Nonlinear Control of Competitive Mixed-Culture Bioreactors via Specific Cell Adhesion

Michael J. Kurtz[†], Michael A. Henson[*] and Martin A. Hjortsø

Department of Chemical Engineering, Louisiana State University, Baton Rouge, LA 70803-7303, USA

Mixed culture fermentations are more versatile than pure culture fermentations because they can use more complex substrates and generate a wider range of products. The competition between two cell populations for a common limiting substrate is the case of greatest practical interest. In a continuous bioreactor, the steady state corresponding to the coexistence of the two populations is unstable if the two cell types have different growth rates (Roos and Hjortso, 1989). Under normal operation, the population with the larger growth rate eventually dominates the reactor.

If the two populations can be separated, a recycle stream enriched in the slower growing cells can be used to alter the population balance. *Specific cell adhesion* can provide a very selective method for separating even closely related cell populations (Hertz et al., 1985). By stabilizing the coexistence steady state in this manner, environmental parameters such as the dilution rate and the inlet substrate concentration are available for optimization of reactor productivity. Experimental results have shown that open-loop control strategies based on specific cell adhesion can temporarily increase the fraction of slower growing cells in the reactor (Roos and Hjortso, 1991). However, an open-loop controller cannot stabilize the unstable coexistence steady state in the presence of external disturbances.

In this paper, a nonlinear control strategy for mixed-culture bioreactors containing two cell populations that differ in their adhesion properties is developed. The technique allows direct control of the fraction of each cell type in the reactor. Nonlinear controller and observer design are based on a continuous dynamic model that neglects periodic operation of the recycle loop. A particularly novel feature of the control scheme is the use of the sampling interval during which material is removed from the reactor as the manipulated input.

The remainder of the paper is organized as follows. First, a brief introduction to competitive mixed-culture bioreactors and specific cell adhesion is given. Then, the dynamic models used to design and evaluate the nonlinear control scheme are presented. The design and implementation of the control strategy are discussed next. Then, a simulation study that demonstrates the performance and robustness of the proposed technique is presented. Finally, a summary and some conclusions are provided.

Competitive Mixed-Culture Bioreactors

The most common type of competitive mixed-culture fermentation involves the production of two cell populations from a common growth-limiting substrate. When grown in a continuous stirred tank reactor (CSTR), such mixed cultures exhibit four steady states corresponding to: (i) the presence of the first population only; (ii) the presence of the second population only; (iii) the coexistence of both populations; and

A nonlinear control strategy is developed for competitive mixed-culture bioreactors in which two cell populations compete for a common growth limiting substrate. A stream is periodically removed from the reactor, and the two cell populations are separated using specific cell adhesion. The steady state corresponding to the desired population fraction is stabilized by discarding faster growing cells and recycling slower growing cells to the reactor. The recycle loop must be operated periodically to allow regeneration of the adhesion column after each separation. As a result, the manipulated input is chosen as the sampling interval during which material is removed from the reactor. The nonlinear controller is designed using a simplified dynamic model that assumes continuous separation of the cell populations. The controller is implemented by calculating the sampling interval that leads to the same amount of material being removed from the reactor as that computed from the continuous control law. A nonlinear, closed-loop observer is used to generate one-time-delay-ahead predictions of the measured cell concentrations and the unmeasured substrate concentration. The efficacy of the proposed control strategy is evaluated via simulation.

On a mis au point une stratégie de contrôle non linéaire pour des bioréacteurs de cultures mixtes en concurrence dans lesquels deux populations de cellules sont en compétition pour un substrat à limitation de croissance commun. Un courant est extrait périodiquement du réacteur et les deux populations de cellules sont séparées en utilisant l'adhésion spécifique des cellules. Le régime stationnaire correspondant à la fraction de population désirée est stabilisé en éliminant du réacteur les cellules à croissance rapide et les cellules à croissance lente en recirculation. La boucle de recirculation doit être actionnée périodiquement pour permettre la régénération de la colonne d'adhésion après chaque séparation. En conséquence, l'entrée manipulée est choisie comme l'intervalle d'échantillonnage pendant lequel le matériau est retiré du réacteur. Le régulateur non linéaire est conçu à l'aide d'un modèle dynamique simplifié qui suppose la séparation continue des populations de cellules. Le contrôleur est implanté en calculant l'intervalle d'échantillonnage qui conduit à la même quantité de matériau retirée du réacteur que celle calculée à partir de la loi de contrôle continu. Un observateur à boucle fermée non linéaire sert à produire des prédictions anticipées d'une durée d'un intervalle des concentrations de cellules mesurées et de la concentration de substrat non mesurée. L'efficacité de la stratégie de contrôle proposée est évaluée par simulation.

Keywords: mixed-culture bioreactors, nonlinear control, nonlinear state estimation, specific cell adhesion.

[*]Author to whom correspondence may be addressed. E-mail address: henson@nlc.che.lsu.edu
[†]Current address: Exxon Chemical Company, 4999 Scenic Hwy., Baton Rouge, LA 70805-3359, USA.

(iv) the presence of neither population (i.e., washout). The desired operating point in many applications is the coexistence steady state. However, this steady state is unstable under most conditions due to the populations having different growth rates (Aris and Humphrey, 1977).

Various ways for stabilizing the coexistence steady state have been proposed. One approach (Aris and Humphrey, 1977; Davison and Stephanopoulos, 1986) involves the manipulation of environmental parameters such as dilution rate and pH to establish operating conditions where the growth rates are equal. This method has several disadvantages, including: (i) the growth rates must be equal at some reactor conditions; (ii) a particular ratio of the two populations may be impossible to obtain; and (iii) the input variable is not available to optimize reactor productivity. An alternate approach is to indirectly manipulate the residence times of the cell populations. This can be achieved by removing a stream from the reactor, separating the two populations, and recycling slower growing cells back to the reactor (Ollis,1982). This method has been used to develop open-loop control strategies in which cell populations that differ substantially in cell size or cell aggregate size are separated due to differences in size (Davison et al., 1985) or flocculation (Davis and Parnham, 1989) properties.

As compared to other techniques, specific cell adhesion provides a very selective means to separate closely related or very similar cell populations (Hertz et al., 1985; Roos and Hjortso, 1989). Adhesion is mediated by interactions between an immobilized ligand on the adhesion surface and a receptor on the outer surface of the adhering cell (Hertz et al., 1985). The formation of ligand-receptor bonds usually is highly specific; therefore, only the cell population that expresses a complimentary receptor for the immobilized ligand adheres to the surface. Cells that are otherwise very similar can be separated as long as they differ in the expression or functioning of an outer surface receptor. Specific cell adhesion can be used to increase the effective residence time of slower growing cells in competitive mixed-culture bioreactors. A stream is periodically removed from the reactor and separated in an adhesion column. The slower growing population is returned to the reactor, while faster growing cells are discarded. The appropriate amount of material to be removed during each separation cycle depends on the difference between the two growth rates, which in turn depends on the current state of the reactor.

The use of specific cell adhesion to control a mixed-culture bioreactor containing two strains of Escherichia coli (E. coli) has been investigated by Roos and Hjortso (1991).The two strains of E. coli used differ in the expression of an outer surface transport protein lamB that displays a binding specificity for starch. This property is used to selectively remove faster growing cells from the reactor by adhesion to a starch/Sepharose support. Slower growing cells, which do not adhere to the support, are recycled to the reactor. A stream is removed from the reactor and sent to the adhesion column every 30 minutes to allow sufficient time to regenerate the column.

The experimental work of Roos and Hjortso (1991) shows that near perfect separation of the two cell populations can be achieved if the stream is removed from the reactor at a particular flow rate. Moreover, separation efficiency is unaffected by the period of time the stream is passed through the adhesion column provided that saturation is not reached. This is demonstrated in Figure 1 (Roos and Hjortso, 1991), which shows the fraction of faster growing cells (23716A) adhering to the column as a function of the stream removal time (i.e., pulse
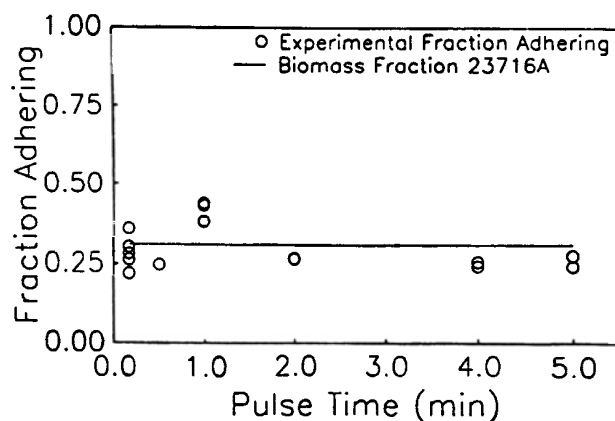

Figure 1. Effect of the pulse time on adhesion column separation efficiency for the optimal column feed flow rate (Roos and Hjortso, 1991). The expected fraction of faster growing cells (23716A) adhering is 0.31.

time) for the optimal stream removal flow rate. Note that the measured values are within experimental error of the value expected for perfect separation (0.31) for all values of the removal time. Figure 2 (Roos and Hjortso, 1991) shows the transient result obtained when pure cultures of slower growing cells (MRC106) and faster growing cells (23716A) are combined to generate a mixed culture with an initial fraction of the slower growing population of approximately 0.99. When the reactor is operated without the recycle loop (w/o recycle), the population of slower growing cells rapidly decreases and the faster growing cells completely dominate the reactor after 23 hours of operation. The introduction of the recycle loop (w/ recycle) substantially increases the fraction of slower growing cells as compared to the case without recycle. The fraction of slower growing cells decreases rapidly when recycle is stopped after 18 hours of operation.

These results demonstrate the plausibility of using specific cell adhesion to control cell populations in competitive mixed-culture bioreactors. However, the proposed open-loop control strategy cannot stabilize a desired coexistence steady state in the presence of external disturbances. In this paper, we propose a nonlinear feedback control strategy based on specific cell
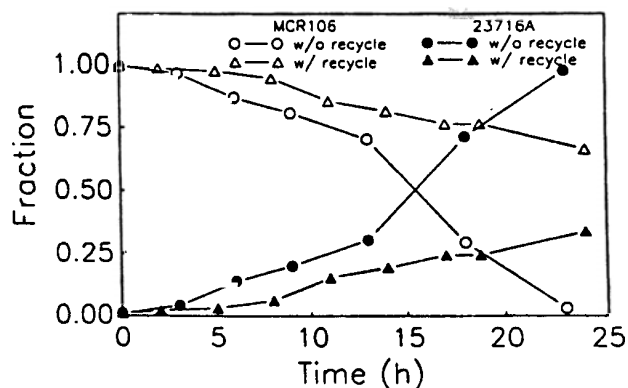

Figure 2. Effect of recycle loop operation on the population balance (Roos and Hjortso, 1991). The initial mixed cultures are obtained by mixing pure cultures of slower growing cells (MRC106) and faster growing cells (23716A). Recycle is stopped after 18 hours of operation.
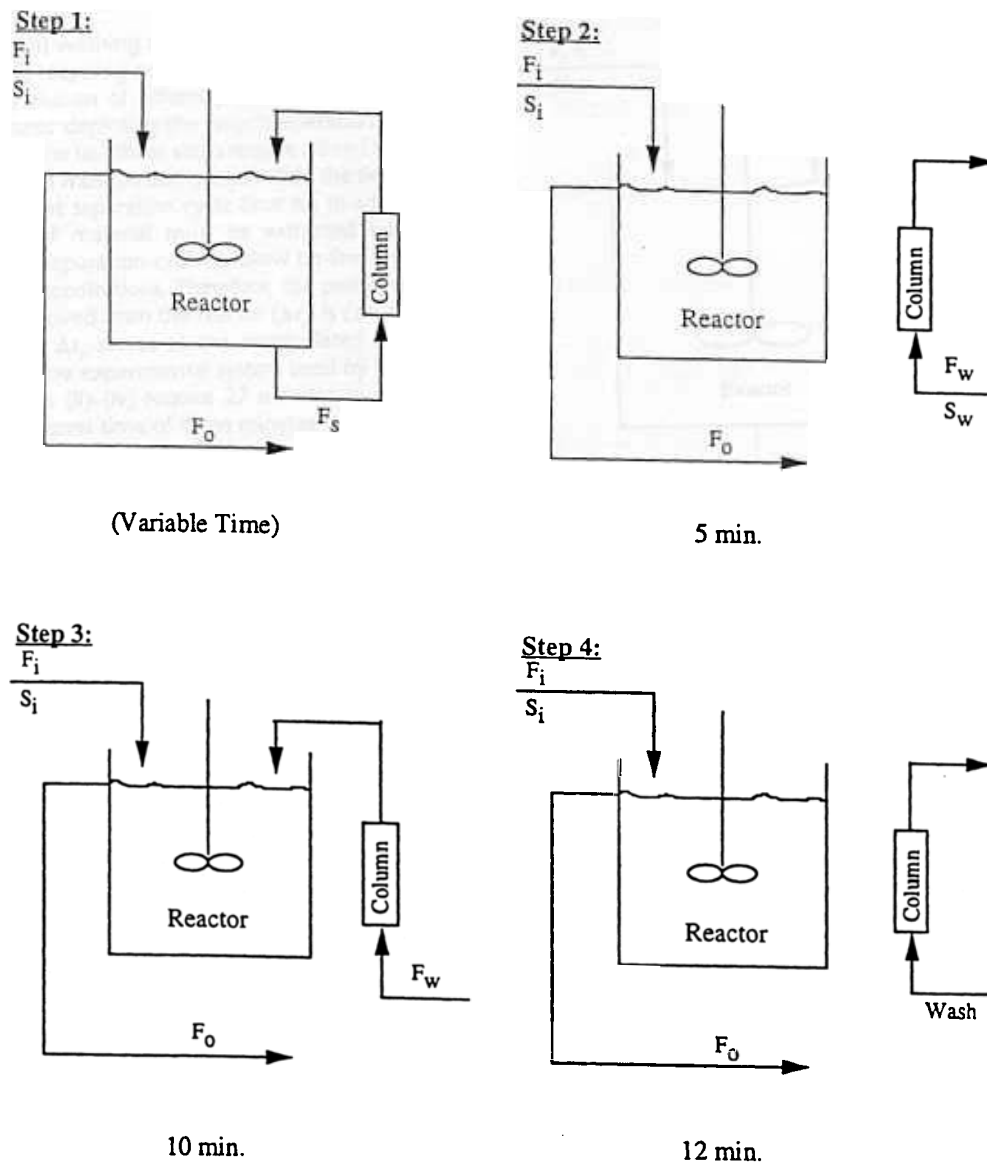
238

Figure 3. Operation of the reactor and adhesion column during recycle operation.

adhesion for a general class of mixed-culture bioreactors. Controller design is challenging not only due to the unstable process dynamics, but also because the recycle loop must be operated periodically with stream removal at a particular flow rate that yields optimal separation. As a result, the standard approach of changing the manipulated input at predetermined, fixed intervals of time is not feasible. Instead, we use the time interval during which material is removed from the reactor as the manipulated input.

Dynamic Modelling

We derive dynamic models of a competitive mixed-culture bioreactor in which specific cell adhesion is utilized to separate and selectively recycle the slower growing cell population. Modelling is complicated by the periodic operation of the recycle loop which is required to regenerate the adhesion column after each sample is processed. The two dynamic models derived differ according to their complexity and ultimate use. The first model includes a detailed description of the periodic operation of the recycle loop and serves as the "process" in closed-loop simulations. This model is referred to as the *detailed model*. The second model assumes continuous operation of the recycle loop and is used for nonlinear controller and estimator design. This model is referred to as the *simplified model*.

Detailed Model

The development of the detailed model is based on the experimental system of Roos and Hjortso (1991). This model explicitly accounts for periodic operation of the recycle loop. For the case where the faster growing cells preferentially adhere, a complete separation cycle involves the following steps (along with the approximate time required for each) (Roos and Hjortso, 1991): (i) continuous removal of cells from the reactor

(variable time Δt_r); (ii) washing non-adhering cells through the column (5 min); (iii) recycling non-adhering cells to the reactor (10 min); and (iv) elution of adhering cells from the column (12 min). A schematic depicting the recycle operation is given in Figure 3. Because the last three steps require a fixed time τ to complete, the first step must be completed within the time $\Delta t - \tau$ to maintain a constant separation cycle time Δt. In addition, a minimum amount of material must be extracted from the reactor during each separation cycle to allow on-line measurements of the cell concentrations. Therefore, the period of time that cells can be removed from the reactor (Δt_r) is constrained. As discussed below, Δt_r serves as the manipulated input for reactor control. For the experimental system used by Roos and Hjortso (1991), steps (ii)–(iv) require 27 minutes to complete and a minimum removal time of three minutes is necessary to acquire enough material to obtain accurate cell measurements. Therefore, to maintain a 45 minute separation cycle the input must be constrained as: 3 min $\leq \Delta t_r \leq$ 18 min.

The differential equations comprising the detailed model are presented in the Appendix. It is assumed that cell growth within the column is negligible as this greatly simplifies model development. This assumption is justified because the average residence time of cells in the adhesion column is significantly less than that in the reactor. A simple method that essentially counts the number of cells drawn into the column during each separation cycle is used to determine the magnitude of the pulse of slower growing cells introduced into the reactor in step (iii) of the recycle operation. Included in the model are separation efficiencies α and β that represent the fraction of slower growing cells entering the column that are recycled to the reactor and the fraction of faster growing cells entering the column that are discarded as waste, respectively. Perfect separation corresponds to $\alpha = \beta = 1$. Substrate concentration variations within the column are described by modelling the column as a simple stirred tank.

Simplified Model

The simplified model is derived from Figure 4 which depicts a competitive mixed-culture bioreactor combined with an adhesion column that allows continuous separation of the cell populations. A nonlinear state-space model is derived from mass balances:

$$V\frac{dX}{dt} = -[F_i + F_w + (1-\alpha)F_s]X + V\mu_1 X$$

$$V\frac{dY}{dt} = -[F_i + F_w + \beta F_s]Y + V\mu_2 Y \tag{1}$$

$$V\frac{dS}{dt} = F_i(S_i - S) + F_w(S_w - S) - \frac{\mu_1}{Y_1}VX - \frac{\mu_2}{Y_2}VY$$

where X, Y, and S are the reactor concentrations of slower growing cells, faster growing cells, and substrate, respectively; S_i and S_w are the substrate concentrations of the feed stream and the stream used to wash non-adhering cells through the column, respectively; V is the reactor volume; and Y_1 and Y_2 are yield parameters. The remaining parameters are defined in the Nomenclature. The specific growth rates are modelled using simple Monod kinetics:

$$\mu_1 = \frac{\mu_1^{max}S}{K_1+S} \quad \mu_2 = \frac{\mu_2^{max}S}{K_2+S} \tag{2}$$
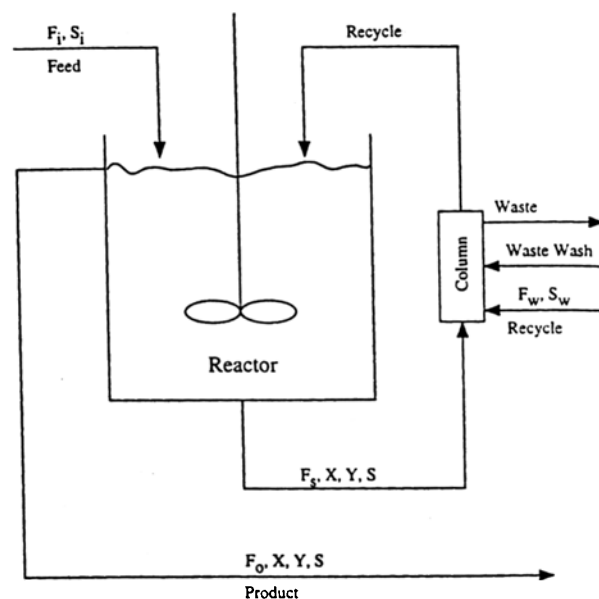

Figure 4. Mixed-culture bioreactor for simplified model development.

where μ_i^{max} and K_i are kinetic parameters. The continuous flow rates (F_i, F_s, F_w) are determined by averaging the actual flow rates over the separation cycle. The flow rate of the stream removed from the reactor (F_s) is utilized as a "fictitious" manipulated input in the subsequent controller design. As discussed below, the F_s value calculated at each time step is used to determine the actual implemented input Δt_r.

Nonlinear Control Strategy

The simplified dynamic model, Equations (1) to (2), is used to design a nonlinear controller that stabilizes the desired coexistence steady state. Initially, controller design is based on two assumptions: (i) online measurements of the state variables (X, Y, S) are available; and (ii) the measurements are undelayed. These assumptions usually are not satisfied in practice. Expensive analytical instruments are required to obtain online measurements of the substrate concentration (S). Consequently, we assume that this variable must be estimated from available measurements. Online measurements of the cell concentrations (X, Y) can be obtained with a spectrometer after the cell populations are separated (Roos and Hjortso, 1991). Because this information cannot be utilized by the controller until the start of the next separation cycle, the cell concentration measurements have an effective delay of one sampling period (45 min). Below, a nonlinear observer that generates one-time-delay-ahead estimates of the three concentrations from delayed measurements of the cell concentrations is derived.

Traditionally, linear techniques such as the Smith predictor (Ogunnaike and Ray, 1994) are used to provide compensation for time delays such as those in the cell concentration measurements. Linearizing the simplified model at the nominal operating point shown in Table 1 yields the following discrete-time transfer function model:

$$G(z) = \frac{z^{-1}(0.1892z^2 - 0.2583z + 0.0938)}{z^3 - 2.4081z^2 + 1.9021z - 0.4940} \tag{3}$$

Table 1. Nominal operating conditions for the simplified bioreactor model.

Variable	Value	Variable	Value
μ_1^{max}	0.75 h^{-1}	S_i	0.5 g/L
μ_2^{max}	0.525 h^{-1}	F_w	40 mL/h
K_1	6.40×10^{-2} g/L	S_w	0 g/L
K_2	7.76×10^{-3} g/L	V	350 mL
Y_1	0.44	F_i	42.9 mL/h
Y_2	0.44	Δt	0.75 h
α	1	X	0.0430 g/L
β	1	Y	0.0430 g/L
F_i	56.8 mL/h	S	0.0450 g/L

The linear model is unstable due to a pole on the unit circle. It can be shown that this unstable pole is present at any steady-state operating point. Therefore, time delay compensation techniques based on the IMC framework (e.g., Smith Predictor) cannot be applied to this class of mixed-culture bioreactors. Additionally, the simplified model contains nonlinearities that can be handled directly using feedback linearization techniques. For these reasons, the proposed method is based on combining a feedback linearizing controller with a nonlinear closed-loop observer that generates estimates of the state variables.

Controller Design

The controlled output (y) is chosen as the fraction of slower growing cells in the reactor. This allows any desired ratio of the two cell populations to be specified in terms of a setpoint. The manipulated input (u) is more difficult to choose because the recycle loop must be operated periodically to allow regeneration of the adhesion column. The underlying objective is to manipulate the number of faster growing cells removed from the reactor. The continuous flow rate of the stream removed from the reactor (F_s) can be employed as the manipulated input for controller *design*. However, periodic operation of the recycle loop must be addressed to actually *implement* the resulting control moves. By defining the manipulated input as $u = F_s$ and the state vector as $x = [X \ Y \ S]^T$, the simplified model can be written as:

$$\dot{x} = f(x) + g(x)u \qquad (4)$$

$$y = h(x)$$

where

$$f(x) = \begin{bmatrix} \dfrac{F_i + F_w}{V} x_1 + \mu_1 x_1 \\[2ex] -\dfrac{F_i + F_w}{V} x_2 + \mu_2 x_2 \\[2ex] \dfrac{F_i}{V}(S_i - x_3) + \dfrac{F_w}{V}(S_w - x_3) - \dfrac{\mu_1}{Y_1} x_1 - \dfrac{\mu_2}{Y_2} x_2 \end{bmatrix}$$

$$g(x) = \begin{bmatrix} \dfrac{1-\alpha}{V} x_1 \\[2ex] \dfrac{\beta}{V} x_2 \\[2ex] 0 \end{bmatrix} \qquad h(x) = \dfrac{x_1}{x_1 + x_2} \qquad (5)$$

Nonlinear controller design is based on input–output linearization (Henson and Seborg, 1991; Isidori, 1989). The simplified model has relative degree one. Therefore, the linearizing control law is:

$$u = \frac{v - L_f h(x)}{L_g h(x)} \qquad (6)$$

where the Lie derivatives are:

$$L_f h(x) = \frac{x_1}{V(x_1 + x_2)^2}[-(F_i + F_w)x_1 + V\mu_1(x_1 + x_2) - V\mu_2 x_2] \qquad (7)$$

$$L_g h(x) = \frac{1}{V(x_1 + x_2)^2}[(1-\alpha)(x_1 + x_2)x_1 + \beta x_1 x_2] \qquad$$

The design of the input v is discussed below.

The periodic nature of the recycle loop is handled by approximating the continuous control moves $F_s(t)$ with implementable flow rate pulses. This is accomplished by determining the removal period Δt_r that results in the same amount of material being extracted from the reactor as that obtained from the discretized version of the continuous control law. This approach is consistent with the requirement that material be extracted from the reactor at a constant, optimal flow rate (as discussed earlier). The resulting control law is:

$$\Delta t_r(k\Delta t) = \frac{u(k\Delta t)}{F_i^*} \Delta t \qquad (8)$$

where Δt is the time required to complete an entire separation cycle (45 minutes); Δt_r ($k\Delta t$) is the time interval during which material is removed from the reactor during the k-th controller iteration; and F_i^* is the constant flow rate of this stream. The signal $u(k\Delta t)$ is computed from the discretized version of the continuous control law (6) which involves state variables that are delayed (x_1, x_2) and unmeasured (x_3). Therefore, the actual state variables $x(k\Delta t)$ are replaced by one-time-delay-ahead predictions $\hat{x}(k\Delta t)$ produced by the nonlinear observer derived below. This procedure yields a discretized version of the linearizing control law (6):

$$u(k\Delta t) = \frac{v(k\Delta t) - L_f h[\hat{x}(k\Delta t)]}{L_g h[\hat{x}(k\Delta t)]} \qquad (9)$$

The continuous input $v(t)$ is designed as:

$$v = k_1(y_{sp} - y) + k_0 \int_0^{t-\Delta t}(y_{sp} - y)d\tau \qquad (10)$$

where y_{sp} is the setpoint and the k_i are controller tuning parameters chosen such that the polynomial $s^2 + k_1 s + k_0$ is Hurwitz. The integral term is included to remove offset in the presence of plant/model mismatch. Note that the upper limit of the integral is $t - \Delta t$ rather than the usual value t. This modification allows actual measurements, rather than predicted values, to be used in the integral. The signal $v(k\Delta t)$ is obtained

by discretizing the continuous input $v(t)$ using the one-time-delay-ahead prediction of the output as necessary. The result is:

$$v(k\Delta t) = v_i[(k-1)\Delta t] + k_1\{\hat{e}(k\Delta t) - \hat{e}[(k-1)\Delta t]\} + k_o \Delta t e[(k-1)\Delta t]$$

$$(11)$$

where $v_i[(k-1)\Delta t]$ is the value of v used to calculate the input $u[(k-1)\Delta t]$ that is actually implemented and:

$$e(k\Delta t) = y_{sp}(k\Delta t) - y(k\Delta t) \tag{12}$$

$$\hat{e}(k\Delta t) = y_{sp}(k\Delta t) - \hat{y}(k\Delta t)$$

The signal $v_i[(k-1)\Delta t]$ is used to include anti-windup compensation (Ogunnaike and Ray, 1994) in the nonlinear controller. Recall that the implemented input must be constrained as 3 min $\leq \Delta t_r \leq$ 18 min to ensure accurate cell concentration measurements and to allow sufficient time for regeneration of the adhesion column. Although not shown here, input constraints seriously degrade closed-loop performance if anti-windup compensation is not included in the controller.

The signal $v_i[(k-1)\Delta t]$ is calculated from Equations (8) and (9) as:

$$v_i[(k-1)\Delta t] = L_f h(\hat{x}[(k-1)\Delta t])$$

$$+ L_g h(\hat{x}[(k-1)\Delta t])\left[\frac{\Delta t_{r,i}[(k-1)\Delta t]F_s^*}{\Delta t}\right] \tag{13}$$

where $\Delta t_{r,i}[(k-1)\Delta t]$ is the implemented removal time that necessarily satisfies the constraints.

Observer Design

Design of the nonlinear observer that provides one-time-delay-ahead predictions of the measured cell concentrations and the unmeasured substrate concentration is now presented. The proposed observer design technique is applicable to nonlinear systems that are affine in the unmeasured state variables (Kurtz and Henson, 1998). While more general techniques such as the Extended Kalman Filter (Muske and Edgar, 1997) may be employed, we utilize the proposed method because the nonlinear oberver is particularly simple to design and implement.

Applying nonlinear observability tests (Isidori, 1989) to the simplified model, Equations (1) to (2), it is easy to show that the substrate concentration is observable from measurements of the cell concentrations. Note that the unmeasured substrate concentration (x_3) appears nonlinearly in the simplified model due to the form of the specific growth rates (μ_1, μ_2). A state-affine model is obtained by linearizing the growth rates about the most recent estimate of the substrate concentration:

$$\mu_i(x_3) \equiv \mu_i(\bar{x}_3) + \frac{\partial \mu_i(x_3)}{\partial x_3}\bigg|_{x_3 = \bar{x}_3}(x_3 - \bar{x}_3) \tag{14}$$

where $\bar{x}_3 = \hat{x}_3[(k-1)\Delta t]$. This yields a bilinear model that can be represented as a state-affine nonlinear system of the form:

$$\dot{x} = \alpha(u, y_m, \bar{x}_3)x + \beta(u, y_m, \bar{x}_3) \tag{15}$$

$$y_m = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \end{bmatrix} x \equiv Cx$$

where y_m represents the measured cell concentrations. The nonlinear functions α and β are shown in the Appendix. Below we discuss two methods for constructing a discrete-time observer from the continuous-time state affine model (15). The first method is based on discretization of a continuous-time observer, while the second method is based on explicit discretization of the continuous-time model followed by discrete-time observer design.

Method 1 — Observer Discretization

In this method, a continuous-time nonlinear observer is constructed and then discretized by assuming the measured outputs and state estimates are constant over the separation cycle. The continuous-time observer based on Equation (15) is (Kurtz and Henson, 1998):

$$\dot{\hat{x}} = \alpha(u, y_m, \bar{x}_3)\hat{x} + \beta(u, y_m, \bar{x}_3) + L(u, y_m, \bar{x}_3)[y_m - C\hat{x}] \tag{16}$$

where $L(u, y_m, \bar{x}_3)$ is an observer gain matrix that depends only on available signals. If the estimation error is defined as $e_x = x - \hat{x}$, the observer yields the following error dynamics:

$$\dot{e}_x = [\alpha(u, y_m, \bar{x}_3) - L(u, y_m, \bar{x}_3)C]e_x \tag{17}$$

The objective is to choose the observer gain L such that the matrix ($\alpha - LC$) has specified eigenvalues that are invariant with respect to u, y_m, and $\bar{x}_3$. This can be achieved if the state-affine system is observable (Kurtz and Henson, 1998).

Note that the observer requires continuous measurement of the cell concentrations (y_m) while only sampled measurements from the previous time step are available. This problem is addressed by assuming the cell concentrations are constant between sampling times. Another approximation is introduced by assuming the state estimates are approximately constant over the separation cycle. For the bioreactor parameters considered in the next section, these assumptions are reasonable because the dominant time constant of the reactor (5.6 h) is much greater than the sampling time (45 min). Nevertheless, the assumption that the cell concentrations and state estimates are constant over the separation cycle is a potential shortcoming of this approach.

The continuous-time observer (16) can be integrated from $t = (k-1)\Delta t$ to $t = k\Delta t$ to yield the discrete-time observer:

$$\hat{x}(k\Delta t) = (I + \Delta t\alpha)\hat{x}[(k-1)\Delta t] + \Delta t\beta \tag{18}$$

$$+ \Delta t L\{y_m(k\Delta t) - C\hat{x}[(k-1)\Delta t]\}$$

where $y_m(k\Delta t) = Cx[(k-1)\Delta t]$ and the dependence of α, β, and L on $u[(k-1)\Delta t]$, $y_m(k\Delta t)$, and $\hat{x}_3[(k-1)\Delta t]$ has been omitted for simplicity. The discrete-time observer produces one-time-delay-ahead predictions of the cell and substrate concentrations from delayed measurements of the cell concentrations. The predictions are used in the nonlinear controller, Equations (9) and (11), to compensate for measurement delays. This observer has been evaluated by the authors in a previous work (Kurtz et al., 1996). It is easy to show that a necessary condition for stability of the discrete-time observer (18) is:

$$-\frac{2}{\Delta t} < \lambda_i[\alpha - LC] < 0 \tag{19}$$

Additional conditions are required to ensure stability even in the continuous-time case (Kurtz and Henson, 1998).

Method 2 — Model Discretization

While the observer discretization method is easy to implement, it suffers from several potential disadvantages, including: (i) the assumption of constant state estimates between sample times may degrade observer performance; and (ii) tuning may be problematic due to the restriction (19) on the eigenvalues of $(\alpha - LC)$. Below we propose an alternative observer design method based on explicit discretization of the nonlinear model that does not suffer from these problems. The assumption that the measured cell concentrations are constant over the separation cycle is still required.

Because the input is held constant between sample times, the continuous-time model (15) can be represented as:

$$\dot{x} = \alpha(u[(k-1)\Delta t], y_m(k\Delta t), \hat{x}_3[(k-1)\Delta t]) \, x \qquad (20)$$

$$+\beta(u[(k-1)\Delta t], y_m(k\Delta t), \hat{x}_3[(k-1)\Delta t])$$

The matrices in Equation (20) are constant between sample times. Therefore, the continuous-time model can be discretized using standard methods for linear systems (Astrom and Wittenmark, 1990):

$$x(k\Delta t) = \alpha_d(u[(k-1)\Delta t], y_m(k\Delta t), \hat{x}_3[(k-1)\Delta t]) x[(k-1)\Delta t] \quad (21)$$

$$+\beta_d(u[(k-1)\Delta t], y_m(k\Delta t), \hat{x}_3[(k-1)\Delta t])$$

The observer is designed directly from the discrete-time model (21):

$$\hat{x}(k\Delta t) = \alpha_d \hat{x}[(k-1)\Delta t] + \beta_d + L\{y_m(k\Delta t) - C\hat{x}[(k-1)\Delta t]\} \qquad (22)$$

where the dependence of α_d, β_d, and L on $u[(k-1)\Delta t]$, $y_m(k\Delta t)$, and $\hat{x}_3[(k-1)\Delta t]$ has been omitted for simplicity. The observer gain matrix L is used to place the eigenvalues of $(\alpha_d - LC)$ at the desired locations within the unit disc in the complex plane. This makes tuning more transparent than in the observer discretization method. The disadvantage of the model discretization approach is the slight increase in computational effort required to perform the discretization. This method is used in the simulation study below.

Simulation Study

The proposed control strategy is evaluated using the detailed bioreactor model derived in the Appendix as the actual process.

Model parameters and nominal operating conditions associated with the simplified model are shown in Table 1. Additional parameters associated with the detailed model are shown in Table 2. The parameters are derived from Roos and Hjortso (1991) with the exception of the flow rates F_w, F_s, F_w^*, and F_s^* and the adhesion column volume V_c whose values have been modified to accommodate a larger column required to achieve sufficient recycle of the slower growing cell population. As shown in Figure 2, experimental data support the assumption of perfect cell separation ($\alpha = \beta = 1$) at the optimal stream removal rate. The initial conditions of the cell concentrations (X, Y) are assumed to be known since the reactor is initially at steady state and cell concentration measurements are available. Note that the initial condition corresponds to a 50/50 mixture of the two populations. By contrast, the initial condition for the unmeasured substrate concentration (S) is approximately 20% larger than the true value (0.0373 g/L). The nominal value of F_s is determined from steady-state relations derived from the simplified model, and therefore it depends on the nominal substrate concentration. As a result, the value reported in Table 1 is approximately 25% lower than the value obtained with the actual substrate concentration (55.4 mL/h).

Figure 5 shows the steady-state growth rates of the two cell populations. Note that the first population (corresponding to concentration X) is slower growing for low substrate concentrations, while the second population (corresponding to concentration Y) is slower growing for high substrate concentrations. The growth rates are equal at $S = 0.12$ g/L, but they differ significantly for substrate concentrations slightly removed from this point. The initial condition in Table 1 corresponds to a steady state to the left of this value. For the control scheme to be successful, the substrate concentration cannot remain in the region where the first population is faster growing for prolonged periods of time. Otherwise, the second population must be recycled to stabilize the coexistence steady state.

The nonlinear controller is designed as described above, while the nonlinear observer is designed using the model discretization method. The controller tuning parameters are chosen as $k_1 = 2$ and $k_0 = 1$, which correspond to a closed-loop time constant of approximately 1 h. The eigenvalues of the matrix $\alpha_d - LC$ are placed at [0.45 0.5 0.55], which makes the dominant time constant of the observer error dynamics approximately equal to 1.4 h. The controller and observer both utilize the initial conditions in Table 1.
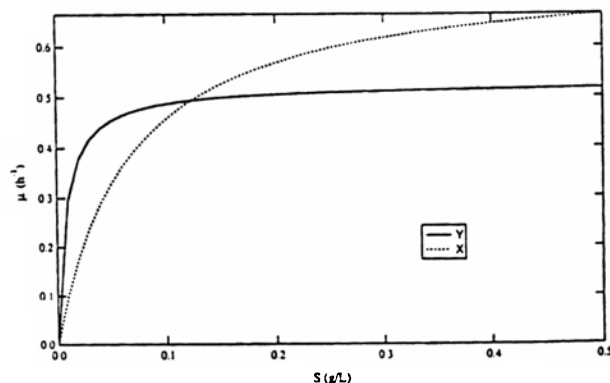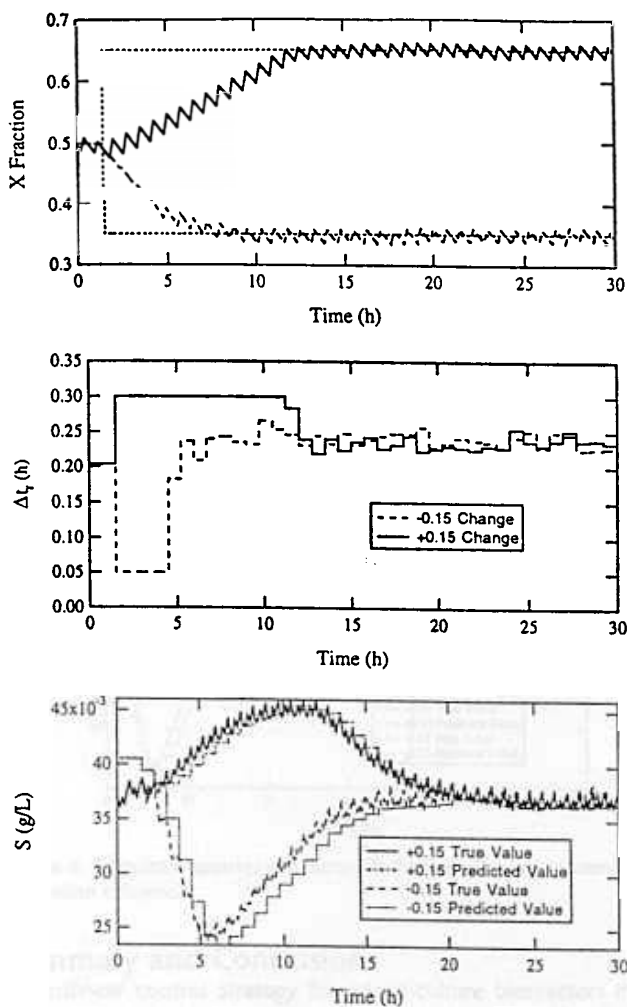
Table 2. Nominal operating conditions for the detailed bioreactor model.

Variable	Value	Variable	Value
F_w^*	180 mL/h	X	0.0430 g/L
F_s^*	180 mL/h	Y	0.0430 g/L
V_c	61.44 mL	S	0.0373 g/L
T	0.1667 h		

Figure 5. Steady-state cell growth rates.

Figure 6. Setpoint responses with no modelling error.

substrate estimates are biased due to the modelling error. The asymmetrical output trajectories for the two setpoint changes are caused by the input constraints.

In Figure 8, the same setpoint changes as shown in Figure 6 are performed with errors in the separation factors α and β. Both parameters are changed in the detailed model from unity to 0.9 to mimic imperfect separation. The modelling error reduces the effectiveness of the nonlinear controller because less slower growing cells are recycled to the reactor than that predicted by the model. The effect of the error is apparent in the time required for the fraction of slower growing cells to reach the target value for the +0.15 setpoint change. The slower response is a direct result of the input being constrained at the upper constraint for a longer period of time than in Figure 6.

The disturbance rejection performance of the controller to a change in the inlet substrate concentration is shown in Figure 9. The inlet substrate concentration is change from its nominal value of 0.5 g/L to 0.75 g/L at $t = 0$ h. The disturbance is rejected very effectively as the output deviates from the setpoint only during the initial phase of the test. As expected, the substrate concentration estimate is biased in this case. Figures 7 to 9 demonstrate the ability of the proposed controller to provide acceptable performance in the presence of significant modelling errors.
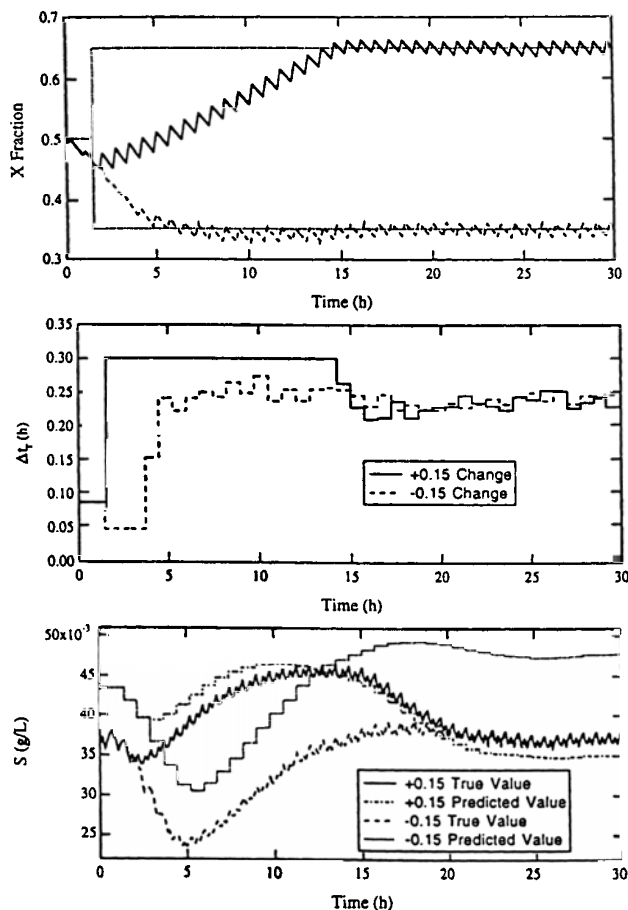
The setpoint tracking performance of the nonlinear controller is shown in Figure 6. The setpoint for the fraction of slower growing cells is changed from the nominal value to 0.35 or 0.65 at $t = 1$ h. Both setpoint changes are tracked effectively. A more sluggish response is obtained for the positive change because the manipulated input remains at the upper constraint for an extended period of time. The observer provides accurate predictions of the substrate concentration for both tests. Note that the variables do not asymptotically converge to constant values due to the periodic nature of the recycle loop.

In Figure 7, the same setpoint changes as shown in Figure 6 are performed with an error in the maximum growth rate parameters (μ_i^{max}). A +25% error in μ_1^{max} and a −25% error in μ_2^{max} are considered. These errors make the intersection of the two steady-state growth curves shown in Figure 5 move to a higher substrate value. As a result, it is more difficult to maintain the desired fraction of slower growing cells due to the increased difference between the growth rates. This causes an initial decrease in the fraction of slower growing cells for both setpoint tests. The fraction of slower growing cells reaches the desired setpoints in a time comparable to that observed in Figure 6 where no modelling error is present. Note that the

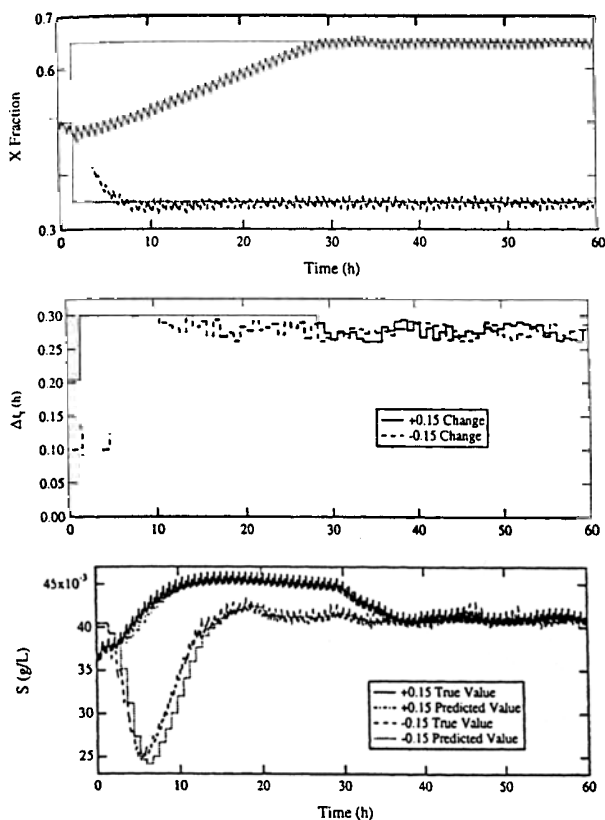Figure 7. Setpoint responses with errors in the cell growth rates.

244

Figure 8. Setpoint responses with errors in the the adhesion column separation efficiencies.

Summary and Conclusions

A nonlinear control strategy for mixed-culture bioreactors in which two cell populations compete for a single growth limiting substrate has been developed. Effective control of these processes is challenging because the steady state corresponding to the coexistence of the two populations is unstable. Specific cell adhesion is proposed as a means to separate the two populations such that slower growing cells can be recycled back to the reactor to stabilize the desired coexistence steady state. Nonlinear control system design is complicated by the requirement that the recycle loop must be operated periodically to allow regeneration of the adhesion column. An approximate dynamic model based on the assumption of continuous separation is used to calculate changes in a "fictitious" manipulated input which is chosen as the flow rate of the stream being removed from the reactor. The continuous control moves are approximated by implementable flow rate pulses. The controller utilizes one-time-delay-ahead predictions of the measured cell concentrations and the unmeasured substrate concentration generated by a nonlinear closed-loop observer. The nonlinear control scheme provides excellent performance when applied to a detailed bioreactor model that accounts for periodic operation of the recycle loop. Future work will focus on experimental evaluation of the proposed control strategy.

Appendix
Detailed Bioreactor Model

During Step 1 of the recycle operation, material is extracted from the reactor and sent to the column. Cells are held in the column, but substrate is allowed to flow through the column
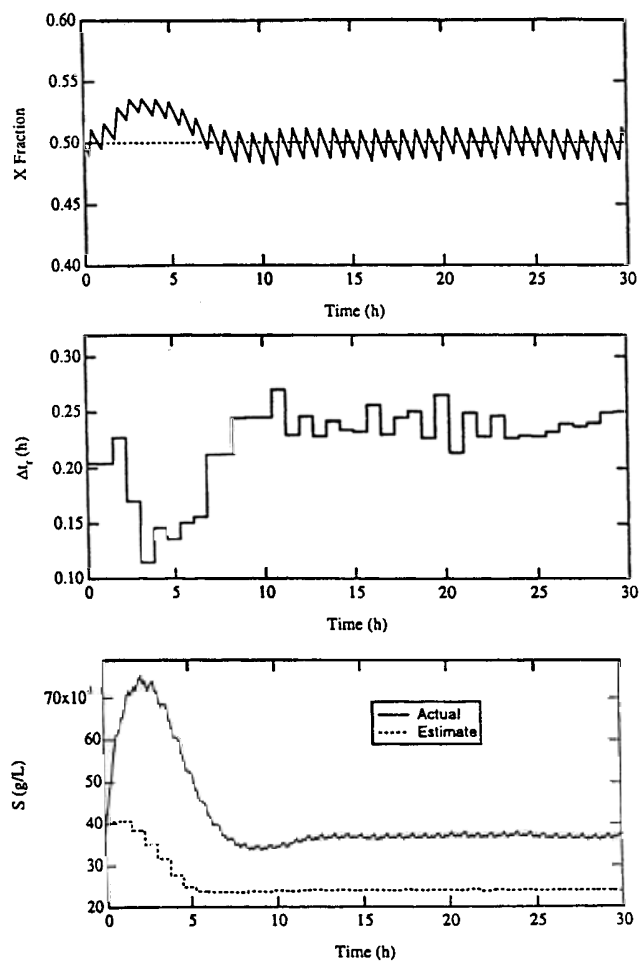

Figure 9. Regulatory response for an unmeasured disturbance in the feed substrate concentration.

and back into the reactor. Cell dynamics are modelled using the mass of cells accumulated within the column rather than the concentration of the cells. Initial conditions correspond to the final conditions from the last recycle operation. During this step the system is modelled as:

$$V\dot{X} = -(F_i + F_s^*)X + V\mu_1(S)X$$

$$V\dot{Y} = -(F_i + F_s^*)Y + V\mu_2(S)Y$$

$$V\dot{S} = F_i(S_i - S) + F_s^*(S_c - S) - \frac{V}{Y_1}\mu_1(S)X - \frac{V}{Y_2}\mu_2(S)Y$$

$$V_c\dot{S}_c = F_s^*(S - S_c)$$

$$\dot{M}_X$$

$$\dot{M}_Y$$

(23)

where M_X and M_Y are the mass of slower growing cells and faster growing cells, respectively, accumulated in the separation column; S_c is the substrate concentration in the separation column; and V_c is the volume of the separation column. The "*" is used to denote actual flow rate values rather than the average values used for the simplified model. The other variables and parameters are defined in the Nomenclature.

During Step 2 of the recycle operation, there is no exchange of material between the reactor and the column. However, a substrate does flow out of the system as waste. The equations for the system during this step are:

$$V\dot{X} = -F_i X + V\mu_1(S)X$$

$$V\dot{S} = F_i(S_i - S) - \frac{V}{Y_1}\mu_1(S)X - \frac{V}{Y_2}\mu_2(S)Y \qquad (24)$$

$$V_c\dot{S}_c = F_w^*(S_w - S_c)$$

$$\dot{M}_X = 0$$

$$\dot{M}_Y = 0$$

Cells are recycled to the reactor in a pulsatile fashion during Step 3. The pulse input of cells into the reactor is used to simulate the plug flow behaviour of the cells within the separation column. Other modelling approaches may result in a large number of non-adhering cells remaining in the column. This would contradict the experimental results of Roos and Hjortso (1991) which show that virtually all the non-adhering cells are eluted and recycled to the reactor. Note that substrate from the column is recycled along with the cells. The equations describing this step are:

$$V\dot{X} = -(F_i + F_w^*)X + V\mu_1(S)X + \frac{\alpha M_X}{T}$$

$$\qquad -(F_i + F_w^*)Y + V\mu_2(S)Y + \frac{(1-\beta)M_Y}{T}$$

$$V\dot{S} = F_i(S_i - S) + F_w^*(S_c - S) - \frac{V}{Y_1}\mu_1(S)X - \frac{V}{Y_2}\mu_2(S)Y \qquad (25)$$

$$V_c\dot{S}_c = F_w^*(S - S_c)$$

$$\dot{M}_X = 0$$

$$\dot{M}_Y = 0$$

where α and β are the separation efficiencies, and T is the interval of time during which Step 3 occurs.

Step 4 is required to regenerate the adhesion column. A wash stream is introduced into the column to elute adhering cells and remove any remaining non-adhering cells. The corresponding equations are:

$$V\dot{X} = -F_i X + V\mu_1(S)X$$

$$V\dot{Y} = -F_i Y + V\mu_2(S)Y$$

$$V\dot{S} = F_i(S_i - S) - \frac{V}{Y_1}\mu_1(S)X - \frac{V}{Y_2}\mu_2(S)Y$$

$$\qquad\qquad\qquad\qquad\qquad (26)$$

$$V_c\dot{S}_c = F_w^*(S_w - S_c)$$

$$\dot{M}_X = -\frac{F_w^*}{\epsilon}M_X$$

$$\dot{M}_Y = -\frac{F_w^*}{\epsilon}M_Y$$

where ϵ is a constant chosen sufficiently small such that essentially no cells remain in the column by the end of Step 4. A detailed description of the decrease in cell mass as the result of Step 3 is not necessary because Step 4 is used only to regenerate the column. Therefore, the initial condition used for M_i at the start of Step 4 is inconsequential as long as ϵ is chosen sufficiently small to bring the cell mass very close to zero by the completion of the recycle operation. The special modelling technique used for the cells is not required for the substrate concentration.

System Matrices

Working from the continuous-time Equation (1) of the simplified model, partial linearization with respect to S around the point $\bar{S}$ yields,

$$= \alpha(u, y_m, \bar{x}_3)x + \beta(u, y_m, \bar{x}_3) \qquad (27)$$

$$y_m = Cx$$

where

$$x \equiv [X\ Y\ S]^T \qquad (28)$$

$$\alpha(u, y_m, \bar{x}_3) = \begin{bmatrix} 0 & 0 & X\frac{\partial\mu_1}{\partial S}\big|_{\bar{S}} \\ 0 & 0 & Y\frac{\partial\mu_2}{\partial S}\big|_{\bar{S}} \\ 0 & 0 & -\frac{F_i + F_w}{V} - \frac{X}{Y_1}\frac{\partial\mu_1}{\partial S}\big|_{\bar{S}} - \frac{Y}{Y_2}\frac{\partial\mu_2}{\partial S}\big|_{\bar{S}} \end{bmatrix}$$

$$\beta(u, y_m, \bar{x}_3) = \begin{bmatrix} \frac{F_i + F_w + (1-\alpha)u}{V}X + \mu_1(\bar{S})X - X\frac{\partial\mu_1}{\partial S}\big|_{\bar{S}}\bar{S} \\ -\frac{F_i + F_w + \beta u}{V}Y + \mu_2(\bar{S})Y - Y\frac{\partial\mu_2}{\partial S}\big|_{\bar{S}}\bar{S} \\ \frac{F_i S_i + F_w S_w}{V} - \frac{\mu_1(\bar{S})X - X\frac{\partial\mu_1}{\partial S}\big|_{\bar{S}}\bar{S}}{Y_1} \\ -\frac{\mu_2(\bar{S})Y - Y\frac{\partial\mu_2}{\partial S}\big|_{\bar{S}}\bar{S}}{Y_2} \end{bmatrix}$$

$$C = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \end{bmatrix}$$

$$\frac{\partial\mu_i}{\partial S}\big|_{\bar{S}} = \frac{u_i^{max}K_i}{(K_i + \bar{S})^2}$$

Nomenclature

C measured output matrix
e output tracking error involving measured output
$\hat{e}$ output tracking error involving estimated output
e_x state estimation error
$f(x)$ drift vector field
F flow rate, (m^3/h)
$g(x)$ input vector field
$G(z)$ discrete-time transfer function
$h(x)$ nonlinear output map
I identity matrix
k discrete time index
k_0, k_1 controller tuning parameters
K growth rate parameter, (kg/m^3)
L nonlinear observer gain matrix
$L_f h(x)$ Lie derivative
$L_g h(x)$ Lie derivative
M_X mass of slower growing cells, (kg)
M_Y mass of faster growing cells, (kg)
S substrate concentration, (kg/m^3)
T time interval during cells are recycled to the reactor, (s)
u manipulated input
v calculated manipulated input for feedback linearized system
v_i implemented manipulated input for feedback linearized system
V volume, (m^3)
x state vector
$\bar{x}_3$ steady-state substrate concentration, (kg/m^3)
$\hat{x}$ estimated state vector
X concentration of slower growing cells, (kg/m^3)
y controlled output
y_m measured output vector
y_{sp} setpoint
Y concentration of faster growing cells, (kg/m^3)
Y_1 cell-mass yield for slower growing cells
Y_2 cell-mass yield for faster growing cells

Greek Letters

α, β nonlinear model functions, separation efficiencies
Δt sampling period, (s)
Δt_r calculated stream removal period, (s)
Δt_{ri} implemented stream removal period, (s)
ϵ constant in detailed bioreactor model
λ_i eigenvalue
μ specific growth rate, (s^{-1})
μ_{max} maximum specific growth rate, (s^{-1})

Subscripts

c adhesion column
d discrete time
i inlet stream
s reactor removal stream
w adhesion column wash stream

0 product stream
     slower growing cell population
2 faster growing cell population

Superscripts

*   detailed model

References

Aris, R. and A.E. Humphrey, "Dynamics of a Chemostat in which Two Organisms Compete for a Common Substrate", Biotechnol. Bioeng. 19, 1375–1386 (1977).

Astrom, K.J. and B. Wittenmark, "Computer Controlled Systems: Theory and Design", Prentice-Hall, Englewood Cliffs, NJ (1990).

Davis, R.H. and C.S. Parnham, "Competitive Yeast Fermentation with Selective Flocculation and Recycle", Biotechnol. Bioeng. 33, 767–776 (1989).

Davison, B.H., K.-Y. San and G. Stephanopoulos, "Stable Competitive Coexistence in a Continuous Fermentor with Size-Selective Properties", Biotechnol. Prog. 1, 260–268 (1985).

Davison, B.H. and G. Stephanopoulos, "Effect of pH Oscillations on a Competitive Mixed Culture", Biotechnol. Bioeng. 28, 1127–1137 (1986).

Henson, M.A. and D.E. Seborg, "Critique of Exact Linearization Strategies for Process Control", J. Process Control 1, 122–139 (1991).

Hertz, C.M., D.J. Graves, D.A. Lauffenburger and F.T. Serota, "Use of Cell Affinity Chromatography for Separation of Lymphocyte Subpopulations", Biotechnol. Bioeng. 27, 603–612 (1985).

Isidori, A., "Nonlinear Control Systems", Springer-Verlag, New York, NY (1989).

Kurtz, M.J. and M.A. Henson, "State and Disturbance Estimation for Nonlinear Systems Affine in the Unmeasured Variables", Comput. Chem. Eng. 22, 1441–1459 (1998).

Kurtz, M.J., M.A. Henson and M.A. Hjortsø, "Nonlinear Control of Competitive Mixed-Culture Bioreactors via Specific Cell Adhesion", in "Proc. IEEE Int. Conf. on Control Applications", Dearborn, MI (1996), pp. 504–509.

Muske, K.R. and T.F. Edgar, "Nonlinear State Estimation", in "Nonlinear Process Control", M.A. Henson and D.E. Seborg, Eds., Prentice-Hall, Englewood Cliffs, NJ (1997) pp. 311–370.

Ogunnaike, B.A. and W.H. Ray, "Process Dynamics, Modelling, and Control", Oxford Press, New York, NY (1994).

Ollis, D.F., "Industrial Fermentations with (Unstable) Recombinant Cultures", Phil. Trans. R. Soc. Lond. B 297, 617–629 (1982).

Roos, J.W. and M.A. Hjortso, "Control of Mixed Microbial Cultures via Specific Cell Adhesion", Biotechnol. Bioeng. 33, 638–649 (1989).

Roos, J.W. and M.A. Hjortso, "Control of an *Escherichia Coli* Mixed Culture via Affinity Binding", Biotechnol. Bioeng. 38, 380–388 (1991).