Analyses of Various Control Schemes for Continuous Bioreactors

Pramod Agrawal and Henry C. Lim
School of Chemical Engineering, Purdue University, West Lafayette, Indiana 47907, U.S.A.

A common framework is laid through which the feasibilities and efficacies of various control schemes for continuous bioreactors can be evaluated using only the steady state information. It is shown that many important and practical conclusion can be drawn based on the steady state growth models. For this purpose two well known steady state growth models are used, the Monod model and the substrate inhibition model. The control schemes that are reviewed and theoretically analyzed in terms of potential advantages as well as disadvantages, include turbidostats, nutristats, pH-auxostats and those based on various rates such as the base addition rate, the oxygen absorption rate, the oxygen uptake rate, and carbon dioxide evaluation rate. The feasibility of these control schemes is tested by cheking the local controllability and/or stability criteria, while the practical effectiveness is evaluated by analyzing the steady state gains. Existence of input multiplicity is also checked to point out potentially poor static and/or dynamic performances. Finally, a new control scheme is proposed, which is superior to the conventional continuous bioreactor operations and which allows for a multivariable control scheme.

1 Introduction

Slow dynamics usually associated with microbial growth in bioreactors, can result in off specification products over a significant time period. Unforseen disturbances in a bioreactor can cause havoc with the control system, perhaps resulting in a failure of the reactor and requiring a new start-up procedure. Due to the increasing cost of raw materials and capital investments, failures in biological processes could have catastrophic economic consequences. Therefore, there is a strong economic incentive for proper control of continuous bioreactors.

A recent development in biotechnology is the computerization of laboratory and industrial scale fermentation processes [1]. Real-time digital computers already in use, are suitable for on-line control of bioreactors. Application of computers [2] is however currently limited mostly to data logging and control of indirect variables, such as dissolved oxygen, pH, and temperature. Therefore, a large fraction of computer time is already available for implementation of sophisticated control schemes.

For large scale commerical processes such as cell mass and primary metabolite productions as well as laboratory scale operation for modeling microbial growth, there is a strong incentive to develop efficient control schemes that would enable rapid startup and stabilization of steady states in continuous bioreactors.

Even though numerous control schemes have been proposed for continuous bioreactors no rigorous analyses nor relative efficacies of these control schemes have been reported.

Different modes of controlling continuous bioreactors, that have appeared in literature are: turbidostats [3-11], nutristats [9], pH-auxostats [8, 12-16], and those based on the oxygen absorption rate [17-19], the oxygen uptake rate [19], and the carbon dioxide evolution rate [20, 12]. In these control schemes a representative variable, which characterizes the steady state balanced growth [22, 23] of microorganisms in a chemostat, is controlled by manipulating the flow of nutrient medium to a culture vessel.

Lack of accurate dynamic models has prevented analysis of dynamic responses of control systems and even when a reasonable dynamic model is available the analysis has been based on extensive simulation and the results have been specific to the particular model used. In this work we lay a common framework through which feasibility and efficacy analyses of various control schemes can be made using only the steady state information and show that important and practical conclusions can be drawn based on the steady state growth models. When appropriate, we cite previous simulation or experimental findings. The present work makes no attempt to simulate the dynamic characteristics of continuous cultures under various control schemes. In particular, various control schemes are reviewed and theoretically analyzed in terms of

a) local controllability,
b) local stability,
c) the steady state gain, and
d) input multiplicity.

The feasibility of control schemes is tested by checking local controllability and/or local stability criteria and the practical effectiveness is evaluated by analyzing the steady state gain.

Two well-known steady state growth models used in this analysis are, the Monod [24] model, and the substrate inhibition model [9, 25, 26].

A new control scheme is proposed. Compared to control schemes for the conventional bioreactor operation, the proposed control scheme a) statisfies the local controllability criterion at all steady states, b) can have high steady state gains at open-loop stable steady states, and c) can be made free from any problem associated with input multiplicity. Also, the new method provides additional flexibility to control continuous bioreactor operation by employing a multivariable control scheme.

2 Continuous Cultures

The steady state models used in this work are listed in Table 1, and also listed are the parameter values for each model.

Table 1. List of models and associated parameter values

Model	Form	Parameter values
1. Monod [24]	$\mu = \frac{\mu_m s}{K_m + s}$	$\mu_m = 0.4\ h^{-1}$, $K_m = 0.05\ g\ l^{-1}$ $s_F = 1\ g\ l^{-1}$, $y = 0.4\ g\ g^{-1}$ $D \leq \frac{\mu_m s_F}{K_m + s_F} = 0.381\ h^{-1}$
2. (a) Substrate inhibition [9]	$\mu = \frac{\mu_m s}{K_S + s + s^2/K_i}$	$\mu_m = 0.53\ h^{-1}$, $K_S = 0.12\%$ w/v
Constant yield	$y = y_0$	$K_i = 2.2\%$ w/v, $y_0 = 0.4\ g\ g^{-1}$
(b) Variable yield	$\frac{1}{y} = \frac{1}{y} + \frac{m}{\mu}$	$s_F = 4\%$ w/v $m = 0.01\ h^{-1}$
3. Substrate Inhibition model with variable yield [11]	$\mu = \frac{\mu_m s(1 - as)}{K_s + s + s^2/K_i}$	$\mu_m = 0.504\ h^{-1}$ $a = 0.204\%$ w/v, $K_3 = 8.49 \times 10^{-4}\%$ w/v
	$y = \frac{y_0(1 - as)}{1 + bs + cs^2}$	$K_i = 2.46$ $y_0 = 0.383\ g\ g^{-1}$, $b = 2.96\ (\%\ w/v)^{-1}$ $c = -0.501\ (\%\ w/v)^{-2}$ $s_F = 1\%$ w/v

2.1 Open-loop Operation: Chemostat

A chemostat, described by Novick and Szilard [27,28] and Monod [29], is a very popular mode of operating a continuous biological reactor. In this open-loop mode, the flow rate of nutrient to the bioreactor is held constant so that the dilution rate is less than the maximum specific growth rate. The medium contains excess of all but one nutrients. A steady state results when the specific growth rate of microorganisms balances exactly with the dilution rate. The steady state is said to be asymptotically stable if the reactor returns to its steady state after small perturbations. However, the attainment and maintenance of the desired steady state may be difficult. This problem, although manifested by many microbial cultures, can be predicted theoretically only for those cultures that exhibit substrate inhibited growth [25,30-31], but not [32-34] for those that follow the Monod [24] or the Monod-Herbert [35] type growth. This is not too surprising, if we consider the limited applicability [36-38] of steady state growth models under dynamic situations.

A continuous culture in its simplest form may be represented by the concentration of substrate, s, and the cell mass concentration, x. The material balances on the cell mass and the substrate of a constant-volume continuous-flow reactor, are

$$dx/dt = (\mu(s) - D)\, x \tag{1}$$

and

$$\frac{ds}{dt} = D(s_F - s) - \frac{\mu(s)}{y(s)} x \tag{2}$$

where μ, y, D, and s_F represent the specific growth rate, the yield of cell mass, the dilution rate, and the feed substrate concentration, respectively.

In a vector form Eqs. (1) and (2) can be written as

$$dX/dt = f(X, D) \tag{3}$$

where

$$X = \begin{bmatrix} x \\ s \end{bmatrix} \tag{4}$$

and

$$f(X, D) = \begin{bmatrix} f_1(X, D) \\ f_2(X, D) \end{bmatrix} = \begin{bmatrix} (\mu - D)\, x \\ D(s_F - s) - \dfrac{\mu x}{y} \end{bmatrix} \tag{5}$$

2.2 Closed-loop Operation

Consider the problem of microbial cell production in a continuous bioreactor (Eqs. (1) and (2)). Now let us suppose that we want to design a scheme for controlling the effluent cell concentration, x(t) to a desired values, x_d by manipulating the dilution rate D(t).

There are two problems, the startup problem of driving x(t) from an initial condition x_0 to the set point x_d and also the problem of maintaining x(t) at x_d. The startup problem, although important, is not considered here because the startup period usually represents only a small fraction of the total operational period of a continuous bioreactor. In this work, emphasis is laid on the control of continuous bioreactor at a desired steady state.

A common feedback controller is the proportional-integral-derivative (PID) controller given by

$$0 \leqq D(t) = D_s + K_c\left[(x_d - x) + \frac{1}{\tau_I}\int_0^t (x_d - x)\,dt' + \tau_d\frac{d(x_d - x)}{dt}\right] \quad (6)$$

where D_s is the steady state operating value of dilution rate, and K_c, τ_I and τ_d are controller parameters. For simplicity the proportional control mode will be used to compare various control schemes.

3 Criteria for Control System Evaluation

3.1 Controllability

Prior to tackling a design of control system, a notion called controllability is very useful in analyzing control systems. A precise definition of controllability is given in Refs. [39,40], but loosely speaking, a system is said to be controllable if there exists a control policy $U(t)$ which will drive the system from any given initial state X_0 to any other desired state X_d in finite time.

Consider a linear system

$$dX/dt = AX + BU \quad (7)$$

$$Y = CX \quad (8)$$

where X is a state vector of dimension n, U is a control vector of dimension m, Y is an output vector of dimensional l, and A, B, and C are constant matrices of dimensions $n \times n$, $m \times n$, and $l \times n$, respectively. This system is said to be completely controllable if and only if the rank of an $n \times nm$ "controllability matrix" L_c is n, where

$$L_c \equiv [B|AB|A^2B|\ldots|A^{n-1}B] \quad (9)$$

The notion of output controllability, which applies to the output Y, means the outputs are controllable if and only if the rank of the $l \times nm$ controllability matrix L_c^0 is l, where

$$L_c^0 \equiv [CB|CAB|...|CA^{n-1}B] \tag{10}$$

Unfortunately, a rigorous controllability criterion can be derived only for the linear system described above. However, local controllability criterion can be obtained through linearization of the nonlinear Eqs. (3) to (5) and application of the linear theory to the linearized equations. Local controllability is necessary, but not sufficient, for controllability of the original nonlinear Eqs. (3) to (5). Nevertheless, for most nonlinear systems good practical conclusions can be drawn by checking the local controllability criterion.

Linearizing Eqs. (3) to (5) around a non-trivial ($x \neq 0$) steady state

$$A = \partial f/\partial X|_{s.s.} = \begin{bmatrix} 0 & \mu' x \\ -\dfrac{\mu}{y} & -\mu - \dfrac{\mu' x}{y} + \dfrac{\mu y' x}{y^2} \end{bmatrix}_{s.s.} \tag{11}$$

where the prime denotes derivative with respect to s, also,

$$b = \partial f/\partial D|_{s.s.} = \begin{bmatrix} -x \\ s_F - s \end{bmatrix}_{s.s.} = \begin{bmatrix} -x \\ x/y \end{bmatrix}_{s.s.} \tag{12}$$

and the local controllability matrix

$$L_c = [b|Ab]_{s.s.} = \begin{bmatrix} -x & \mu' x^2/y \\ x/y & -\mu' x^2/y^2 + \mu y' x^2/y^3 \end{bmatrix}_{s.s.} \tag{13}$$

The system of Eqs. (3) to (5) satisfies the local controllability criterion if and only if the rank of L_c is two, or $\det L_c \neq 0$, where

$$\det L_c = -\frac{\mu y' x^3}{y^3} \tag{14}$$

For steady states of practical interest $x \neq 0$ and μ and y are non-zero quantities. Thus, L_c is singular only when $y' = 0$. Therefore, it can be concluded that the system of Eqs. (3) to (5) is locally controllable at all the non-trivial steady states, except at a steady state where the yield coefficient is constant or goes through local minima or maxima, $dy/ds = 0$.

3.2 Stabilizability [39,40]

A system is said to be stabilizable if the unstable states of the system can be made stable by controller action. Stabilizability condition for a system is much weaker than the controllability condition. A system which is controllable is automatically stabilizable.

The linear system of Eq. (7) is stabilizable, if the real parts of every eigenvalue of A can be made negative by controller action. If the real parts of every eigenvalue of A is negative, the system is automatically stabilizable (even without controller action).

Consider a proportional controller fed back on the state variables

$$U(t) = -KX(t) \tag{15}$$

where K is an $m \times n$ constant gain matrix, then Eq. (7) becomes

$$dX/dt = (A - BK)\,X \tag{16}$$

If elements of K can be chosen such that the real parts of all the eigenvalues of (A — BK) are negative, then the system is stabilizable by the above feed back controller.

With a proportional controller fed back on the output variables (Eq. (8)), we have

$$U(t) = -KCY(t) \tag{17}$$

and

$$dX/dt = (A - BKC)\,X \tag{18}$$

The output stabilizability requires that the real parts of all the eigenvalues of (A — BKC) be negative for some choice of the feed back gain matrix K.

For nonlinear systems local stabilizability conditions can be obtained by linearizing the system about a steady state and applying the results obtained for linear systems.

From Eq. (14) we conclude that a microbial system that exhibits a constant cell-mass yield ($y' = 0$) is not completely controllable in a continuous bioreactor. However, by manipulating D, it is possible to control x or s, but not both.

For the case of constant yield $y' = 0$, let

$$z = x - y(s_F - s) \tag{19}$$

Equations (1) and (2) can be reduced to

$$\frac{dx}{dt} = (\mu - D)\,x \tag{1}$$

and

$$\frac{dz}{dt} = -Dz \tag{20}$$

The last equation yields

$$z = z(0)\, e^{-\int_0^t D(t_1)\,dt_1} \tag{21}$$

Since, x can be controlled by manipulating D and the eigenvalue associated with z is always negative (hence z is stable), the system of Eqs. (1) and (20) (or (1) and (2)) is stabilizable. Even though the system is not completely controllable, it is possible to design a successful control system in which x is controlled and would take on the value given by $s = s_F + x/y$.

This type of continuous bioreactor control scheme is termed a turbidostat. Similarly, it is possible to design a control system in which s is controlled by manipulating D and x automatically reaches a steady state. Nutristat is a name given to this control scheme.

In general controllability and stabilizability have the following implications [40] in the design of control systems. If a system is completely controllable, a control scheme can always be designed. It may be impossible to design a control scheme for a system which is not completely controllable. But if such a system is stabilizable and the uncontrollable eigenvalues of the system are sufficiently large and negative, then it is possible to design an acceptable control scheme. However, it would generally be impossible to design a control scheme for a system which is not stabilizable.

3.3 Steady State Process Gain

The steady state process gain, K_y, is defined as the change in the controlled (output) variable resulting from a unit change in the manipulated variable, dY/dD. For a (scalar) proportional control, the proportional gain K_c is inversely proportional to the steady state process gain K_y, since the ultimate gain, K_cK_y, is the system property and may be assumed to be constant.

The steady state gain, K_y, is an important parameter in determining the effectiveness of a control scheme. A small value of K_y indicates an insensitivity of the manipulated variable to changes in the controlled variable. For example, a small value of K_y implies that a large change in the manipulated variable (controller action) is needed to correct for a small change in the controlled variable. This can cause saturation in the manipulated variable and can result in poor control action. Also, if there is a small and constant error in the measurement or estimation of the controlled variable, the manipulated variable, because of low K_y, would settle at a value entirely different from its designed steady state value. On the other hand, a high value of K_y implies that a small change in the manipulated variable is needed to correct for the controlled variable, thus avoiding the problem of potential valve saturation and poor control. Consequently, the steady state gain plays an important role in controller tuning.

3.4 Input Multiplicity [41]

A control system has multiple steady states when more than one set of manipulated (input) variables U can produce the desired steady state outputs Y. This type of multiplicity in a closed-loop system is called the input multiplicity. This is to distinguish from the output multiplicity in open-loop systems where one value of input U could produce more than one set of steady state values Y. In the presence of input multiplicity, a control system may exhibit poor static performance or dynamic

performance, or both. The static performance suffers from the fact that the controlled system may find an alternate steady state (since there is more than one value of the manipulated variable which maintain the same controlled variable) instead of finding the designed. The dynamic performance suffers due to poor controllability in the presence of multiple steady states. The necessary condition [41] for existence of input multiplicity in a nonlinear multivariable control system is the singularity of process gain matrix $\partial Y/\partial U$ at some steady states, or

$$\det (\partial Y/\partial U) = 0 \tag{22}$$

For a scalar control system Eq. (22) reduces to

$$K_y = \partial Y/\partial U = 0 \tag{23}$$

Equation (22) is also called the catastrophe condition [41].

We shall now analyze and compare various control schemes in terms of local controllability, local stabilizability, steady state gain, and input multiplicity.

4 Continuous Culture Control Schemes

4.1 Turbidostat

The evolution of laboratory scale chemostat and turbidostat [3] took place at the same time. In turbidostat the optical density, which is related to the cell mass concentration, is controlled by manipulating the medium flow rate. At a steady state the optical density or the turbidity in the bioreactor is held constant and hence the name "turbidostat". Turbidostat was introduced for a variety of reasons. Unlike a chemostat in which the growth is nutrient limited, the turbidostat provides opportunities to study growth under excess of nutrients [3]. It is also used to study the selection of mutants surviving in excess, toxic nutrient environments [5]. A turbidostat could be used to operate a bioreactor at near the maximum specific growth rate, a task for which chemostat is found to be unsuitable. It can also be used to study the development of mutants adaptable to higher growth-rate conditions [4]. Zines and Rogers [7] studied the effect of external addition of product inhibitor, ethanol, on the growth of *Klebsiella aerogenes* in a turbidostat. The turbidostat response to the above disturbance was oscillatory. Edwards et al. [9] proposed the turbidostat scheme to control a continuous bioreactor at open-loop stable steady states, when growing microorganisms that exhibit substrate-inhibited kinetics. Whaite and Gray [10] used a turbidostat to maximize the productivity, (μxv), of *Candida utilis*. However, the control was implemented in a feed-forward fashion which partly could be the reason for the process instabilities observed in their work. DiBiasio et al. [11] working with a methanol utilizing bacterium *L3*, which exhibits the substrate-inhibition kinetics, employed turbidostat to study the growth kinetics at open-loop unstable steady states. Natural instability and control of unstable steady states in continuous cultures was demonstrated in their work. Figure 1 presents a representative set of experimental profiles of x and

s for growth of *L3* in a turbidostat. Batch growth of *L3* occurred until an elapsed time of 15 hours. Thereafter, a proportional control was used to stabilize x near its set-point value. Within the accuracy of the experimental analysis, s also reached a steady state value. After sufficient time at the steady state the controller was disconnected as indicated by the arrows in Fig. 1 and the dilution rate was left at its steady state value. The washout which began shortly thereafter demonstrated instability of the steady state.

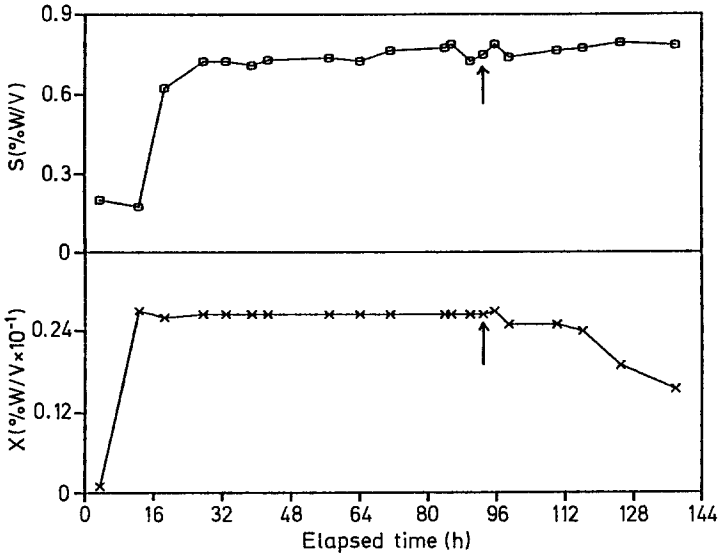

Fig. 1. Demonstration of control of unstable steady state and natural instability in a continuous culture of *L3* (after: DiBiasio et al. [11])

Assuming a proportional control on x in a turbidostat, we have

$$D(t) = D_s + K_{ct}(x_d - x) \geqq 0 \tag{24}$$

where k_{ct} is the proportional controller gain.

With the above proportional controller, the linearized closed-loop system of Eqs. (3)—(5) becomes

$$dX/dt = (A - bK_t^T)\,X \tag{25}$$

where A and b are given by Eqs. (11) and (12), respectively, and

$$K_t^T = [K_{ct}, 0] \tag{26}$$

Thus,

$$(A - bK_t^T) = \begin{bmatrix} K_{ct}x & \mu' x \\ -K_{ct}\dfrac{x}{y} - \dfrac{\mu}{y} & -\mu - \dfrac{\mu'}{y}x + \dfrac{\mu}{y^2}y'x \end{bmatrix}_{s.s.} \tag{27}$$

For local stability of the closed-loop system, K_{ct} should be chosen such that the real parts of the eigenvalues of $(A - bK_t^T)$ are negative, or,

$$\det(A - bK_t) = \left[\left\{\mu' - K_{ct}\left(y - \frac{y'x}{y}\right)\right\}\frac{\mu x}{y}\right]_{s.s.} > 0 \tag{28}$$

and

$$\mathrm{tr}(A - bK_t^T) = \left[K_{ct}x - \frac{\mu' x}{y} - \frac{\mu}{y}\left(y - \frac{y'x}{y}\right)\right]_{s.s.} < 0 \tag{29}$$

At open-loop steady states, from Eqs. (1) and (2) we obtain

$$x = y(s_F - s) \tag{30}$$

Therefore, the steady-state process gain, $K_y = K_x = dx/dD$ is obtained by differentiating Eq. (30) and recognizing $\mu = D$ at steady states

$$K_X = \frac{dx}{dD} = [(s_F - s)\,y' - y/\mu']_{s.s.}$$

$$= -[(y - y'x/y)/\mu']_{s.s.} \tag{31}$$

Upon substituting Eq. (31) into Eqs. (28) and (29), we obtain

$$\det(A - bK_t^T) = \left[\{\mu'(1 + K_{ct}K_x)\}\frac{\mu x}{y}\right]_{s.s.} > 0 \tag{32}$$

and

$$\mathrm{tr}(A - bK_t^T) = \left[K_{ct}x - \frac{\mu' x}{y} + \frac{\mu\mu'}{y}K_x\right]_{s.s.} < 0 \tag{33}$$

Table 2 gives a typical classification of steady states in continuous bioreactor under turbidostat control. It also indicates the proper range of K_{ct} values for stable closed-loop operation.

For the constant yield case ($y' = 0$) the stability conditions given by Eqs. (28) and (29) reduce to a single condition given by

$$[\mu' - K_{ct}y]_{s.s.} > 0 \tag{34}$$

If $\mu' > 0$, the steady state is open-loop stable, and by choosing $K_{ct} < 0$ the stability characteristics of the closed-loop system can be improved. For $\mu' < 0$ the open-loop steady state is unstable and by choosing K_{ct} such that

$$K_{ct} < \left[\frac{\mu'}{y}\right]_{s.s.} < 0 \tag{35}$$

the steady state can be stabilized.

For the Monod model with a constant cell mass yield the steady state gain K_x is given by

$$K_x = -\frac{\mu_m K_m y}{(\mu_m - D)^2} \tag{36}$$

Table 2. A typical classification of steady state in turbidostat

$\mu' > 0$		$\mu' < 0$
$K_x > 0$	$K_x < 0$	$K_x > 0$
*$K_{ct} \lesseqgtr 0$	$K_{ct} < 0$	$K_{ct} < \frac{-1}{K_x} < 0$

* Depending on the operating conditions

At low to moderate dilution rates where the growth is substrate limited, the steady state gains are very low and the turbidostat scheme may not be reliable. However, at dilution rates close to the maximum specific growth rate, where the growth is not substrate limited, the cell density falls rapidly with the dilution rate and the steady-state gains are high. Thus, the turbidostat would be effective. For given values of μ_m and s_F, higher the values of $K_m y$ larger will be the effectiveness range of turbidostat operation.

Figure 2 shows plots of (a) the cell concentration x and (b) the steady state gain K_x vs. the dilution rate D for the Monod model (1) and a substrate inhibition model (2a). The low conversion steady state in the substrate inhibition model is open-loop unstable [30,42]. In Fig. 2 and subsequent figures, the solid lines represent the open-loop stable steady states, while the dotted lines represent the open-loop unstable steady states.

For the substrate inhibition model the region of rapidly falling cell concentration does not represent the open-loop stable steady states. Therefore, K_x at stable steady states has low values, except in a narrow range of dilution rates near the maximum specific growth rate. However at the unstable steady states, K_x values are high, making the turbidostat scheme effective as was experimentally demonstrated by DiBiasio et al. [11].

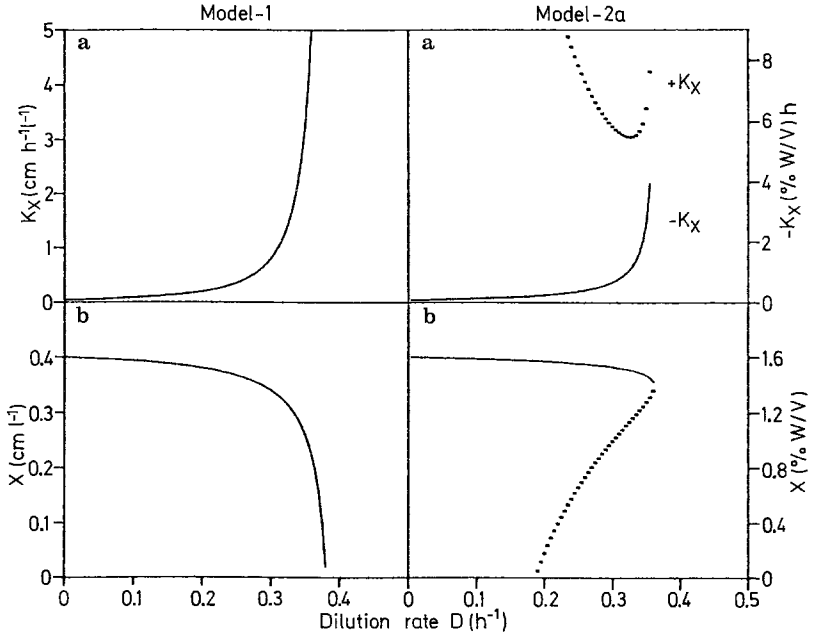

Fig. 2a and b. Plots of (a) steady state gain, K_x, and (b) cell mass concentration, x, vs. dilution rate, D, for the Monod model (1) and a substrate inhibition model 2a

If the cell mass yield varies as a result of the maintenance requirement (Model 2b), K_x is obtained from Eq. (31) as

$$K_x = \frac{my_0^2 s_F}{(\mu + my_0)} - \frac{\mu y_0}{(\mu + my_0)\,\mu'} \tag{37}$$

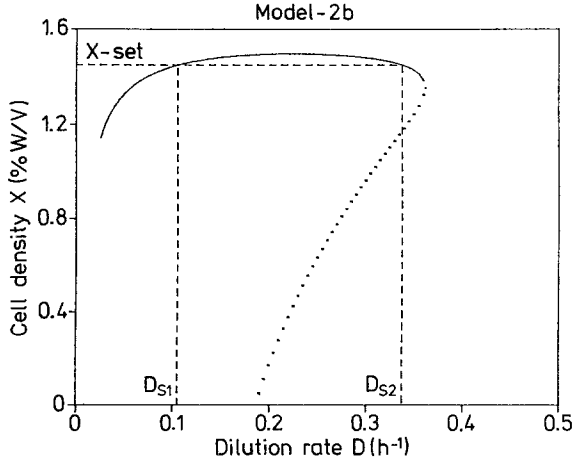

Fig. 3. Plot of cell mass concentration, x, vs. dilution rate, D, for a maintenance model 2b

Thus, K_x changes sign (goes through zero) at an open-loop stable steady state and satisfies Eq. (23). Therefore, the maintenance term may lead to the problems associated with input multiplicity. This is so illustrated in Fig. 3 where two different dilution rates yield the same cell concentration.

The controller in turbidostat scheme is constrained. When $x < x_d$, the controller reduces the nutrient flow rate and therefore, indirectly increases the cell concentration by washing out less cells rather than directly adding cells to the bioreactor. If the integral action is also used in the control algorithm, the error builds up forcing D to be zero in Eq. (6). Furthermore, if the transient cell mass yields are low, it is not difficult to conceive of a situation where $x < x_d$, $s = 0$ and $D = 0$. When this happens, the controller will fail. This is another shortcoming of a turbidostat.

4.2 Nutristat

As early as 1957 Fuld and Dunn [43] proposed automatic control of fermentors based on the residual substrate concentration. Edwards et al. [9] used the term nutristat for such a type of bioreactor control scheme. Unavailability of a measurement device capable of continuously monitoring the residual substrate concentration in a bioreactor, hindered the application of nutristat. Much effort is made by many [44–49] to develop sensors for industrially important substrates. A successful report dealing with direct nutristat control of continuous bioreactors is yet to appear. However, control of nutrients in fed batch fermentors has been reported [43,47].

For a proportional control on the residual substrate concentration in a nutristat, we have

$$D(t) = D_s + K_{cn}(s_d - s) \geqq 0 \tag{38}$$

$$dX/dt = (A - bK_n^T)\,X \tag{39}$$

where K_{cn} is the proportional controller gain, s_d is the set point value of the substrate concentration, and

$$K_n^T = [0 \;\; K_{cn}] \tag{40}$$

Thus,

$$(A - bK_n^T) = \begin{bmatrix} 0 & \mu' x + K_{cn} x \\ -\dfrac{\mu}{y} & -\mu - \dfrac{\mu' x}{y} + \dfrac{\mu y' x}{y^2} - \dfrac{K_{cn} x}{y} \end{bmatrix}_{s.s.} \tag{41}$$

and K_{cn} should be chosen such that

$$\det(A - bK_n^T) = \left[\frac{\mu x}{y}(\mu' + K_{cn})\right]_{s.s.} > 0 \tag{42}$$

and

$$\mathrm{tr}(A - bK_n^T) = \left[- K_{cn} \frac{x}{y} - \frac{\mu' x}{y} - \frac{\mu}{y} \left(y - \frac{y' x}{y} \right) \right]_{s.s.} < 0 \tag{43}$$

Clearly, from Eqs. (42) and (43), a positive K_{cn} should be chosen for a proper nutristat control at all steady states in continuous bioreactor.

For a nutristat the steady state gain K_s is given by

$$K_s = \left[\frac{ds}{dD} \right]_{s.s.} = \frac{1}{[\mu']_{s.s.}} \tag{44}$$

For the case of constant yield ($y' = 0$) K_{cn} should be such that

$$[\mu' + K_{cn}]_{s.s.} = \frac{1}{K_s} + K_{cn} > 0 \tag{45}$$

For control at open-loop stable steady states ($\mu' > 0$), $K_{cn} > 0$; and for stabilization of the open-loop unstable steady states ($\mu' < 0$),

$$K_{cn} > (-\mu')_{s.s.} = - \frac{1}{K_s} > 0 \tag{46}$$

From Eqs. (31) and (44) for constant yield case

$$K_s = - \frac{1}{y} K_x \tag{47}$$

In general the cell mass yield (g of cells per g of substrate) is less than one, therefore, the steady state nutristat gains are higher than the turbidostat gains. Thus, the nutristat response is expected to be better than the turbidostat response, consistent with the extensive simulation results of Edwards et al. [9]. The cell mass yield of most microorganisms is in the neighborhood of 0.5 g g^{-1}, hence the steady state gains of nutristat would be about twice the turbidostat gains. This moderate increase in K_s over K_x may not be sufficient to ensure a feasible nutristat control in the region where turbidostat is ineffective. Also, the substrate concentrations at substrate limited growth conditions are usually very low so that even small measurement errors would adversely affect the nutristat operation. Nutristats, however, have other advantages over turbidostats: a) absence of input multiplicity and b) absence of problems associated with the constrained controller, as found in turbidostats; contrary to turbidostat, a nutristat requires positive controller action ($K_{cn} > 0$, whereas K_{ct} is generally less than zero).

4.3 pH-Auxostat

The pH has a marked influence on the rate of microbial growth. Characteristically there is a pH value at which the growth rate is optimal and on each side of this optimum the growth rate is lower. The pH is controlled by the addition of suitable buffers.

In bioreactors, the pH is usually controlled at an optimum value by the addition of acid or base. Changes in the pH occur as a result of nutrient uptake or due to acid production by microorganisms. Manipulation of the nutrient flow based on the pH change in continuous culture is possible, if there exisits a definite correlation between the microbial growth and the pH change. Watson [8] indicated the possibility of continuous-culture control based on pH as the controlled variable. Martin and Hempling [12] gave a brief analysis of a pH-auxostat, and since then few reports have appeared on successful operations of pH-auxostats [12-17]. Interestingly a pH auxostat was shown by Martin and Hempling [12] to cause a smooth transition of a continuous culture of *E. coli B* from aerobic to anaerobic growth condition and vice versa. Their experimental results are reproduced in Fig. 4. When the steady state aerobic growth

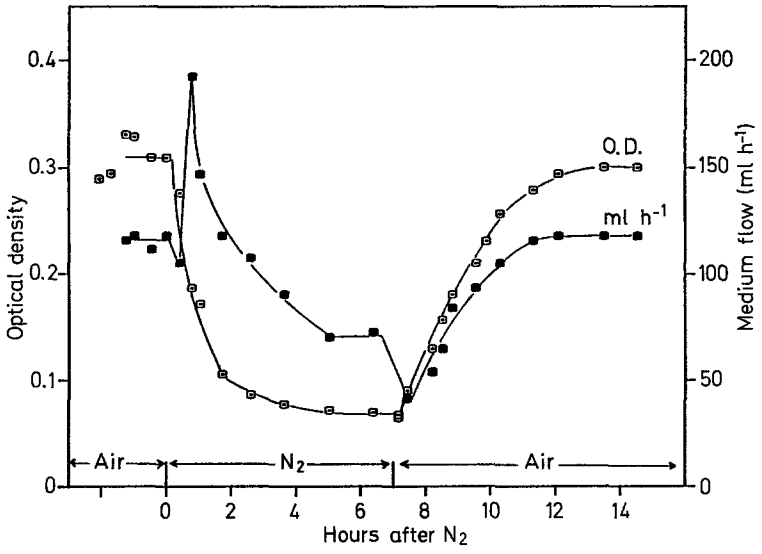

Fig. 4. Population density and medium flow rate under aerobic and anaerobic growth of *E. coli B* in a pH-auxostat (after: Martin and Hempling [12])

of microorganisms is perturbed by cessation of aeration, the population density falls and reaches a new steady level corresponding to the anaerobic growth condition. On the other hand, the medium flow rate increases sharply in the beginning stage of anaerobic growth and gradually decreases to a steady state value. Upon reaeration of the continuous culture at 7 hours the population density increases to the original steady state level corresponding to the aerobic growth condition, while the medium flow rate decreases sharply first and then increases to the original steady state value.

Consider a microbial growth system where the growth is associated with the production of acid (H^+ ions). In pH-auxostat, the pH is controlled by feeding an appropriate amount of medium containing either a buffer or base [13, 15]. We define the buffering capacity, BC, of a medium as BC moles of H^+ ions absorbed by a liter of

medium to change its pH to the set point pH (pH_d) in the bioreactor. The H^+ ion balance in a continuous-biological reactor yields

$$\frac{d(H^+)}{dt} = \mu x y_{H^+/x} - D(BC) - D(H^+ - H_d^+) \tag{48}$$

where $y_{H^+/x}$ is the yield of acid (moles of H^+ per g of cell mass) and H_d^+ is the set point pH, pH_d. At a steady state, $dH^+/dt = 0$, $H^+ = H_d^+$, and $\mu = D$, and therefore, from Eq. (48) we obtain

$$BC = x y_{H^+/x} \tag{49}$$

Thus, at a controlled steady state the buffering capacity of nutrient medium determines the cell mass concentration x, which in turn determines the steady state value of the specific growth rate.

The steady state gain of pH-auxostat is related to the steady state gain of turbidostat by $y_{H^+/x}$,

$$K_{H^+} = y_{H^+/x} K_x \tag{50}$$

An interesting situation occurs when the specific rate of acid production μ_{H^+}, follows Luedeking and Piret [50] form (Eq. (51)) as in the lactic acid production [16] from lactose under nitrogen or energy limited conditions. The specific rate of acid production is given by

$$\mu_{H^+} = \mu y_{H^+/x} = \alpha\mu + \beta \tag{51}$$

and therefore,

$$y_{H^+/x} = \alpha + \beta/\mu \tag{52}$$

where α and β are constants. The steady state gain K_{H^+} is obtained by substituting Eq. (52) to Eq. (50)

$$K_{H^+} = (\alpha + \beta/\mu)\, K_x \tag{53}$$

The K_{H^+} values are high at low dilution rates ($\mu = D$) and therefore, unlike the turbidostat scheme the pH-auxostat scheme would work well at low dilution rates.

4.4 Rate Controlled Continuous Cultures

4.4.1 Base-Addition Rate (BAR) Controlled Bioreactor

In a pH-auxostat the pH in the bioreactor may vary substantially from the setpoint value during the transient to the desired steady state. Variations in the pH could have deleterious effects on microbial growth [51, 52]. This could make the pH-auxostat

undesirable. The pH-auxostat also has another disadvantage due to its operational limit caused by the buffering capacity of the nutrient medium [13, 15]. These drawbacks of pH-auxostats suggest a new mode of control in which the pH is independently controlled by the addition of a base, and the base addition rate, (BAR), is controlled by manipulating the dilution rate. A BAR controlled bioreactor is currently under experimental investigation in our laboratory.

For simplicity we assume the pH of the nutrient medium to be the same as the steady-state pH in the bioreactor. The rate of acid production by microorganism is given by

$$vr_{H^+} = \mu_{H^+} vx = \mu y_{H^+/x} vx \tag{54}$$

The pH is maintained constant usually by the addition of a base or an acid. To maintain a constant pH in a continuous culture, the base addition rate must be equal to the acid production rate. Thus,

$$BAR = vr_{OH^-} = vr_{H^+} = y_{H^+/x}(\mu vx) \tag{55}$$

At a controlled steady state the base addition rate determines the cell mass productivity (μvx).

4.4.1.1 Output Controllability

We consider BAR as an output variable for the system of Eqs. (3) to (5), therefore,

$$Y = BAR = y_{H^+/x}\mu vx \tag{56}$$

and for simplicity we assume $y_{H^+/x}$ to be constant. The linearized output controllability matrix L_c^0 is given by Eq. (10), or

$$L_c{}^0 = [cb \mid cAb]_{s.s.} \tag{57}$$

where A and b are given by Eqs. (11) and (12), respectively, and

$$c^T = \left[\frac{\partial y}{\partial x} \frac{\partial y}{\partial s}\right]_{s.s.} = y_{H^+/x} v[\mu \mid \mu' x]_{s.s.} \tag{58}$$

Substituting Eqs. (11), (12) and (58) into Eq. (57) we obtain

$$L_c^0 = y_{H^+/x} v \left[x\left(-\mu + \frac{\mu' x}{y}\right) \middle| \frac{\mu' x^2}{y}\left(\mu - \frac{\mu' x}{y} + \frac{\mu y' x}{y^2}\right)\right]_{s.s.} \tag{59}$$

The output variable BAR is locally controllable, if and only if at least one of the two terms in L_c^0 is non-zero. The two terms are simultaneously zero only when

$$\left[\frac{\mu' x}{y} - \mu\right]_{s.s.} = 0 \quad \text{and} \quad y' = 0 \tag{60}$$

For a microbial system that exhibits a constant cell-mass yield ($y' = 0$) at steady states

$$\frac{d(\mu x v)}{dD} = \frac{vy}{\mu'}\left[\frac{\mu' x}{y} - \mu\right] \tag{61}$$

Thus, the condition given by Eq. (60) states that the output (BAR) fails to satisfy the local controllability criterion only at a steady state that corresponds to the maximum cell mass productivity in a continuous microbial system.

From Eq. (55) the steady state gain, K_b, for a BAR controlled bioreactor is

$$K_b = \frac{d(BAR)}{dD} = y_{H^+/x} v \left[x + D\frac{dx}{dD}\right]_{s.s.} \tag{62}$$

Figure 5 shows the splots of a) K_b and b) BAR vs. the dilution rates for the Monod model (1) and the substrate inhibition model (3). An interesting feature of the rate

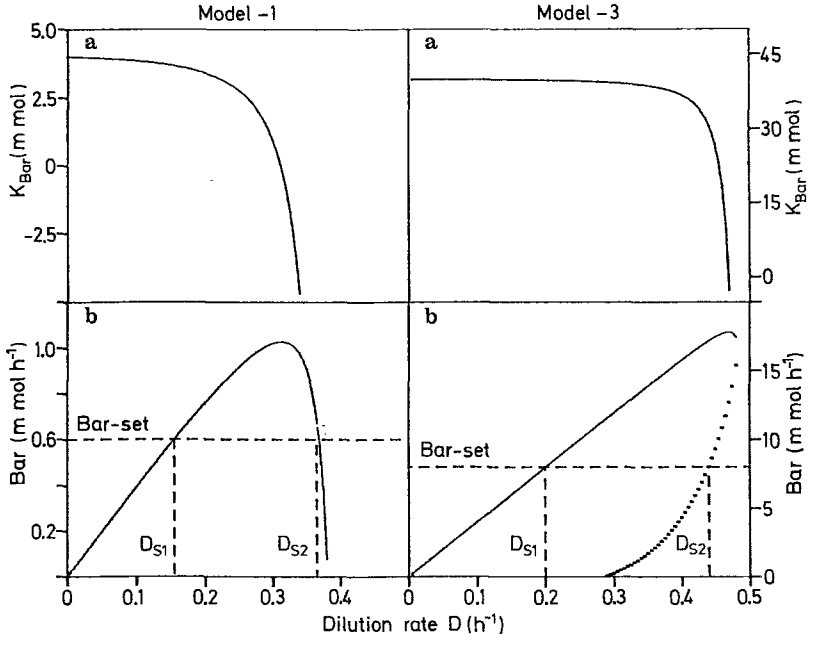

Fig. 5a and b. Plots of (a) steady state gain, K_{BAR}, and (b) base-addition rate, BAR, vs. dilution rate, D, for the Monod model 1 and a substrate-inhibition model 3. V = 1.0 l and $Y_{H^+/x}$ = 10.0 mmol (OH^-) per g cell-mass

controlled operations, as opposed to the control modes considered previously, is that the steady state gain depends not only on the growth associated term (i.e. term involving dx/dD) but also on xv. Therefore, the steady state gain depends on the operating conditions of a bioreactor, such as s_F and v. As a result, the K_b values are higher than the corresponding K_x values in the substrate limited growth region (where dx/dD is low). In this region a rate controlled operation would be effective.

If a proportional controller is employed in a BAR-controlled bioreactor, we have

$$D(t) = D_s + K_{cb}(BAR_d - BAR) \geqq 0 \tag{63}$$

$$dX/dt = (A - b^T K_{cb} c)\, X \tag{64}$$

where K_{cb} is the controller gain. For local stability of the closed loop system the choice of K_{cb} should be such that the eigenvalues of $(A - b^T K_{cb} c)$ have negative real parts. From Eqs. (11), (12), (58), (62) and (64) we obtain the following stability conditions

$$\det(A - b^T K_{cb} c) = \left[\mu' \left\{1 + \frac{K_{cb}}{y_{H^+/x}} K_b\right\} \frac{\mu x}{y}\right]_{s.s.} > 0 \tag{65}$$

$$\mathrm{tr}(A - b^T K_{cb} c) = \left[\mu - \frac{\mu' x}{y}\left(1 + \frac{K_{cb}}{y_{H^+/x}} K_b\right) + \frac{\mu y' x}{y^2}(1 - K_{cb} v x)\right]_{s.s.} < 0 \tag{66}$$

For constant yield ($y' = 0$) we have a simplified stability condition given by

$$\left[\mu'\left(1 + \frac{K_{cb} K_b}{y_{H^+/x}}\right)\right]_{s.s.} > 0 \tag{67}$$

Table 3 gives a typical classification of steady states in a rate-controlled continuous bioreactor. The table also gives proper K_{cb} limits for a stable closed-loop operation. At the maximum cell mass productivity, $d(\mu x v)/dD = 0$, the steady state gain K_b changes its sign and is equal to zero. Therefore, from Eq. (67) (and also Eq. (60)) control near the maximum cell productivity is very difficult.

Table 3. A typical classification of steady states in the BAR controlled continuous bioreactor

$\mu' > 0$		$\mu' < 0$
$K_b > 0$	$K_b < 0$	$K_b > 0$
$K_{cb} > 0$	$K_{cb} < 0$	$K_{cb} < \dfrac{-y_{H^+/x}}{K_{cb}} < 0$

The rate-controlled bioreactor operation exhibits input multiplicity because the steady state gain K_b changes sign and satisfies the condition given by Eq. (23). As shown in Fig. 5, for both models (Monod and Substrate Inhibition) there exist two steady state values of dilution rates for a given set point value of the base addition rate (except at the maximum productivity). Let D_{S1} and D_{S2} ($D_{S2} > D_{S1}$) represent

the two steady state dilution rates for a set point value of BAR (Fig. 5). In general, for a particular choice of K_{cb}, say $K_{cb} > 0$, the steady state corresponding to the low dilution rate D_{S1} would be stable, while the steady state corresponding to the higher dilution rate D_{S2} would be unstable. Therefore, the input multiplicity would not cause poor static performance of the closed-loop system. Ultimately, the system would settle at the designed steady state.

As opposed to the turbidostat scheme, the BAR-controlled bioreactor operation would be little, if at all, affected by a) variations in the cell-mass yield due to the maintenance requirement, and b) constraints on the controller.

4.4.2 Oxygen Absorption Rate (OAR) and Oxygen Uptake Rate (OUR) Controlled Bioreactors

For aerobic cultures the two most important substrates are the carbon source in the nutrient medium and oxygen in the fermentor gas stream. In these modes of operations, the absorption/uptake rate of oxygen is used to regulate the feed flow rate of the nutrient medium containing the carbon source. The dissolved-oxygen concentration (DOC) which is related to OAR and the off-gas oxygen concentration (OOC) which is related to OUR, can be used as controlled variables in OAR and OUR controlled schemes, respectively. The control based on OAR was used by Hospodka [17] to monitor a continuous culture of *Saccharomyces cerevisiae*. The regulation of pulse feed [18,53] of the limiting nutrient by a control based on the DOC level has been reported also. Yamada et al. [19] controlled the continuous fermentation of sorbital to sorbose by *Acetobacter suboxydans* using the partial pressure of oxygen in effluent gas and also the dissolved oxygen concentration in culture broth as controlled variables. The control scheme based on the partial pressure of oxygen gave a better performance than the one based on the dissolved oxygen concentration. Their typical experimental results on continuous culture control based on the measure-

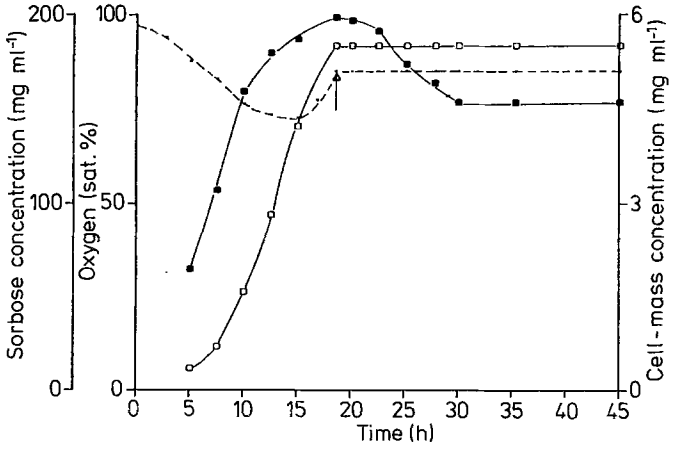

Fig. 6. Time profiles of cell mass and sorbose concentrations and partial pressure of oxygen in effluent gas in a OUR controlled continuous culture of *Acetobacter suboxydans* (after: Yamada et al. [19].) (——— oxygen, —□— sorbose, and —■— cell mass)

ment of partial pressure of oxygen in effluent gas are reproduced in Fig. 6. This figure shows time courses of cell mass and sorbose concentrations, and partial pressure of oxygen in effluent gas. Batch growth of microorganisms occurred until an elapsed time of 18.5 hours. Thereafter, the feedback control was initiated as indicated by an arrow in Fig. 6. The sorbose concentration reached a constant value very quickly, while cell mass concentration took about 12 hours to reach the steady state value.

4.4.3 Carbon Dioxide Evolution Rate (CER) Controlled Bioreactors

Almost all microorganisms derive the energy required to convert the substrate into cell mass or product by oxidizing a portion of the supplied carbon source to CO_2. At steady state balanced growth conditions the energy producing pathways are coupled with the energy requiring pathways of cellular metabolism. There exisit a constant relationship between the substrate uptake rate and the CER. This idea is used in the CER-controlled bioreactors, in which the off gas CO_2 concentration (which is related to CER) is used to manipulate the nutrient feed rate to the bioreactor. Control based on CER was first demonstrated by Watson [8,20]. The operation was also found feasible for the continuous culture control of a methanol utilizing bacterium (*L3*) [21]. Experimental results of Lee [21] on the control of continuous culture of *L3* based on the CO_2 concentration in the effluent gas are presented in Fig. 7. As indicated by an arrow in Fig. 7, a PID control was initiated during a batch growth of *L3*. After a brief transient growth period of 30 hours the controller was able to stabilize the continuous culture as represented by the constant values of feedflow rate and cell mass and CO_2 concentrations.

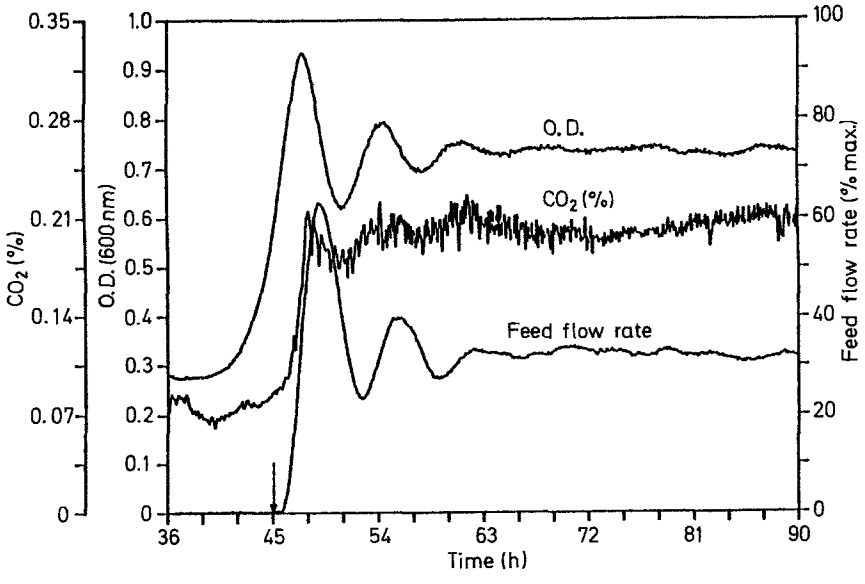

Fig. 7. Time profiles of optical density, CO_2 concentration in effluent gas, and feedflow rate in a CER controlled continuous culture of *L3* (after: Lee [21])

Bioreactor controls based on OAR, OUR and CER are similar to the BAR-controlled bioreactor. In each case the set value of the controlled variable determines the cell mass productivity, (μxv). General conclusions derived from the theoretical analysis of the BAR-controlled bioreactor also applay to OAR, OUR and CER controlled bioreactors.

5 A Novel Continuous Culture Operation

The turbidostat scheme for a conventional bioreactors is found to be unsuitable when the growth is substrate limited. This is not too surprising, because the control of cell density in a turbidostat is carried out by manipulating the flow rate of medium that contains the limiting substrate. In a turbidostat when the cell concentration is greater than the desired value, $x > x_d$, the controller would increase the dilution rate to increase the washout of cells, but this also increases the nutrient addition rate, Ds_F. Under the substrate limited growth condition, higher growth rates due to increased nutrient addition rates would slow the decrease in the cell concentration. Similarly, when $x < x_d$ the controller would decrase the dilution rate to slow down the washout of cells, but this also decreases the nutrient addition which would slow the increase in x. As a result of the two opposing effects, larger changes in the dilution rate are required to bring about smaller changes in the x value. This, obviously, is an undesirable characteristic of a controller. This drawback can be overcome in a continuous bioreactor with two feed streams. One feed stream contains the limiting substrate and the other stream contains all but the limiting nutrients. Alternatively, a pure water stream and a stream containing the concentrated amounts of the limiting substrate and other nutrients can be used. This novel continuous bioreactor operation is schematically shown in Fig. 8.

The cell mass and substrate balance equations for a bioreactor with two feed streams are

$$dX/dt = f_m(X, U) \tag{68}$$

$$X = \begin{bmatrix} x \\ s \end{bmatrix} \tag{69}$$

$$U = \begin{bmatrix} D_m \\ D_c \end{bmatrix} \tag{70}$$

and

$$f_m = \begin{bmatrix} -(D_m + D_c)\, x + \mu(s)\, x \\ D_C s_c - (D_m + D_c)\, s - \dfrac{\mu(s)\, x}{y(s)} \end{bmatrix} \tag{71}$$

At a non-trivial steady state ($x \neq 0$)

$$\mu = D_m + D_c = D \tag{72}$$

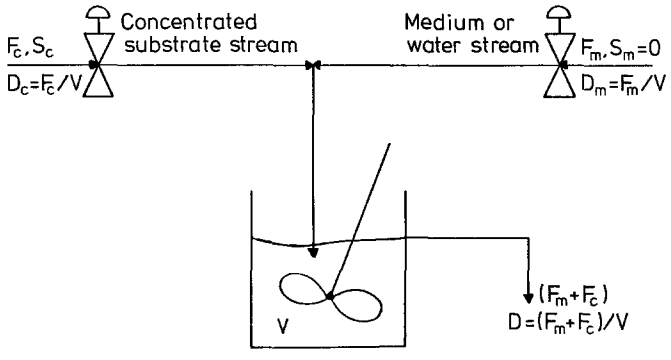

Fig. 8. A novel continuous culture operation

and the effective feed substrate concentration is

$$s_E = \frac{D_c s_c}{D_m + D_c} \tag{73}$$

and

$$x = y(s_E - s) = y\left(\frac{D_c s_c}{D_m + D_c} - s\right) \tag{74}$$

In this scheme both D_m and D_c can be controlled independently. Linearizing Eqs. (68) to (71) around a non-trivial steady state, we obtain

$$dX/dt = A_m X + BU \tag{75}$$

where

$$A_m = \partial f_m/\partial X|_{s.s.} \tag{76}$$

$A_m = A$ and is given by Eq. (11), and

$$B = \partial f_m/\partial U|_{s.s.} = \begin{bmatrix} -x & -x \\ -s & s_c - s \end{bmatrix}_{s.s.} \tag{77}$$

since det $B = -xs_c$ is non-zero, the rank of matrix B is two. Therefore, the rank of local controllability matrix for the system of Eq. (68) through (72), is also two. Hence, it can be concluded that all non-trival steady states are locally controllable. It should be noted that the local controllability criterion is met for all microbial systems whose steady state growth in continuous bioreactors can be modelled in terms of variables $\mu(s, x)$ and $y(s, x)$.

Consider the novel continuous bioreactor scheme in which the feed stream containing the limiting substrate is supplied at a constant rate, and the flow rate of other

stream is manipulated to control the cell density. This scheme is called 'modified turbidostat' and is schematically shown in Fig. 9.

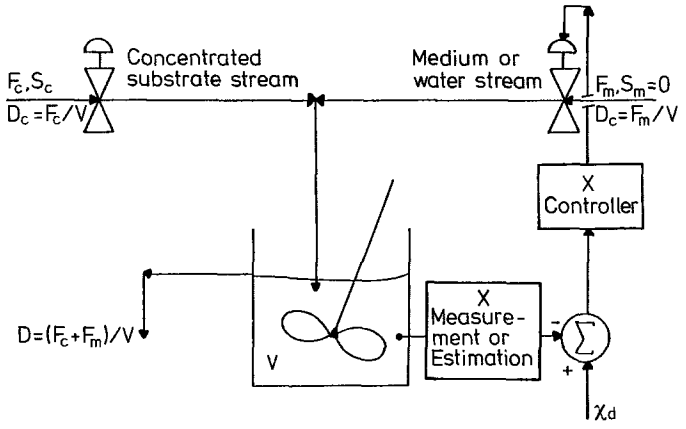

Fig. 9. A modified turbidostat control scheme

If a proportional control is used on D_m we have

$$D_m = D_{ms} + K_{cmx}(x_d - x) \geqq 0 \tag{78}$$

where K_{cmx} is the proportional gain. The steady state gain for the modified turbidostat operation is obtained from Eq. (74) as

$$K_{mx} = \frac{dx}{dD_m} = \frac{dx}{dD} = -\left[y \frac{D_c s_c}{\mu^2} + (y - y'x/y)/\mu' \right]_{s.s.} \tag{79}$$

Comparing Eqs. (31) and (79) we find that the steady-state gain of the modified turbidostat is higher than that of conventional turbidostat by yD_cs_c/μ^2. In the modified scheme the high steady state gains result at an expense of a lower saturation limit on the manipulated variable, D_m. For a constant μ the lower saturation limit on the manipulated variable for the conventional turbidostat is $D = \mu$, while for the modified turbidostat is $D_m = \mu - D_c$. In the modified scheme we choose D_c such that the product of the increase in the steady state gain yD_cs_c/μ^2, and the saturation limit on the manipulated variable, $D_m = \mu - D_c$, is maximized for a given μ. Therefore, D_c should be such that

$$\frac{\partial}{\partial D_c} \left\{ (\mu - D_c)\, y \frac{D_c s_c}{\mu^2} \right\} = 0 \tag{80}$$

or

$$D_c = \mu/2 \tag{81}$$

As an example, for a microbial system described by model (3), to operate a fermentor at $\mu = 0.3\ h^{-1}$ and $s_E = 1.0\%$ w/v we choose $s_c = 2.0\%$ w/v, $D_c = 0.15\ h^{-1}$ and $x_d = 0.4\%$ w/v in the modified scheme. This situation is illustrated in Fig. 10 where the conventional turbidostat is compared with the modified turbidostat. In the region near $\mu = 0.3\ h^{-1}$ the conventional turbidostat would be ineffective because of very low steady-state gains, but the modified turbidostat scheme would be feasible since it has high steady state gains. For stable operation of the closed loop system K_{cmx} should be chosen positive.

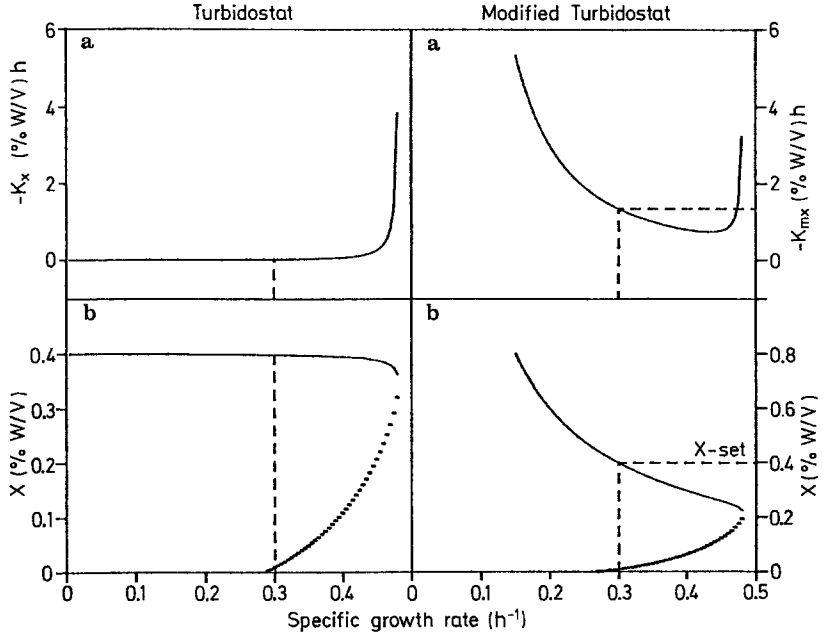

Fig. 10a and b. Plots of (**a**) steady state gain, K_x or K_{mx}, and (**b**) cell mass concentration, x, vs. specific growth rate, μ, for turbidostat and modified turbidostat control schemes based on model 3

Clearly, by properly choosing D_c and s_c, the modified turbidostat could be used to control continuous cultures at all conceivable steady states. In addition this mode of operation is free of input multiplicity and hence does not suffer from difficulties associated with it.

In the conventional bioreactor there are many variables that can be controlled (x, s, pH, BAR, OUR, OAR, and CER), but only one manipulated variable D. However in the novel continuous bioreactor operation proposed above, D_m as well as D_c can be manipulated. This possibility of multivariable control of bioreactors is currently being investigated by us.

6 Discussion

The conventional continuous bioreactor control schemes can be divided into two categories. Turbidostats, nutristats and pH-auxostats would comprise one category, in which the control schemes are feasible only at those conditions where growth is not substrate limited. Control schemes based on rates such as BAR, OUR, OAR and CER would constitute a second category. These control schemes are effective at almost entire steady state growth region, except around the maximum cell productivity for a microbial system that exhibits a constant cell mass yield. The rate controlled schemes also exhibit input multiplicity.

A novel continuous bioreactor operation is proposed. It is shown to satisfy the local controllability criterion at all steady states and for all microbial systems whose growth can be modeled in terms of variables $\mu(x, s)$ and $y(x, s)$. As a simple example of a control scheme for the novel bioreactor, a modified turbidostat is considered. As opposed to the conventional turbidostat, the modified turbidostat can operate under all growth conditions and is free of input multiplicity. Possibility of multivariable control of continuous bioreactors is also indicated. There are other control schemes that have not been analyzed here. These are, either restricted to a specific type of microbial growth as in a viscostat [54] for the production of xanthan bipolymers, or those that are still at a developmental stage, for example, a bioreactor control based on the redox potential [55]. There are also reports on the closed loop operation of bioreactors based on the respiratory quotient [56–57]. However, these have been applied to fedbatch or semibatch bioreactor operations only.

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7 Nomenclature

A	system matrix or the Jacobian matrix [Eqs. (7) and (11)]
A_m	the Jacobian matrix [Eq. (76)]
a	constant in model-3
B	$m \times n$ matrix [Eqs. (7) and (77)]
BAR	base addition rate, mmoles per h
BAR_d	set point value of BAR
BC	buffering capacity, mmoles per l
b	$\partial f/\partial D$ [Eq. (12)]
b	constant in model (3)
C	$1 \times n$ matrix [Eq. (8)]
c^T	vector defined in Eq. (58)
CER	carbon dioxide evolution rate, mmole per l
c	constant in model (3)
D	dilution rate h^{-1}
D_s, D_{s1}, D_{s2}	steady state dilution rates, h^{-1}
D_c	$F_c/v\ h^{-1}$

D_m	$F_m/v\ h^{-1}$
DOC	dissolved oxygen concentration
F	total flow rate of medium, $l\ h^{-1}$
F_c	flow rate of concentrated stream, $l\ h^{-1}$
F_m	flow rate of medium or water stream, $l\ h^{-1}$
f	defined in Eq. (5)
f_m	defined in Eq. (71)
H^+	hydrogen-ion concentration, moles per h
H_d^+	set point value of H^+
K	proportional controller matrix [Eqs. (15)—(18)]
K_t^T	defined by Eq. (26)
K_n^T	defined by Eq. (39)
K_b	steady state gain of BAR-controlled bioreactor, mmoles
K_c, K_{cb}, K_{cn}, K_{ct}	proportional controller constants
K_m	constant in the Monod Model, $g\ l^{-1}$
K_{mx}	steady state gain of the modified turbidostat, (% w/v), h
K_s	steady state gain of a nutristat, (% w/v), h
K_x	steady state gain of a turbidostat, (% w/v), h
K_y	steady state gain
L_c	controllability matrix [Eqs. (9) and (13)]
L_c^0	output controllability matrix [Eqs. (10), (57) and (59)]
m	maintenance constant, h^{-1}
OAR	oxygen absorption rate, moles per h
OOC	off gas O_2 concentration
OCC	off gas CO_2 concentration
OUR	oxygen uptake rate, moles per h
(OH^-)	hydroxide-ion concentration, moles per l
r_{H^+}	rate of acid production per unit volume moles H^+ per h
r_{OH^-}	rate of base production per unit volume moles OH^- per h
s	substrate concentration, $g\ l^{-1}$ or % w/v
s_c	concentration of substrate in the stream F_c, $g\ l^{-1}$
s_d	setpoint value of S
s^E	effective feed substrate concentration, $g\ l^{-1}$
s_F	feed substrate concentration, $g\ l^{-1}$
t	time, h
U	vector of controls
v	volume, l
X	vector of states
x	cell mass concentration, $g\ l^{-1}$ or % w/v
x_d	setpoint value of x
Y	vector of outputs
y	cell mass yield, g cell per g substrate
y_0	yield constant in the maintenance model
$y_{H^+/x}$	yield of acid, moles H^+ per g cell mass
z	defined in Eq. (19)
z(0)	initial value of z
α, β	constants

μ	specific growth rate, h^{-1}
μ_m	maximum specific growth rate, h^{-1}
τ_i, τ_d	controller constant

8 References

1. Rolf, M. J., Lim, H. C.: Enzyme and Microbial Technology *4*, 370 (1982)
2. Dorby, D. D., Jost, J. L.: in: Annual Reports on Fermentation Processes (Perlman, D., ed.), Vol. 1, p. 95, New York: Academic Press 1977
3. Novick, A.: Ann. Rev. Microbial. *9*, 97 (1955)
4. Gerhardt, P.: J. Bact. *52*, 283 (1946)
5. Bryson, V.: Science *116*. 48 (1952)
6. Moss, F. J., Bush, F. E.: Biotech. Bioeng. *9*, 585 (1967)
7. Zines, D. O.: Biotech. Bioeng. *12*, 561 (1970)
8. Watson, T. G.: J. Appl. Chem. Biotechnol. *22*, 229 (1972)
9. Edwards, V. H., Ko, R. C., Balogh, S. A.: Biotech. Bioeng. *15*, 939 (1972)
10. Whaite, P., Gray, P. P.: Biotech. Bioeng. *19*, 575 (1977)
11. DiBiasio, D., Lim, H. C., Weigand, W. A.: AIChE J. *27*, 284 (1981)
12. Martin, G. A., Hempling, W. P.: Arch. Microbiol. *107*, 41 (1976)
13. Stouthamer, A. H., Bettenhaussen, C. W.: Arch. Microbiol. *111*, 21 (1976)
14. Driessen, F. M., Ubbels, J., Stadhouders, J.: Biotech. Bioeng. *19*, 821 (1977)
15. Oltmann, L. F., Schoenmaker, G. S., Reijndero, W. N. M., Stouthamer, A. M.: Biotech. Bioeng. *20*, 921 (1978)
16. McBean, R. D., Hall, R. J., Linklater, P. M.: Biotech. Bioeng. *21*, 1517 (1979)
17. Hospodka, J.: Biotech. Bioeng. *8*, 117 (1966)
18. Yano, T., Kobayashi, T., Shimiju, S.: J. Ferment. Technol. *56*, 416 (1978)
19. Yamada, S., Wada, M., Chibata, I.: J. Ferment. Technol. *57*, 210 (1979)
20. Watson, T. G.: J. Gen. Microbiol. *59*, 83 (1969)
21. Lee, C.: Ph. D. Thesis, Purdue University, W. Lafayette, IN 47907, USA 1981
22. Frederickson, A. G., Ramkrishna, D., Tsuchiya, H. M.: Mathematical Biosciences *1*, 327 (1967)
23. Fredrickson, A. G., Megee III, R. D., Tsuchiya, H. M.: Adv. Appl. Microbiol. *13*, 419 (1970)
24. Monod, J.: Ann. Rev. Microbiol. *3*, 371 (1949)
25. Andrews, J. F.: Biotech. Bioeng. *10*, 707 (1968)
26. Chen, B. J., Lim, H. C., Tsao, G. T.: Biotech. Bioeng. *18*. 1629 (1976)
27. Novick, A., Szilard, L.: Science *112*, 715 (1950)
28. Novick, A., Szilard, L.: Proc. Natl. Acad. Sci. *36*, 708 (1950)
29. Monod, J.: Ann. Inst. Pasteur *79*, 390 (1950)
30. Yano, T., Koga, S.: Biotech. Bioeng. *11*, 139 (1969)
31. Edwards, V. H.: Biotech. Bioeng. *12*, 679 (1970)
32. Spicer, C. C.: Biometrics *11*, 225 (1955)
33. Moser, M.: Proc. Natl. Acad. Sci. *113*, 222 (1956)
34. Koga, S., Humphrey, A.: Biotech. Bioeng. *9*, 375 (1967)
35. Herbert, D., Elsworth, R., Telling, R. C.: J. Gen. Microbiol. *14*, 601 (1956)
36. Mateles, R. I., Ryu, D. Y., Yasuda, T.: Nature *208*, 263 (1965)
37. Storer, F. F., Gaudy, A. F.: Enviro. Sci. Technol. *3*, 143 (1969)
38. Chi, C. T., Howell, J. A.: Biotech. Bioeng. *18*, 63 (1976)
39. Lee, E. B., Markus, L.: Foundations of Optimal Control, New York: Wiley 1967
40. Ray, W. H.: Advanced Process Control, New York: McGraw-Hill 1981
41. Koppel, L. B.: AIChE J. *28*, 935 (1982)
42. Agrawal, P., Lee, C., Lim, H. C., Ramkrishna, D.: Chemical Engineering Science *37*, 453 (1982)
43. Fuld, G. J., Dunn, C. G.: Ind. Eng. Chem. *49*, 1215 (1957)
44. Bach, H. P., Woehrer, W., Roehr, M.: Biotech. Bioeng. *20*, 797 (1978)
45. Neujahr, H. Y., Kjellen, K. G.: Biotech. Bioeng. *21*, 671 (1979)
46. Hikuma, M., Kubo, T., Yasuda, T., Karube, I., Suzuki, S.: Biotech. Bioeng. *21*. 184 (1979)

47. Kobayashi, T., Yano, T., Mori, H., Shimizu, S.: Biotech. Bioeng. Symp. *9*, 73 (1979)
48. Puhar, E., Guerra, L. M., Lorences, I., Fiechter, A.: Eur. of Appl. Microb. and Biotech. *9* (3), 227 (1980)
49. Hewetson, J. W., Jong, T. H., Grey, P. P.: Biotech. Bioeng. Symp. *9*, 125 (1979)
50. Luedeking, R., Piret, E. L.: J. Biochem. Microbial. Techn. Eng. *1*, 393 (1959)
51. Gaudy, Jr., A. F.: Biotech. Bioeng. *17*, 1051 (1975)
52. Brown, D. E., Halsted, D. J.: Biotech. Bioeng. *17*, 1199 (1975)
53. Hopkins, T. R.: Biotech. Bioeng. *23*, 2137 (1981)
54. Silman, R. W., Bagley, E. B.: Biotech. Bioeng. *21*, 173 (1979)
55. Kjaergaard, L., Joergensen, B. B.: Biotech. Bioeng. Symp. *9*, 85 (1979)
56. Wang, H. Y., Cooney, C. L., Wang, D. I. C.: Biotech. Bioeng. *27*, 975 (1979)
57. Wang, H. Y., Cooney, C. L., Wang, D. I. C.: Biotech. Bioeng. Symp. *13* (1979)