebacterium glutamicum* (Kole, *et al.*, 1986). This feedback control system was based on the use of a nitrate-ion-selective electrode to monitor the nitrate levels in the fermentor, and an automatic controller to activate the nitrate feed pump. Kole *et al.* (1986) observed that the optimal controlled nitrate concentration for growth and glutamic acid production by *Corynebacterium glutamicum* was 0·05 M, far below the level that would be appropriate for the initial concentration in a batch fermentation.

Nitrogen sources to antibiotics

Ammonia has been used as a nitrogen source and for controlling the pH during penicillin fermentations (Hosler and Johnson, 1953; Hockenhull and MacKenzie, 1968; Wright and Calam, 1968; McCann and Calam, 1972). Maximum yields of streptomycin (8500 mg/l) in 5 l fermentors were achieved (Singh, Bruzelius and Heding, 1976) by the addition of glucose and ammonium sulphate, at different feed rates, during fermentations. In oxytetracycline production, the intermittent addition of carbon and nitrogen sources during growth (Orlova and Pushkina, 1972) prolonged the period of biosynthesis of the antibiotic. Carbon, phosphorus and nitrogen concentrations were maintained at constant levels in tetracycline fermentation by making additions every 3–6 h, increasing tetracycline yields by over 500%. However, slow feeding of glucose, sucrose, fructose, ammonium salt, urea, and a vitamin mixture during novobiocin production did not stimulate production (Smith, 1956). Periodic addition of a complex nitrogen source in association with slow feeding resulted in lower yields of candidin production than in fermentations with only slow feeding of glucose (Martin and MacDaniel, 1974).

Nitrogen sources to citrate and organic acids

Addition of ammonia, ammonium hydroxide or ammonium carbonate during the fermentation greatly improved citric acid production by *Aspergillus niger* (Miles Laboratories, 1962). In this case, it was important to add the additional nitrogen to the medium when the rate of citric acid production was beginning to decrease from the maximum. An increase in yield was also claimed by use of a combined feed of ammonia and concentrated sugar syrups.

An optimized batch fermentation process for the conversion of cattle feedlot waste filtrate, supplemented with cheese whey, into a nitrogenous feed supplement for ruminants is described (Erdman and Reddy, 1986). Ammonium hydroxide was added from time to time to the fermentation not only to maintain a constant pH but also to produce the ammonium salts of organic acids, compounds which have been shown to be valuable as nitrogenous feed supplements for ruminants.

During the butanol-acetone process, attention was given to the medium components. Maize flour was found to be the best organic carbon source, with ammonia, urea and amino acids as the nitrogen source. These components could be added at intervals or continuously (Distillers Company Ltd and Hutchinson, 1928). Beesch (1952), using a 1 000 000 l process, described the dual function of the ammonium hydroxide, which both supplied the nitrogen source and controlled pH.

Nitrogen source to yeast and biomass production

During yeast biomass production, it was found that improved yeast production was obtained in a medium which initially contained between 10% and 50% of nitrogen in the form of an ammonium salt. The rest of the inorganic nitrogen was added gradually to the medium during the growth cycle. A six-vessel continuous system was described (Olsen, 1961) with different feed rates of molasses, nitrogen source and phosphate in different vessels. Butschef and Kautzmann (1962) described the additions of molasses, nitrogen, phosphate and magnesium made at hourly intervals during large-scale yeast production.

Methanol and ammonium have been fed for the production of biomass from *Pseudomonas* AM-1 culture. At 24 h growth, this system gave a yield of 15 g/l and at 85 h after inoculation, a final biomass of 36 g/l was obtained (Nishio *et al.*, 1977).

Hill and Thommel (1982) developed an ammonium control system to maintain a relatively constant ammonium concentration during fed-batch fermentations for yeast production. This monitoring system was based on an ammonia gas electrode, but their attempts to use it for control resulted in oscillations in ammonium concentration. Thompson, Kole and Gerson (1986) described an improved version of the ammonia control system with which ammonium concentration was maintained at nearly constant concentrations throughout fermentations of *E. coli*. This control system is based on an ammonia gas-sensing electrode that monitors a pH-adjusted effluent stream

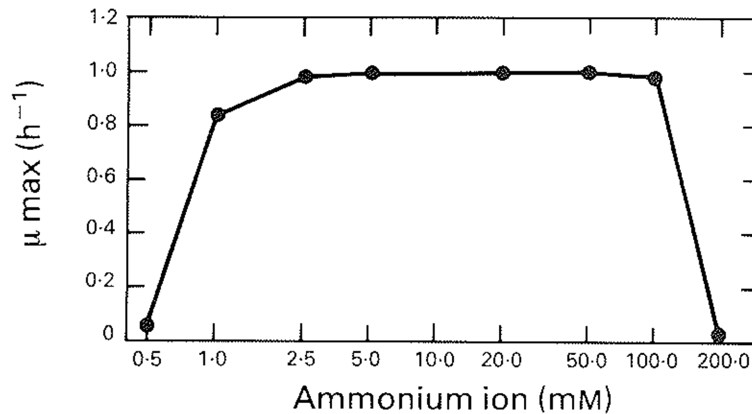

Figure 3. Effect of controlled ammonium levels in *E. coli* fermentations showing the apparent maximum specific growth rate (μ max) as a function of ammonium concentration.

from the fermentor. To overcome the time lag between the fermentor and the electrode, the feedback–feedforward control included metered flows of ammonium to both the fermentor and the electrode vessel. In this experiment, ammonium was controlled at a constant concentration over the set-point range from 5 mM to 170 mM.

A feedforward control technique was used to control the concentration of ammonium during the fed-batch cultivation of *Saccharomyces cerevisiae* (Kole, Thompson and Gerson, 1985). In a complex medium, as the controlled level of ammonium increased in separate fermentations from 38 to 300 mM, the ammonium consumption increased, the protein content of the cells increased and the biomass yield decreased.

Thompson, Kole and Gerson (1986) determined the apparent specific growth rates, biomass and protein production, and glucose yield of fed-batch cultures of *E. coli*. As the concentration of ammonium decreased from 200 mM to 5mM, the yields increased from 1 to 24 g cell dry weight/g ammonium utilized.

The ammonium control system of Kole, Thompson and Gerson (1985) allowed ammonium concentration to be controlled to within ± 5% of a set point value for fed-batch cultures of *E. coli* at ammonium concentrations from 0·5 mM to 170 mM. The growth yields for ammonium and glucose increased approximately 20-fold as the ammonium concentrations increased from 0·5 mM to 2·5 mM. At ammonium concentrations less than 1 mM, growth rate, biomass production, glucose utilization, protein production and ion utilization were all suboptimal, indicating that the minimum ammonium concentration necessary for normal metabolism in *E. coli* fermentations is around 1–5 mM. *Figure 3* describes the apparent maximum specific growth rate (μmax) as a function of ammonium concentration from 0·5 to 200 mM ammonium.

During ammonium-controlled fermentations, instead of feeding ammonium, a mixture of glucose and ammonium was fed (Kole, Ward and Gerson, 1986). This is an open-loop approach to the control of glucose concentration

Table 4. Biomass production by fed-batch culture

Additive	Biomass	Reference
Methanol	25g/l	Yamane, Kishimoto and Yoshida, 1976
Methanol	16·3 g/l	Andriantsoa *et al.*, 1984
Ethanol	64 g/l	Huang and Chu, 1981
Ethanol	85 g/l	Yano, Kobayaski and Shimizu, 1978
Ethanol	125 g/l	Mori *et al.*, 1979
Ethanol; ammonium	268 g/l	Yano, Kobayashi and Shimizu, 1985
Ethanol; mineral	150 g/l	Yano *et al.*, 1980
Mineral; ethanol; ammonia	138 g/l	Suzuki *et al.*, 1985
Cellulose	—	Miller and Srinivasan, 1983
Glucose	30 g/l	Ghoul, Ronat and Engasser, 1986

during ammonium-controlled fermentations of *E. coli*. With this control system (*Figure 4*), the ammonium and glucose concentrations were kept quite constant throughout the fermentation. The maximum apparent specific growth rate increased when both glucose and ammonium concentrations were controlled.

Oguztoreli, Ozum and Gerson (1986) developed a mathematical model and computer simulation of the optimal control of substrate concentrations in bioreactors with separated sensors, such as is required for ammonium concentration control with ammonium gas electrode based controllers.

Table 4 summarizes the use of fed-batch fermentations with a nitrogenous feed.

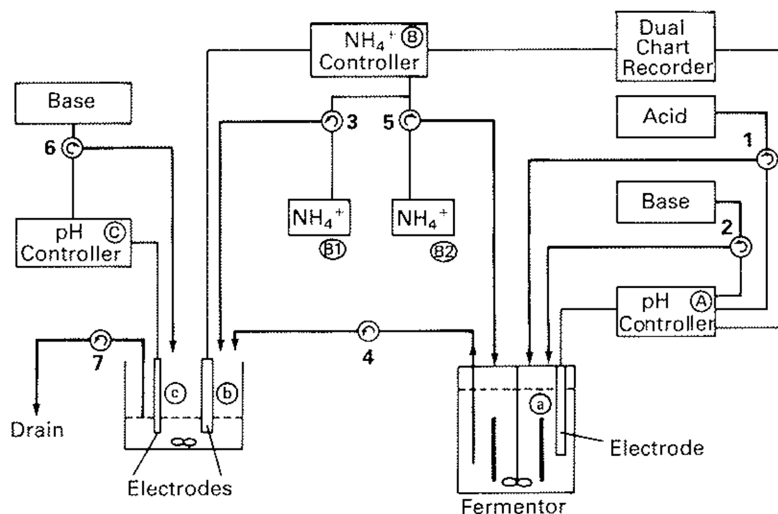

Figure 4. A Chemcadet pH/millivolt on–off controller was used with an Orion 95-12 ammonia gas electrode (electrode b) to determine the ammonium concentration in a continuous sample stream from the fermentor. The sampling rate was maintained by a pump (flow 4). The contents of the measurement vessel were maintained at pH 11·0–11·5 by another Chemcadet controller (controller C, electrode c and flow 6). The measurement vessel was maintained at a volume of 10 ml by continual fluid withdrawal with a Master-flex peristaltic pump (flow 7). Control of the ammonium concentration in the fermentor was effected by the simultaneous operation of 2 pumps (flows 3 and 5) when the electrode output indicated that the ammonium concentration in the measurement vessel had decreased below the set-point. A Master-flex peristaltic pump delivered $(NH_4)_2SO_4$ and glucose from reservoir B2 to the fermentation vessel (flow 5) and syringe pump delivered $(NH_4)_2SO_4$ from reservoir B1 to the measurement vessel (flow 3). Controller A controlled pH in the fermentor by acid and base (flows 1 and 2).

OXYGEN CONCENTRATION CONTROL

Microbial dependence on oxygen

Many, if not most, fermentations used in industrial applications require oxygen; the low solubility of oxygen in water prevents batch operation. The power requirements for the air compression and mixing required to dissolve oxygen continuously and effectively throughout a fermentation constitute a significant fraction of the cost of the fermentation step of an aerobic process (Mudgett, 1980).

Aerobic cells generally have a high affinity for oxygen and the K_s for oxygen is typically quite low. Chen, Tannahill and Shuler (1985) have recently re-evaluated critical oxygen concentrations for growth in *E. coli* and *Azotobacter vinelandii*. The critical oxygen concentration for growth is defined as the oxygen concentration giving 95% of the growth rate obtained at oxygen saturation, and is 19 times the value of K_s. The K_s for *E. coli* was found to be 0·013 mg/l and the K_s for *A. vinelandii* was found to be 0·017 mg/l. One early oxygen concentration control system was developed by Siegell and Gaden (1962). More recently, Chen, Tannahill and Shuler (1985) developed an oxygen concentration control system using a standard dissolved oxygen probe and a proportional mixing valve to supply variable N_2/O_2 mixtures to the fermentation at a constant gas flow rate.

Dissolved oxygen concentration control

Oxygen concentration control schemes have been used successfully to develop methods for high cell-density cultivation. To achieve high cell density, the rate of oxygen demand by the culture must be matched to the rate of oxygen supply to the fermentor. The oxygen transfer rate is physically limited by the solubility of oxygen and the physical characteristics of the fermentor. Gleiser and Bauer (1981) adjusted oxygen demand by decreasing the concentration of substrate (sucrose) and adjusted oxygen transfer by supplying the culture with pure oxygen. Using this approach, it was possible to maintain oxygen at approximately 14% saturation, and they were able to obtain a final cell density of 42 g cell dry weight/litre, compared with approximately 22 g/l without the open-loop control of oxygen concentration. Mori, Kobayashi and Shimuzu (1981) developed an open-loop oxygen-control system to produce a high concentration of sorbose from sorbitol using *Gluconobacter suboxydans*. In this system, sorbitol was fed intermittently and a DO-stat operated with the options of mixing speed adjustment and pure oxygen supply. Dissolved oxygen concentrations were maintained between 2 and 3 ppm until oxygen demand exceeded oxygen transfer rate. Under these conditions, a final sorbose concentration of 460 g/l was obtained.

Dissolved oxygen concentration was used as a nutrient feed indicator by Miskiewicz (1981) for the production of baker's yeast. Oxygen concentration was controlled between 10% and 60% saturation. As the dissolved oxygen

concentration exceeded 60%, sugar was added to the fermentor. The concentration of sugar in the feed was adjusted to provide a range of average substrate concentrations between 0·5 and 3·5 mM sucrose. The optimal specific growth rate of 0·19 h^{-1} was obtained at a sucrose concentration of 2 mM. Hopkins (1981) also describes a feed-on-demand controller for dissolved oxygen concentration, but with additional control to minimize the likelihood of reaching toxic substrate concentrations. This controller allowed the delivery of a timed pulse of methanol to a culture of *Hansenula polymorpha* when the dissolved oxygen concentration reached an upper set-point. If, for reasons including the build-up of substrate to toxic levels, the dissolved oxygen concentration did not decrease following a substrate feeding period, further feeding was interrupted.

Hale, Teasca and Mitchell (1985) developed an automatic control system for wort oxygenation. In this system, a microprocessor system controlled air or oxygen flow based on the dissolved oxygen concentration measured in the wort. The algorithm used for process control allowed for the various time delays in the system, and was successful in maintaining dissolved oxygen concentration at 16 ppm unless the system was disturbed by rapid flow rates.

Dissolved oxygen concentration can also be controlled by manipulation of agitator speed. This approach has a tendency to be non-linear as it also affects the availability of substrate to the cells. Clark, Hesbeth and Seddon (1985) describe a PI (Proportional-Integral) control system capable of accurately maintaining dissolved oxygen concentration during fungal steroid hydroxylation. Using this system, the dissolved oxygen concentration was maintained at 30 ± 2% saturation in fermentations of *Corticium practicola* and *Pellicularia filamentosa*.

Cultivations of microaerophillic organisms offer the greatest opportunities for improved productivities through oxygen concentration control. Van Hemert (1974) investigated the usefulness of oxygen concentration control in vaccine production. In these investigations, dissolved oxygen concentration was used to control either the rate of agitation, the rate of gas flow or the mixture of gases supplied to the fermentor. *Vibrio cholerae* and *S. typhi* were grown with dissolved oxygen concentration controlled at 15 torr. Under these conditions, cell density increased nearly threefold for either organism. *Corynebacterium diphtheriae* requires a dissolved oxygen concentration greater than 0·3 torr and less than 30 torr to produce toxin. Growth of *Bordetella pertussis* is inhibited by high oxygen concentrations and optimal cell densities were achieved by cultivations at a dissolved oxygen concentration of approximately 1·0 torr. In the production of viral vaccines, it was found that growth of the host mammalian cells could be optimized by dissolved oxygen control at approximately 120 torr, but that, once infected, production of virus was relatively independent of dissolved oxygen concentration.

Heinzle *et al.* (1986) have developed techniques to control dissolved oxygen concentration below 100 ppb. Standard oxygen electrodes suitable for fermentation service were used in this work. A cascade controller was designed to overcome the drastic changes in system dynamics which occur as glucose is exhausted. It was found that highly turbulent conditions contributed to the

accuracy of control. With this system, the dissolved oxygen concentration of *B. subtilis* fermentation was held within a range between 60 and 80 ppb.

MAINTENANCE AND YIELD FACTORS

Maintenance factors

Maintenance energy was originally proposed as a part of the theory of cell cultivation by Pirt (1965). All cells require a certain rate of substrate consumption to maintain life in the absence of growth. Maintenance energy requirements provide the cell with resources for a variety of physiological and biochemical functions including such activities as the maintenance of introcellular ionic concentrations and DNA repair. The maintenance requirements are generally considered to be relatively constant, but recent studies have shown them to be functions of both the immediate chemical environment and the recent history of the cells.

Watson (1970) found that sodium chloride decreased the growth rate of *Saccharomyces cerevisiae* by increasing the maintenance requirements for glucose to accommodate the energetic requirements for maintaining low intracellular sodium levels. With *Zymomonas mobilis*, Fieschko and Humphrey (1983) found that temperature had a significant effect on maintenance requirements. The maintenance coefficient, m, was found to increase as either the concentration of glucose or ethanol in the medium increased. However, it was found that glucose consumed for maintenance was also converted to ethanol.

Several methods have been developed to determine the maintenance coefficient. A simple method for maintenance coefficient determination was proposed by Beyeler, Rogers and Fiechter (1984). They suggested that the maintenance coefficient could be determined efficiently through the use of a cell recycle system to maximize biomass accumulation at a given dilution rate. A steady state is reached when all incoming nutrients are consumed for maintenance, and no further growth occurs. Fieschko and Humphrey (1984) provide a statistical comparison of methods for the determination of the maintenance coefficient.

Maintenance energy requirements depend on the growth rate, especially at low growth rates. Pirt (1987) has proposed a model for maintenance energy requirements at slow growth rates. In this model, it is proposed that the cell population consists of two sub-populations, one with a normal maintenance requirement and one with no maintenance requirements. The dormant sub-population contributes to biomass but does not contribute to the utilization of substrate or to viable counts. In cultures of *K. aerogenes*, the viable fraction declined from approximately 95% at a specific growth rate of 0·1 to approximately 50% at a specific growth rate of 0·01. Thus, there is a relation between the specific growth rate and the maintenance coefficient, as well as a

separation of the maintenance coefficient into two terms, one for the viable and one for the dormant sub-population.

Verseveld *et al.* (1984) have further subdivided the relation between the maintenance coefficient and the specific growth rate, although it may be possible to describe the general relation as a continuous function. Discontinuities in the relation are supported by genetic studies indicating the possibility of a genetic response to conditions of starvation (Arbige and Chesbro, 1982a). Evidence for a three-phase response to starvation by the adjustment of maintenance requirements has been obtained for *E. coli* (Chesbro, Evans and Eifert, 1979) and *Bacillus polymyxa* (Arbige and Chesbro, 1982b). The ecological significance of variable maintenance energy requirements may be that this system provides for increased long-term viability under conditions of severe nutrient limitation (Flint, 1987).

The relation of maintenance energy requirements to growth rate may become an important consideration in the development of fermentation processes involving the establishment of a productive steady-state cell population in which growth is limited but metabolic activity is relatively high. Under these conditions, substrate would be consumed at relatively high rates although growth would be minimal, with a significant fraction of the substrate flow being to product.

Yield factors

The usual initial assumption in fermentation process analysis is that yield factors are relatively constant. Yield is the quantity of product or biomass produced from a given quantity of substrate. The yield is often confused with final concentrations obtained in a fermentation system. Yields are a measure of the efficiency of a fermentation process and are intimately involved in the stoichiometry of the overall process, they are usually defined in terms of a single product and a single substrate, although this is clearly an oversimplification. Yields may more properly be considered to be partial differentials of the quantity of a particular product produced with respect to the quantity of a particular substrate consumed during the process.

Physiologically, yields are not constant over wide ranges in substrate concentration, although they do tend to be relatively constant over narrow, intermediate concentration ranges. Fieschkco and Humphrey (1983) investigated the effects of temperature and ethanol concentration on the growth yield factor of *Zymomonas mobilis*. Over a temperature range from 30 to 35°C, the growth yield on glucose, Y_G. was constant. Ethanol concentration, however, significantly affected Y_G: as ethanol concentration increased from 9 to 27 g/l, Y_G decreased from 0·0142 to 0·0108 g cells/g glucose. Growth rate also affected Y_G: yields changed dramatically if specific growth rate exceeded 0·08 h^{-1}. Paredes-Lopez, Camargo-Rubioe and Ornelas-Vale (1976) also studied the effects of growth rate on the yield of *Candida utilis* on a complex medium. Yield of biomass on sugar was relatively constant between pH 3·5 and 4·5, but decreased significantly outside that range. The optimal temperature for growth

yield was between 27 and 33°C, and the optimal specific growth rate range for growth yield was between 0·2 and 0·4 h^{-1}. In most fermentation processes, yield optima for various possible products are fairly independent of optima for growth rate.

Solomons *et al.* (1982) describe a maximum likelihood technique for the estimation of growth yields. This statistical technique provides both yield and maintenance parameters, and allows for the design of efficient experimental plans for the determination of these important relations between growth conditions, substrate concentrations and biomass or product yields.

Theory of substrate concentration control

INTRODUCTION

Bioreactor models

Bioreactors may be modelled as three-component systems consisting of media, micro-organisms and metabolic products. Mathematical models adequately describing the behaviour of bioreactors are needed for bioreactor scale-up, reactor optimization and control. These models should properly represent the bioreactor system under a variety of conditions and should express quantitative relationships between the state variables (metabolic product concentration, number of microbial cells) and the control variables (substrate concentrations in the media, operating conditions, etc.) of the reacting system.

Model equations describing the basic system are needed to study any bioreactor system. The effects of reactor geometry, reactor operating mode and reactor hydrodynamics on the performance of bioreactor should also be represented by the mathematical model in as realistic a form as possible.

Bioreactor model equations

A biochemical process may take place in a batch reactor (BR), in a continuous flow stirred tank reactor (CSTR), in a tubular reactor (TR) or in a reactor system which is composed of some combination of any of these reactors. In this section, mathematical expressions will be developed to describe relationships between substrates present in the medium and the products produced by the culture under consideration. The media is assumed to consist of the substrates $[S = (S_1, S_2, \ldots S_s)]$, and biological cells $[X = (X_1, X_2, \ldots X_x)]$. The product consists of chemical species, $P = (P_1, P_2, \ldots P_p)$. In the present study, X and P are considered as the state variables, while S is considered as the control variable. Actually, operating variables effecting the reactor hydrodynamics such as mixing rate, liquid and gas flow velocities through the reactor, reactor diameter and length, reactor orientation, and finally the temperature of the reacting system should be included as control variables.

In most bioreactor applications, the reactor temperature is maintained constant due to the small exothermicity involved in biochemical reactions, slow reaction rates and good temperature control. Therefore, in the present

formulations the temperature of the bioreactor system is assumed to be constant. If temperature changes are allowed in space and time, the problem may become extremely difficult because the dependence of biochemical reactions on temperature is basically nonlinear.

In formulating the model equations, the most general case is considered. Substrate, cell and product are considered to be vector quantities. Unsteady-state model equations are developed first, and are essential to study bioreactor dynamics and control. In all of these formulations the sign of the generation term is considered to be positive if the component is produced by the reaction.

BATCH REACTOR

In the case of a batch bioreactor operation (*Figure 5a*), the system is closed. Assuming perfect mixing in the bioreactor, the model equations representing the substrate, cell and product balances become:

$$\frac{d[VS]}{dt} = Vf(S, X, P) \tag{10}$$

$$\frac{d[VX]}{dt} = Vg(S, X, P) \tag{11}$$

$$\frac{d[VP]}{dt} = Vh(S, X, P) \tag{12}$$

with corresponding initial conditions at $t = 0$:

$$S(0) = S_o; \quad X(0) = X_o; \quad P(0) = P_o \tag{13}$$

Generally speaking, Eqs (10–12) form a system of coupled, non-linear, ordinary differential equations. If the biochemical reaction does not affect the volume of the reacting media, volume will be considered as a constant.

CONTINUOUS-FLOW TANK REACTOR

In the case of CFTR operation (*Figure 5b*), the model equations for substrate, cell and product balances take the following form:

$$\frac{d[VS]}{dt} = v_{in}S_{in} - v_{out}S + Vf(S, X, P) \tag{14}$$

$$\frac{d[VX]}{dt} = v_{in}X_{in} - v_{out}X + Vg(S, X, P) \tag{15}$$

$$\frac{d[VP]}{dt} = v_{in}P_{in} - v_{out}P + Vh(S, X, P) \tag{16}$$

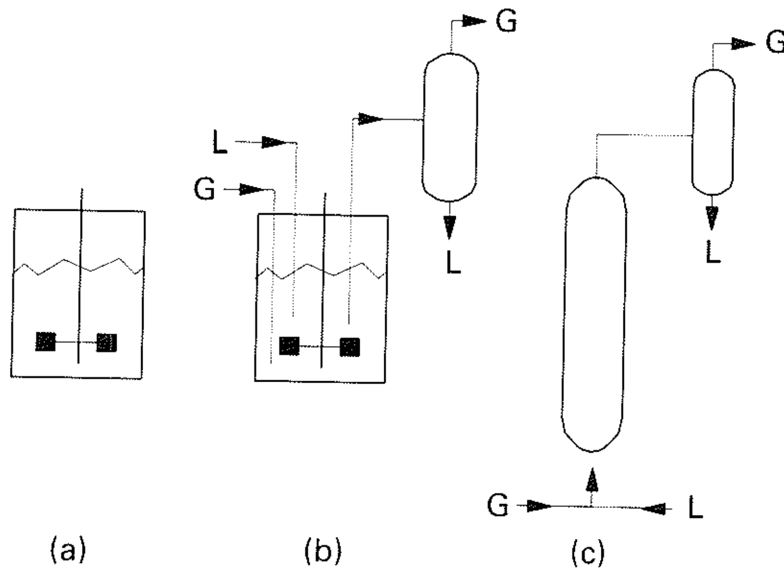

Figure 5. Three bioreactor configurations, with indicated flows of gas (G) and liquid (L). (a) Batch reactor; (b) continuous-flow tank reactor; (c) tubular reactor.

where V is the liquid volume of the reacting system, v_{in} and v_{out} are the liquid flow rates of the feed and exit streams of the reactor.

Many bioreactions involve gaseous substrate and products. Biochemical reactions in these systems take place in the liquid phase. Material balances for gaseous substrates or products should also be formulated considering gas solubility and gas-liquid interfacial transport mechanism. For example, if the j-th substrate is in the gaseous state, mass balance for the j-th substrates must be formulated for the liquid and gas phases. The liquid phase mass balance for the j-th substrate takes the following form:

$$\frac{d[VS_j]}{dt} = v_{in}S_{j,in} - v_{out}S_j + V_T K_m a(\frac{S_{j,g}}{H_j} - S_j) - Vh_j(S, X, P) \quad (17)$$

where V is the liquid volume in the reactor, V_T is the total volume of the reacting system ($V = \varepsilon_l V_T$, ε_l is the liquid hold-up) K_m is the interfacial mass transfer coefficient (cm/s), a is the gas–liquid interfacial area per reactor volume (cm^{-1}) and H_j is the Henry's constant of the j-th substrate. In Eq. (17) the sign of the reaction term $h_j(S, X, P)$ is taken as negative, since j-th substrate is consumed by the reacting system.

Gas phase material balance for j-th substrate takes the following form:

$$\frac{d[V_j S_{j,g}]}{dt} = v_{g,in}S_{j,g,in} - v_{g,out}S_{j,g} - V_T K_m a(\frac{S_{j,g}}{H_j} - S_j) \quad (18)$$

Since no biochemical reaction takes place in the gas phase, the biochemical reaction term disappears. In Eq. (18), V_g ($V_g = (1 - \varepsilon_l) V_T$) is the gaseous volume in the bioreactor, $v_{g,in}$ and $v_{g,out}$ are the gas feed and exit volumetric flow rates, $S_{j,g,in}$ is the concentration of j-th substrate in the gas feed. If the m-th substrate is a gaseous substrate and produced by biochemical reactions taking place in liquid phase, liquid and gas phase balance equations become:

$$\frac{d[VP_m]}{dt} = v_{in}P_{m,in} - v_{out}P_m + V_T K_m a\left(\frac{P_{m,g}}{H_m} - P_m\right) + Vh_m(S, X, P) \quad (19)$$

and

$$\frac{d[V_g P_{m,g}]}{dt} = v_{g,in}P_{m,gin} - v_{g,out}P_{m,g} - V_T K_m a\left(\frac{P_{m,g}}{H_m} - P_m\right) \quad (20)$$

In the above formulations ε_l and $K_m a$ are assumed to be known. Both ε_l and $K_m a$ are dynamic properties of the reacting system, and their values strongly depend on reactor hydrodynamics (geometry, gas and liquid physical properties, mixing rate, impeller type etc.).

Equations (14–20) are unsteady-state equations; considering the liquid phase volume V, and gas phase volume V_g to be constant, they can be taken outside of the derivative operations. If the process reaches its steady-state operating conditions, the time derivative terms in equations (14–20) vanish. Under the steady-state operating conditions, balance equations form a system of algebraic equations. The time required for a bioreactor system operating in CFTR mode to reach its steady-state operating conditions generally depends on the variations in the velocities and concentrations of the feed streams, the kinetics of the growth and product formation, and the reactor volume. If the mean residence time of the feed stream in the reactor is τ_m (= V/V, assuming that $v_{in} = v_{out} = v$) and the characteristic reaction time $\tau_r = 1/k$ (k is some approximate rate constant for any state variable), the system reaches its steady-state operating conditions faster as the ratio of characteristic times τ_m/τ_r gets smaller.

TUBULAR REACTOR

Tubular reactor operation may be beneficial for certain types of bioreactor processes, such as continuous-flow cheese manufacturing. In many practical operating conditions, a tubular reactor does not operate as an ideal tubular reactor. Deviations from the ideal (plug flow) tubular reactor can be handled by introducing an axial dispersion term (like molecular diffusion term in mass transfer) into the balance equations. In this section tubular reactor balance equations are developed for an ideal tubular reactor, and for a tubular reactor with axial dispersion or mixing (*Figure 5c*).

Ideal (plug flow) tubular reactor

In an ideal TR operation, fluid elements in the reactor are assumed to flow with an uniform axial velocity at a given cross-section of the reactor. Therefore, the

axial flow velocity is independent to the radial position. For an ideal tubular reactor, balance equations for the substrates, cells and products become:

$$\frac{dS}{dt} + \frac{d[US]}{dx} = f(S, X, P) \quad (21)$$

$$\frac{dX}{dt} + \frac{d[UX]}{dx} = g(S, X, P) \quad (22)$$

$$\frac{dP}{dt} + \frac{d[UP]}{dx} = h(S, X, P) \quad (23)$$

where U (= v/A, A is the cross-sectional area of the reactor) is the liquid phase axial flow velocity and x is the axial distance from the inlet of the reactor.

In many bioreactor applications, substrate or products may be in the gaseous state. Under these conditions substrate and product balances must be developed for both liquid and gas phases. For example if the j-th substrate is in gaseous state and being consumed in the reacting system, balance equations for the j-th substrate for the liquid and gas phases take the following forms:

$$\frac{d(\varepsilon_l S_j)}{dt} + \frac{d(\varepsilon_l U S_j)}{dx} = K_m a\left(\frac{S_{j,g}}{H_j} - S_j\right) - fj(S, X, P) \quad (24)$$

$$\frac{d(\varepsilon_g S_{j,g})}{dt} + \frac{d(\varepsilon_g U_g S_{j,g})}{dx} = -K_m a\left(\frac{S_{j,g}}{H_j} - S_j\right) \quad (25)$$

If the m-th substrate is in gaseous state and produced by biochemical reactions which are taking place in liquid phase, the balance equations for the liquid and gas phases become:

$$\frac{d(\varepsilon_l P_m)}{dt} + \frac{d(\varepsilon_l U P_m)}{dx} = K_m a\left(\frac{P_{m,g}}{H_m} - P_m\right) + h_m(S, X, P) \quad (26)$$

$$\frac{d(\varepsilon_g P_{m,g})}{d^t} + \frac{d(\varepsilon_g U_g P_{m,g})}{d^x} = -K_m a\left(\frac{P_{m,g}}{H_m} - P_m\right) \quad (27)$$

where ε_l and ε_g are the liquid and gas hold-ups, $U(=v/A)$ and U_g $(=v_g/A)$ are the linear axial flow velocities of the liquid and gas phases respectively.

Initial and boundary conditions for the balance equations (12–14) are:

$$\text{at } x = 0{:}\ S|_{x=0} = S_o(t);\quad X|_{x=0} = X_o(t);\quad P|_{x=0} = P_o(t) \quad (28)$$

and naturally solutions for S, X, and P should satisfy the following conditions at $x = L$:

$$\left.\frac{dS}{dx}\right|_{X=L} = 0; \left.\frac{dX}{dx}\right|_{X=L} = 0; \left.\frac{dP}{dx}\right|_{X=L} = 0 \quad (29)$$

where $S_o(t)$, $X_o(t)$ and $P_o(t)$ are some functions of time.

All of the balance equations described above are time dependent or partial differential equations of the first order. Time derivative terms disappear if the

system reaches its steady-state operating conditions if the properties of the reactor feed can be kept constant for a long period of time. Under these conditions time derivative terms in Eqs (21–27) vanish, and initial conditions expressed in Eq. (19) become time independent i.e.

$$\text{at } x = 0 \quad S|_{x=0} = S_o; \quad X|_{x=0} = X_o; \quad P|_{x=0} = P_o \tag{30}$$

which reduces the problem into a set of first order coupled nonlinear equations. Therefore, Eq. (30) can be considered as a set of initial conditions.

Tubular reactor with axial mixing

Tubular bioreactors (TR) in laboratory, pilot or industrial scale exhibit axial mixing to some extent, depending on the reactor length to diameter ratio and flow rates. Axial mixing in tubular reactors may be modelled by introducing an effective dispersion term. Balance equations for axially dispersed TR become:

$$\frac{dS}{dt} + \frac{d(US)}{dx} = D_e \frac{d^2S}{dx^2} + \varepsilon_l f(S, X, P) \tag{31}$$

$$\frac{dX}{dt} + \frac{d(UX)}{dx} = D_e \frac{d^2X}{dx^2} + \varepsilon_l g(S, X, P) \tag{32}$$

$$\frac{dP}{dt} + \frac{d(UP)}{dx} = D_e \frac{d^2P}{dx^2} + \varepsilon_l h(S, X, P) \tag{33}$$

If one of the substrates is gaseous, model equations must be developed for two phases. Assuming that the j-th substrate is gaseous and consumed in bioreactor, balance equations become:

$$\frac{d(\varepsilon_l S_j)}{dt} + \frac{d(\varepsilon_l U S_j)}{dx} = \varepsilon_l D_e \frac{d^2S_j}{dx^2} + K_m a\left(\frac{S_{j,g}}{H_j} - S_j\right) - \varepsilon_l f_j(S, X, P) \tag{34}$$

and

$$\frac{d(\varepsilon_g S_{j,g})}{dt} + \frac{d(\varepsilon_g U_g S_j)}{dx} = \varepsilon_g D_{e,g} \frac{d^2S_{j,g}}{dx^2} - K_m a\left(\frac{S_{j,g}}{H_j} - S_j\right) \tag{35}$$

Similarly, for the m-th substrate which is in gaseous state and produced by biochemical reactions, the balance equations for the liquid and gas phase become:

$$\varepsilon_l \frac{dP_m}{dt} = \varepsilon_l \frac{d(UP_m)}{dx} = \varepsilon_l D_e \frac{d^2P_m}{dx^2} + K_m a\left(\frac{P_{m,g}}{H_m} - P_m\right) + \varepsilon_l h(S, X, P) \tag{36}$$

$$\varepsilon_g \frac{dP_{m,g}}{dt} = \varepsilon_g \frac{d(U_g P_{m,g})}{dx} = \varepsilon_g D_{e,g} \frac{d^2P_{m,g}}{dx^2} - K_m a\left(\frac{P_{m,g}}{H_m} - P_m\right) \tag{37}$$

Eqs (36) and (37) are unsteady-state balance equations and each of them requires two boundary conditions and one initial condition. For engineering applications, we may assume that the reactor is operating under the

steady-state operating conditions. To reach the steady-state, the system must be fed by steady-state inputs. Therefore, initial conditions become:

$$S|_{t=0} = S_{s,in}; \quad X|_{t=0} = X_{s,in}; \quad P|_{t=0} = P_{s,in} \tag{38}$$

and

$$S|_{x=0} = S_0(t); \quad X|_{x=0} = X_0(t); \quad P|_{x=0} = P_0(t) \tag{39}$$

Boundary conditions must satisfy the continuity condition as the reactor inlet (at x = 0):

$$(US)|_{x=0-} = (US)|_{x=0+} - D_e \frac{dS}{dx}|_{x=0+} \tag{40}$$

$$(UX)|_{x=0-} = (UX)|_{x=0+} - D_e \frac{dX}{dx}|_{x=0+} \tag{41}$$

$$(UP)|_{x=0-} = (UP)|_{x=0+} - D_e \frac{dP}{dx}|_{x=0+} \tag{42}$$

Another set of boundary conditions would be the reactor exit conditions. If the reactor is long enough, at the reactor exit the composition of the reaching media will reach a steady platform. Therefore, the reaction exit boundary conditions at x = L become:

$$\frac{dS}{dx} = \frac{dX}{dx} = \frac{dP}{dx} = 0 \tag{43}$$

If the reactor reaches its steady-state operating conditions time derivative terms in Eqs (31–38) disappear. Balance equations become a set of second order ordinary differential equations. Boundary conditions of such equations will be the same as Eqs (40–42) with ordinary derivatives instead of partial derivatives.

BASIC CONTROL THEORY

Introduction

In the control of a biochemical reactor, one must control the performance of the reactor considering several factors such as economics, production specification, environmental regulations, safety, etc. The first step in bioreactor control is to formulate the problem in terms of the state variables, the control variables and the controller with consideration of the constraints that are affecting the process.

For linear control systems, the application of Laplace transforms provides solutions to the control systems in the time domain. Let us consider a simple control system which involves the control of a state variable that is to be controlled at a desired level by adjusting one or more control variables in the bioreactor system (*Figure 6*). Suppose that we want to control the

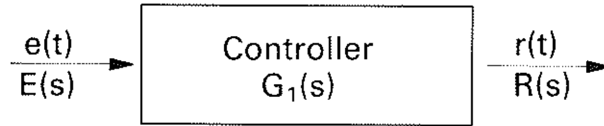

Figure 6. A simple controller with error signal inputs, E(t) and E(s), and proportional outputs, P(t) and R(s).

Figure 7. A simple sub-system with inputs, x(t) and X(s), a function, G(s) and outputs, y(t) and Y(s).

concentration of a substrate, S, in a bioreactor at a concentration of S_R. The measured value of S at the process exit is fed back to a comparator which feeds the information e($e = S_R - S_m$) to the controller. The controller makes the action decision and when the decision is made, a feed of substrate S_F is added to the reactor to keep the substrate concentration in the reactor as close as possible to a concentration of S_R.

Let us consider the simple system given in *Figure 7.* The system can be represented by a linear first-order differential equation in time. If x(t) is the input (forcing function) to that system, y(t) would be the response of the system to the forcing function x(t). Therefore, y(t) is the solution of the differential equation describing the system with given initial conditions under the influence of disturbances x(t). In the control system, x(t) is considered to be the forcing function with respect to some steady state value, and y(t) is the response of the system with respect to some reference (i.e. steady-state) value. The transfer function G(S) of this simple step is defined as:

$$G(s) = \frac{Y(s)}{X(s)} \tag{44}$$

where X(s) and Y(s) are the Laplace transforms of x(t) and y(t), respectively.

If two linear non-interacting systems are combined in series (*Figure 8*), the transfer function between the output and input takes the form:

$$\frac{Y(s)}{X(s)} = G(s) = G_1(s)G_2(s) \tag{45}$$

The rule expressed by Equation (45) can be applied to determine the transfer function between each level of the control system described in *Figure 8.* The transfer function between E(s) ($E(s) = S_R(s) - S_m(s)$) and S(s) become:

$$\frac{S(s)}{E(s)} = \frac{G_1(s)G_2(s)G_3(s)}{1 + G_1(s)G_2(s)G_3(s)G_4(s)} \tag{46}$$

Figure 8. Two non-interacting systems, $G_1(s)$ and $G_2(s)$, in series, with inputs x(t) and X(s) and outputs y(t) and Y(s).

and transfer function between $S_F(s)$ and S(s) becomes:

$$\frac{S(s)}{S_F(s)} = \frac{G_3(s)}{1 + G_1(s)G_2(s)G_3(s)G_4(s)} \quad (47)$$

In control literature, the product of all transfer functions of the loop G(s):

$$G(s) = G_1(s)G_2(s)G_3(s)G_4(s) \quad (48)$$

is called 'open-loop transfer function', because G(s) relates the measured value of the state variable $S_m(s)$ to the set point of the state variable $S_R(s)$:

$$G(s) = G_1(s)G_2(s)G_3(s)G_4(s) = \frac{S_m(s)}{S_R(s)} \quad (49)$$

The response of s(t) to e(t) (or to $s_F(t)$) can be calculated if all transfer functions are known in Equation (46). Because of the properties of inverse Laplace transforms, the roots of the expression

$$1 + G_1(s)G_2(s)G_3(s)G_4 = 0 \quad (50)$$

are sufficient to calculate the response of the system s(t) to e(t) (or to $s_F(t)$). The expression in Equation (50) is known as the characteristic equation, and it depends only on the transfer functions of individual elements in the loop. The roots of the characteristic equation are also the pools of the closed-loop transfer functions (Equations (46) and (47)).

Controller action

In a simple process control system (*Figure 9*), the set point of state variable $s_R(t)$ will be maintained at a desired value by loading the system as a function of time with $s_F(t)$. The control problem described in *Figure 9* is called the 'regulator problem'. To regulate the s(t) as close as possible to $s_R(t)$, the controller plays an important part. In this section the action of the controller in response to an error e(t) is summarized.

On–off controller On–off controller is a special case of proportional controller. If the gain is kept very high, the controller will move from one extreme position to the other, if e(t) deviates slightly from the set point. This very sensitive action of controller will fully load the $s_F(t)$ on to the system (on

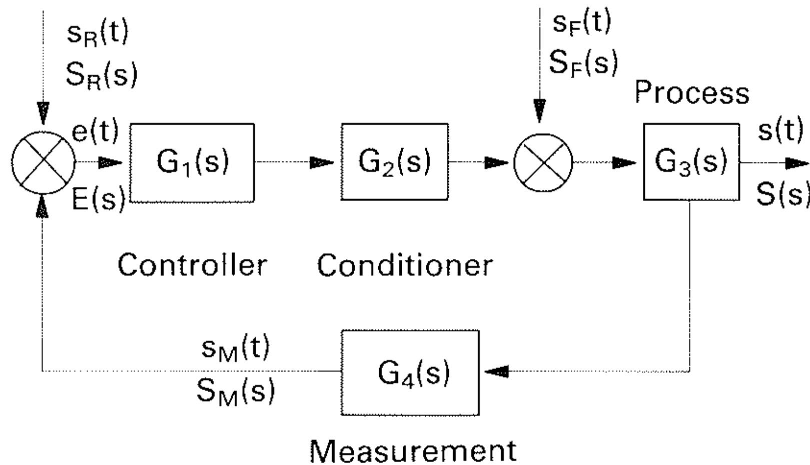

Figure 9. Basic feedback control of a bioreactor: $s_R(t)$ and $S_R(s)$ define the desired substrate concentration, $s_M(t)$ and $S_M(s)$ are the measured conditions in the bioreactor. The error signals, e(t) and E(S) are the input to a controller, $G_1(s)$. The controller output is conditioned, $G_2(s)$, to operate the actuators providing a feed, $s_F(t)$ and $S_F(s)$, to the process, $G_3(s)$. The process output is s(t) and S(s), and is sensed by the measurement module, $G_4(s)$.

action) or fully closed (off action). Note that the bandwidth of an on–off controller is practically zero.

Proportional controller A proportional controller (*Figure 9*) responds to an error signal in a linear fashion with a linearity coefficient K_c:

$$p(t) = K_c e(t) \tag{51}$$

The transfer function $G_1(s)$ for a proportional controller takes the following form:

$$G_1(s) = \frac{P(s)}{E(s)} = K_c \tag{52}$$

K_c is called the 'gain'.

Proportional–integral (P-I) controller The response of a P-I controller to an error signal e(t) is expressed as follows:

$$p(t) = K_c[e(t) + \frac{1}{\tau_I}\int_0^t e(t)dt] \tag{53}$$

where K_c and τ_I are the gain and integral time of the controller. As seen from Equation (51), the controller action is the summation of a proportional action and an integral action to an error signal e(t). Parameters K_c and τ_I can be adjusted by the user of the controller. The transfer function for P-I controller is (by Laplace transform of Equation (53)):

$$G_I(s) = \frac{P(s)}{E(s)} = K_c(1 + \frac{1}{\tau_I s}) \quad (54)$$

P-I controllers can be adjusted in the laboratory by setting e(t) to a unit step function, and observing the slope and the jump of the controlled state variable ('reset rate' is defined as $1/\tau_I$, which is used by some manufacturers).

Proportional–derivative (PD) controller The response of P-D controller to an error signal e(t) is expressed in the following:

$$p(t) = K_c e(t) + K_c \tau_D \frac{de(t)}{dt} \quad (55)$$

where K_c and τ_D are the gain and derivative time of the controller. As seen from Equation (55), the controller action is summation of a proportional action and a derivative action to an error signal e(t). The values of K_c and τ_D can be adjusted by the user. Transfer function for P-D controller is:

$$G_1(s) = \frac{P(s)}{E(s)} = K_c(1 + \tau_D s) \quad (56)$$

Proportional–derivative–integral (PID) controller The response of a PID controller to an error signal *e(t)* is in the form of:

$$p(t) = K_c e(t) + K_c \tau_D \frac{de(t)}{dt} + \frac{K_c}{\tau_I} \int_0^t e(t)dt \quad (57)$$

where K_c, τ_D and τ_I are the adjustable parameters which have been described previously. Transfer function for a PID controller is:

$$G_1(s) = \frac{P(s)}{E(s)} = K_c(1 + \tau_D s + \frac{1}{\tau_I s}) \quad (58)$$

REACTOR OPTIMIZATION

Optimal control of bioreactors

Optimization, as an act of obtaining the best result under given conditions, can be expressed as a mathematical problem of maximizing or minimizing a certain function with several variables under certain constraints imposed on the variables. Since the aim of the optimizer is to operate the system in the best way, such a function representing the performance criterion of the system is called the objective function. The maximization or minimization of the objective function leads to the optimization of the system. The optimizer has several variables under his control which are called control variables. The optimization problem consists of the finding the values of the control variables

within the allowed region determined by the constraints which maximize (or minimize) the value of the objective function. This sequence of decisions is called an optimal policy and is of fundamental importance in the commercializations of bioreactors. In the following we briefly describe the mathematical foundation of optimal control of bioreactors.

We now assume that the state at time t of a bioreacting system is described by n 'state variables' $y_1(t), y_2(t), \ldots, y_n(t)$ satisfying n first-order differential equations of the form

$$\frac{dy}{dt} = f_i(y_1, y_2, \ldots, y_n; u_1, u_2, \ldots, u_r) \quad (59)$$

for $t_0 \leq t \leq T$, subject to the 'initial conditions'

$$y_i(t_0) = y_{i0} \quad (i = 1, 2, \ldots, n) \quad (60)$$

where y_{i0}'s are certain given numbers, $u_1 = u_1(t), u_2(t), \ldots, u_r(t)$ are the 'control variables', t_0 is the starting (initial) time and T is the termination (terminal) time of the process under consideration. We assume, in addition to the initial conditions Eq. (60), that at time t = T the 'terminal conditions'

$$y_i(T) = y^0_i \quad (i = 1, 2 \ldots, n) \quad (61)$$

are satisfied, where y_{i0}'s are certain prescribed numbers.

Let $y = y(t)$ and $u = u(t)$ be n- and r-dimensional vectors with components $y_1 = y_1(t), y_2 = y_2(t), \ldots, y_n = y_n(t)$, and $u_1 = u_1(t), u_2 = u_2(t), \ldots, u_r = u_r(t)$, respectively. We assume that a control $u = (u_1, u_2, \ldots, u_r)$ is 'admissible' if controllers $u_j = u_j(t)$ $(j = 1, 2, \ldots, r)$ are bounded and piecewise continuous in the interval $t_0 \leq t \leq T$ and satisfy the 'constraints'

$$Q_k(u_1, u_2, \ldots, u_r) \leq 0 \quad (k = 1, 2, \ldots, m) \quad (62)$$

and are such that the terminal conditions are also satisfied. The region Ω in the r-dimensional space defined by the inequalities (62) is called the 'control region' of the process. The set of all admissible controls is denoted by *U*. We assume that the set U is not empty.

According to the notation introduced in the preceding sections, the state variables $y_1, y_2, \ldots, y_n$ represent the sets of substrates $S_1, S_2, \ldots, S_s$, biological cells $X_1, X_2, \ldots, X_x$, and products $P_1, P_2, \ldots, P_p$ with $n = s + x = p$; similarly, the control variables $u_1, u_2, \ldots, u_r$, represent the feed-substrates $S_{1F}, S_{2F}, \ldots, S_{sF}$ with $r \times s$. Clearly, the state equations (Eq. 59) depend also on a number of constant parameters such as K_m, a, V_T, v_{in}, v_{out}, $v_{g,in}$, $v_{g,out}$, ... characterizing certain features of the bioreacting system. To simplify our presentation we assume that all of these constant parameters are fixed.

Now, let the performance of the bioreacting system under an admissible control $u = (u_1, u_2, \ldots, u_n)$ be measured by the performance criterion

$$J = J(u) = \int_{t0}^{T} f_0(y_1, y_2, \ldots, y_n; \quad u_1, u_2, \ldots, u_n)dt \quad (63)$$

Let the functions $f_i = f_i(y;u)$ $(i = 1, 2, \ldots, n)$ be continuous for all values

of y and for all $u \varepsilon \Omega$. Further, let the function $f_0(y;u)$ be continuous with its partial derivatives

$$\frac{df_0(y;u)}{dy_i} \quad (i = 1, 2, \ldots n) \tag{64}$$

for all values of y and for all $u \varepsilon \Omega$. Then, for each admissible control u the system of differential equations (Eq. 59) has a solution $y = y(t;u)$ for $t_o \leqslant t \leqslant T$ satisfying the conditions (Eq. 60) and (Eq. 61). This solution is called a 'trajectory'. To every admissible control u there corresponds a trajectory

$$y = y(t;u).$$

Consider now the 'functional' $J = J(u)$ defined by Eq. (63) which assigns the number $J(u)$ to each admissible control u. An admissible control $u^* = u^*(t)$ is said to be 'optimal' if the inequality

$$J(u^*) \leqslant J(u) \quad (\text{or } J(u^*) \geqslant J(u)) \tag{65}$$

holds for any other admissible control $u = u(t)$. By the 'optimal trajectory' we mean the trajectory which corresponds to the optimal control $u^* = u^*(t)$, i.e. $y = y(t;u^*)$. Then, optimization problem consists of the finding the conditions characterizing optimal controls and optimal trajectories.

It is convenient to treat the performance criterion functional defined by Eq. (63) as the final value $y_0(T)$ of an additional state variable $y_0 = y_0(t)$ satisfying the additional state equation

$$\frac{dy_0}{dt} = f_0(y_1, y_2, \ldots, y_n; \quad u_1, u_2, \ldots, u_r) \tag{66}$$

and the initial condition

$$y_0(t_0) = 0. \tag{67}$$

Since $y_0(T) = J(u)$ by definition, we can reformulate the above stated optimization problem as follows: Find an admissible control $u^* = u^*(t)$ which minimizes (or maximizes) $y_0(T;u)$ over all admissible controls $u = u(t)$:

$$\min_{(u \varepsilon U)} \; y_0(T;u) = y_0(T;u^*), \; u^* \varepsilon U. \tag{68}$$

The conventional procedure of solving the above stated optimization problem is to introduce (i) an (n+1)-dimensional vector $z = z(t)$, $z = (z_0, z_1, z_2, \ldots, z_n)$, so-called the 'adjoint vector' or 'co-state vector' and (ii) a 'Hamiltonian' $H = H(y,z,u)$ $(= H(y_1, y_2, \ldots, y_n, z_0, z_1, \ldots, z_n, u_1, u_2, \ldots, u_r))$, which satisfy the following relations:

$$H(y(t), z(t), u(t)) = \Sigma_{i=0}^{n} \; z_i(t) f_i(y_1, y_2, \ldots, y_n; \; u_1, u_2, \ldots, u_r) \tag{69}$$

$$\frac{dz_i}{dt} = -\frac{dH}{dy_i} = -\Sigma_{j=0}^{n} z_j \frac{df_j}{dy_i} \quad (i = 0, 1, 2, \ldots, n; \quad t_0 \leqslant t \leqslant T) \tag{70}$$

(co-state equations)

and

$$z_0(T) = 1 \tag{71}$$

Then, necessary conditions for optimal control are summarized in the theorem set out below.

Pontryagin's Maximum Principle

Let $u = u(t)$, $t_0 \leq t \leq T$, be an admissible control. In order that the functional $J = J(u)$ defined by Eq. (63) may be minimum (maximum) for the process described by the equations (59) and (66) with the given initial conditions at $t = t_0$,

$$y_i(t_0) = y_{i0} \quad (i = 0, 1, 2, \ldots, n)$$

and the given terminal conditions at some 'undetermined' time, $t = T$,

$$y_i(T) = y_{i0} \quad (i = 1, 2, \ldots, n)$$

it is necessary that there be a non-zero continuous co-state vector function $z = z(t)$ that satisfies equations (70) and (71) and that the control $u(t)$ be chosen so that the Hamiltonian $H(y(t), z(t), u(t))$ is zero and minimum at every t, $t_0 \leq t \leq T$.

There are other forms of the maximum principle and other optimization techniques, such as dynamic programming and discrete optimization, on which a large number of publications exist in the literature. A simple application of the maximum principle on a biotechnological process is given in Oguztoreli, Ozum and Gerson (1986). For the optimal control of lumped systems, i.e. systems described by ordinary differential equations, we refer the reader to Athans and Falb (1966), Bellman (1957), Boudarel, Delmas and Guichet (1971), Fan, (1966), Gumowski and Mira (1968), Lapidus and Luus (1967), Lee and Markus (1967), Petrov (1968) and Pontryagin *et al.* (1962); for time-lag control systems *see* Oguztoreli (1966); for distributed parameter systems, i.e. systems governed by partial differential equations, *see* Ahmed and Teo (1981), Aziz, Wingate and Balas (1977), Butkovskiy (1969) and Lions (1971).

Technology of substrate concentration control

SENSORS

Feedback controllers require three main components: sensors, control units and actuators. Sensors for many physical parameters, such as temperature and pressure, are plentiful, accurate and reliable. Sensors that determine the concentration of substrates are either marginally reliable or in experimental phases of development. Sensors for fermentation processes must either be sterilizable or separated from the fermentor by a sterile break, such as an ultrafiltration membrane. The use of separated sensors for substrate concentration control systems generates a time delay that must be compensated

for in the design of the control system (Kole, Thompson and Gerson, 1985; Oguztoreli, Ozum and Gerson, 1986).

The most reliable sensor for the determination of substrate concentration in fermentation media is the polaragraphic oxygen electrode. Commonly, these electrodes are temperature compensated and stable through sterilization. These electrodes employ silver electrodes, and are thus sensitive to fermentation by-products which react with silver, such as sulphides. These electrodes can be used to provide closed-loop control of dissolved oxygen concentration in conjunction with appropriate amplifiers, controllers and actuators (mass flow controllers regulating the rate of sparging, for instance). Dissolved-oxygen probes can also be used for the development of open-loop controllers in which dissolved oxygen concentration is controlled by substrate feed or by a combination of substrate feed and aeration rate control.

There have been numerous attempts to develop glucose sensors based on enzymes such as glucose oxidase (EC 1.1.3.4). These sensors cannot be sterilized and must be used to measure the glucose concentration of sterile ultrafiltrates of fermentation media. Typically, these would be collected continuously by subjecting a small sidestream from the fermentation vessel to tangential flow ultrafiltration, although probes employing integral filtration membranes have been developed. Cleland and Enfors (1983) describe an enzyme electrode for glucose and its use in glucose-fed batch fermentations of *E. coli*. Automated HPLC (high pressure liquid chromatography) systems (Dinwoodie and Mehnert, 1985) and automated analysers similar to those used for medical diagnostic applications (Imming, Schaller and Meiners, 1982) have also been used as the sensor component of substrate concentration control systems.

Sensors for other substrates are rare. Ammonium has been detected in a continuous sidestream through the use of ammonia gas electrodes (Kole, Thompson and Gerson, 1985). Nitrate concentration has been controlled by using a nitrate sensor (Kole *et al.*, 1986). Low molecular weight gases can be sensed either by specialized sensors such as that used by Mandenius and Mattiasson (1983) for ethanol, or by mass spectrometry.

Sensor development programmes are well established and promise to provide reliable substrate concentration detection systems for use in fermentation systems over the next few years.

CONTROL UNITS

Industrial process controllers are plentiful and are constantly undergoing improvement. Generally, these common devices are sensitive, stable and accurate. The current trend is towards increased adaptability to and compatibility with computer systems. At the present state in the development of the technology of fermentation process controllers, emphasis has shifted away from devices and towards complete control systems. In this section, controllers, control systems and some examples of their application are briefly reviewed.

Controllers consist of sensor input modules, control algorithms, however

simple or complex, and output modules which condition the signal for the activation of actuators. One major group of considerations is the relevance, accuracy and precision of the information provided to the controller by the process instrumentation (Flynn, 1982). Some data are easily obtained in a highly accurate and precise form (pressure and temperature, for example), but are not generally relevant to effective and useful process control in fermentation systems (the process is not highly sensitive to either temperature or pressure, and both are easily maintained at a constant level). Unfortunately, the current situation still is that the most important data are obtained with the greatest uncertainty and least reliability. For example, temperature can be accurately measured with equipment having a very low failure rate (0·35 faults/year), but pH, dissolved oxygen concentration or carbon dioxide concentration are at best measured to within a 0·1 log range and experience failure rates as high as 5–10 faults/year (Flynn, 1982). Most of the failures were in the sensors rather than in the controllers, indicating the focus future development should take.

Veres *et al.* (1981) and Nyeste and Sevella (1981) describe an integrated computer-controlled fermentation system having many of the general characteristics of integrated systems now available from a variety of manufacturers. Many considerations are applied to the selection of control units for fermentation systems, and two of paramount importance are tuning and the likelihood of error. One common procedure for tuning a PID controller is to trace the consequences of a step or pulse change in the operating conditions. In fermentation systems, the response to the step change depends not only on the conditions of the system at the time of the change, but also on the response of the organisms to the change. In some instances, a step change in dissolved oxygen concentration that is large enough to be useful in tuning a PID controller is also large enough to alter significantly the metabolism of the cells being cultivated. In other cases, the response time is sufficient to permit significant changes in the cell density. Once a PID controller has been tuned to the time constant of the system, it rapidly goes out of tune as cell density increases by the two orders of magnitude common to many fermentations.

The likelihood and consequences of controller error are also difficult to determine. Types of errors encountered with PID controllers include overshoot (pump continues operation past the set-point), creep (pump continues operation at a very low level when it should be off) and set-point offset. All can overload a culture with substrate, increasing response times and detuning the system with respect to the controller. Controller errors of even relatively short duration can kill the majority of cells in a culture. On–off controllers are less prone to failures due to time-constant changes during cultivation than are PI or PID controllers. Unlike many engineered systems, a fermentation system displays a variety of time constants ranging over approximately two orders of magnitude from hours (doubling times) to seconds (response to substrate depletion), and it may be desirable to develop specialized types of control algorithms designed to span this wide range of time constants.

Actuators convert the signals from the control unit to a rate of substrate

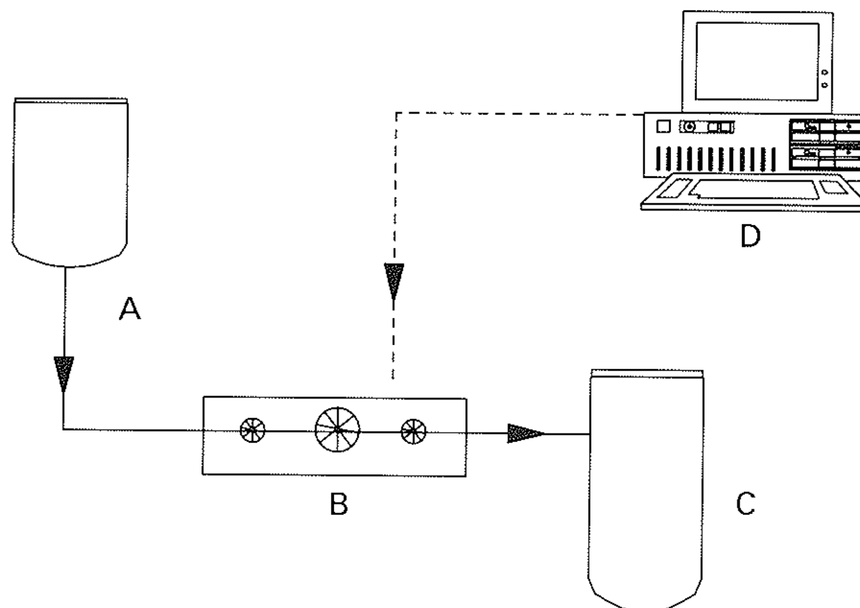

Figure 10. Kobio Pump, produced by Bioengineering AG, Wald, Switzerland. This system consists of a feed tank (A) and three pneumatic valves (B), and delivers metered volumes of feed solutions to a fermentor (C) regardless of backpressure. The system can be operated under microprocessor control (D).

addition to the fermentation system. Most commonly, substrates are added by peristaltic pumps or, when vessel pressure becomes a problem, by control valves regulating flow into the fermentation vessel from pressurized tanks. Peristaltic pumps with either continuous or stepping drives have been used, although the latter generally are more accurate and reliable, especially as part of proportional control systems.

Control valves regulating the flow from pressurized reservoirs to the fermentor can also operate in either continuous or discontinuous mode. On–off solenoid valves have the advantage of positive closure, but the disadvantage of allowing a direct connection between the fermentor and reservoir while open. Proportional control valves smoothly increase the rate of flow but also suffer from the direct connection problem. A three-valve system has recently been developed (personal communication, Bioengineering, AG) which allows punctuated dosage of the fermentor while preventing backflow (*Figure 10*).

COMPUTER MONITORING AND CONTROL OF FERMENTATION SYSTEMS

Introduction

The general design for a computer monitoring and control system is summarized in *Figure 11*, which presents an overall view of the flow of

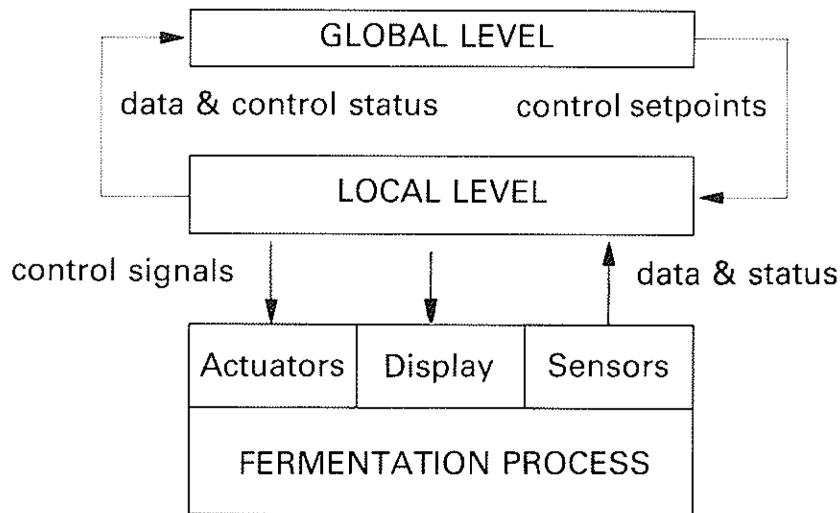

Figure 11. Control system design for a fermentation facility. The Global Level determines setpoints, archives data and provides data manipulation and finished display materials. The Local Level provides control signals to actuators to effect the desired setpoints, receives data from sensors in the fermentation process and provides real-time display. The Fermentation Process consists of a bioreactor system, sensors for all relevant parameters, actuators for all control actions and a real-time display sub-system.

information and control. In general outline, this schematic system is similar to some existing installations (Hatch, 1982; Rolf *et al.*, 1982; Titus *et al.*, 1984; Gerson and Kole, 1987; Kole and Gerson, 1988).

The design has the form of a hierarchy, with a number of fermentor stations at the lowest level, each with a local control and data acquisition facility. Some display capability is required at a secondary level, to monitor the current status of experiments. Data logging display of the long term of the fermentation behaviour is best done at the global level of the hierarchy, probably in a single host computer. Supervisory control algorithms exerting set-point control should be run at the global level to modify the parameters of on–off, PID or other control algorithms running at the local level. Hierarchical, adaptive controllers can be programmed in such a system, increasing the dynamic range of the overall control system.

In a typical system implementation, the control and data acquisition mechanisms should be largely independent of the characteristics of particular fermentation, but control strategies would be dependent on the scale of the fermentation, the type of equipment used, the nature of the substrate, the characteristic of the organism involved, and the detectability of the product produced.

In most cases, the system architecture would be implemented in stages, starting from the lowest level. At the lowest level, several fermentors with local control and data logging would be grouped, allowing implementation of a second hierarchical level. This level would consist of data logging and display

Table 5. Experimental parameters

Parameter	Measurement	Control
pH	Probes	On/off, PID
Pressure	Gauges	
Temperature	Thermistors, RTD	On/off, PID
Volume	Load cells, level sensing	On/off
Agitation speed	Tachometers	Proportional
Airflow	Flowmeters	Proportional
Redox potential	Probes	
Dissolved O_2	Probes	Cascade
CO_2	Probes	
NH_3	Probes	
Feed substrate	Load cells, flow rate	On/off
Output gases (O_2, H_2, CO_2, N_2, EtOH)	Mass spectrometer	
Organism growth	Turbidity, sampling	
Products	Probes, sampling Mass spectrometer	
Foam level	Probes	On/off

functions tied into the global level. The supervisory control algorithms are the most complex parts of the system and would be introduced when the other parts of the system are in place and working.

Experimental design

The measured parameters of each experiment are summarized in *Table 5*, together with indications of how each parameter will be measured and controlled.

The majority of the parameters are being measured in the usual manner (Hatch, 1982; Mor, 1982). For instance, a mass spectrometer is the best instrument to monitor effluent gases. This technique has proved to be very useful, as the ratio of O_2 to CO_2 in the effluent gases is a valuable indicator of the metabolic activity and state of the organisms in the fermentor (Buckland and Fastert, 1982). A mass spectrometer usefulness can be extended to monitoring the gases in solution within the fermentor as well (Pungor *et al.*, 1983).

Measurement of organism growth and product levels remains one of the more difficult aspects of fermentation monitoring. Cell growth can be estimated by turbidity measurements, but sampling will be necessary as well because few turbidometer probes perform well. The samples are also required to determine amounts of products, although it is hoped that enzyme-based sensors can be introduced to measure product levels directly (Danielsson and Mosbach, 1982). Assay results from samples should be added to the experimental logs with appropriate chronological information when the sample processing has been completed to permit co-ordinated analysis of electronically and manually recorded data.

Local control

The responsibility of each local control system is to measure and control many

of the measured parameters listed in *Table 5*. The control algorithms at this level should be primarily standard, hard-wired on–off, proportional or PID algorithms. The set-points and gains of these controllers should be capable of being modified by the global control centre. Local control should be implemented with either analogue controllers or direct digital controllers, depending on cost, simplicity and reliability in a given situation.

The local control level of the hierarchy should include appropriate amplifiers for conditioning sensor signals, and drivers to activate actuators within the fermentation system. There should also be sufficient communication capability at the local level for sending data and control status information to the global level, and for receiving control directives in return.

Local control should be relatively autonomous, having the ability to continue running the fermentation systems (at the currently established set-points) in the event of a failure at the global level. Local over-ride capability should also be available for some experimental parameters, but the use of manually defined set-points should cause the termination of global control, while still being monitored at the global level.

Data display capability at the local level is most profitably limited to the display of the state variable and control status throughout the time course of the current process. Display capability for historical comparisons or data analysis should be added at the global level.

Global level

At the global level, data should be collected in relational databases for display and analysis. A master display programme should be able to give a graphic or numeric representation of all the parameters measured either electronically or manually during the course of any given experiment. Ideally, analytical comparisons between experiments should be possible, with statistical analyses designed to aid in process optimization.

Overall control of a particular experiment should reside at the global level, and strategies to handle changes in scale, organism or desired product should be implemented at the highest level of the hierarchy. At this stage in the development of fermentation technology, this will be the most innovative part of the system, as control algorithms for fermentation processes are in their infancy.

As seen elsewhere in this chapter, many fermentation control algorithms are available in the literature (Zabriskie, 1979; Rolf and Lim, 1982); but the activity has only recently moved from the theoretical to the practical level. One form of control which has been successful in a number of experiments is based on materials balancing (Cooney, Wang and Wang, 1977). Effluent gases are monitored to measure the activity of organisms in the fermentor, and a substrate feed rate is controlled to maintain the culture in the desired metabolic state. Another approach depends on multivariate analyses of previous fermentation runs (Nyeste and Sevella, 1980). The desired parameters can be used to define an optimum trajectory for the fermentation. This is largely an offline approach, but a dynamic programming procedure can be employed to

correct, in real time, for variations from expected behaviour (Kishimoto, Yoshida and Taguchi, 1981). Adaptive techniques have also been tried with some success (Yousefpour and Williams, 1981).

More complete models of cell behaviour (Domach *et al.*, 1984) offer in principle, the most powerful tool for control, as an accurate simulation of a given organism would allow optimal control with a minimum of time-consuming and costly experimentation. Such simulations are very complex, and are only beginning to be applied to the control of real fermentation systems.

Conclusions

Substrate concentration control provides a realistic and practical route to increased productivities in the fermentation step of biotechnological processes. The effectiveness and efficiency of fed-batch fermentation systems either with or without feedback control of substrate concentration has been repeatedly demonstrated. With improved and more readily available sensors and controllers, the ease with which reliable substrate concentration control systems can be implemented will steadily increase. With improved techniques for planning experiments through process simulation and optimization, computer-controlled fermentations will be developed more easily and will become the norm in industrial fermentation processes.

As it becomes more possible to apply computer-based control to fermentation processes, the reproducibility and consistency of fermentations will steadily improve. As computer-controlled fermentations become a reliable component of manufacturing processes, computer-integrated manufacturing systems will perform as well in fermentation-based processes as in chemical or assembly-based manufacturing industries. Combined with manufacturing resource planning, these techniques should be capable of greatly increasing the efficiency and profitability of a wide variety of biotechnological manufacturing industries.

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