Optimization of Fermentation Processes Using Recombinant *Escherichia coli* with the Cloned *trp* Operon

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Optimal operating conditions have been determined for recombinant *Escherichia coli* cells in a fed-batch and two-stage continuous fermentors. The model expression system used in this article was the *E. coli trp* promoter cloned on plasmids. Model equations for cell growth and cloned-gene expression have been formulated and used to evaluate process performances under different operating modes. The operating variables manipulated for maximum performance include the timing of IAA addition to derepress transcription from the *trp* promoter, the total operating period and the nutrient concentration profile during fermentations. For a fed-batch mode, the performance was significantly improved by adjusting the IAA addition (environmental switch) time relative to the total operation period. It was found that the optimal switching time exists for a given total operation period. For a two-stage continuous fermentation system, the productivity is more sensitive to the combination of the dilution rates than to the volume ratio of two reactors. In general, as long as the down time is less than the total operation time in the fed-batch mode, the fed-batch mode gives higher productivity than the two-stage continuous system.

INTRODUCTION

The productivity of a recombinant cell fermentation system is primarily dependent upon host cell growth rate and cloned gene expression rate. Overproduction of cloned-gene proteins are deleterious and even fatal to the growth and the overall protein synthesis activity of host cells. This is presumably due to the competitive interactions between the plasmids and host chromosome for the common biosynthetic machinery of the host cells.

A number of research groups have found that decoupling of host cell growth and cloned-gene expression during fermentation is essential in utilizing most effectively the limited biosynthetic potential, leading to an increase in the overall process productivity. Regulated promoters are most commonly used to separate the cell growth stage from the cloned-gene expression stage since they allow control of the cloned-gene expression level by manipulation of environmental parameters. Siegel and Ryu[1] have studied a two-stage continuous fermentation system for recombinant *E. coli* cells containing the temperature-sensitive p_L promoter cloned on plasmids. The first fermentor is operated at a low temperature to support a high cell growth rate while keeping the cloned-gene expression level low. The second fermentor is manipulated at a higher temperature to induce the production of a cloned protein. Seressiotis and Bailey[2] have determined the optimal operating strategies for batch and continuous recombinant cell cultures. They have used the expression system featuring regulated promoters and plasmid replicons, both subject to environmental manipulation.

This study represents theoretical calculations of the performance of recombinant cells in a batch, a fed-batch and a two-stage continuous fermentors. The model system chosen in this article is recombinant *E. coli* containing the cloned *trp* promoter. The expression system has been investigated extensively. A unique feature of the *trp* promoter is that a cloned gene product level can be controlled by tryptophan and tryptophan analogs such as 3β-indoleacrylic acid (IAA) or indolyl-3-propionic acid (IPA).[11] The operational parameters manipulated to maximize the performance of fermentation include the timing of environmental switching from a cell growth stage to a gene expression stage, the total operating time, and the nutrient concentration profile during the course of fermentation. Finally, fed-batch and two-stage continuous models were compared in terms of productivity. In general, a fed-batch culture is superior, as long as the down time is less than the total operation time.

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MATHEMATICAL MODEL AND OPTIMIZATION

The kinetic model for cell growth and product formation is based on the previous experimental observation of recombinant *E. coli* containing a cloned *trp* promoter.[3] The 3β-indoleacrylic acid (IAA), a structural analog of tryptophan, was used to derepress the *trp* operon. Addition of IAA to a culture medium enhances production of a cloned protein while reducing the specific growth rate of recombinant cells and the stability of recombinant plasmids represented here in terms of a segregation coefficient. The course of fermentations of *trp* operon-harboring cells can be separated into two stages: a growth stage followed by a production stage. The growth stage designates the period prior to the addition of IAA, in which the cells are allowed to grow fast while the *trp* promoter is repressed so that the cloned gene is expressed at a negligible rate. The production stage is the period after IAA addition which renders the product formation and segregation coefficient higher but the specific growth rate lower.

A well-posed optimization problem exists with respect to the switching time from the growth stage to the production stage by the addition of IAA.

Model equations before and after IAA addition were formulated by using Monod model for cell growth and a Leudecking–Piret equation for product formation.[2] In this study, the recombinant cells are assumed to respond instantaneously to changes in the growth conditions and hence the kinetic parameters are also assumed to change instantaneously to new values upon IAA addition. In reality, transient period may occur before the new values are attained, though, it is presumed that these transient periods are short to have insignificant effects on the optimization characteristics.

Batch Operation

The mass balance equations for growth phase before IAA addition (with a negligible plasmid reversion), $0 < t < t_s$, are

$$\frac{dx^+}{dt} = \mu_1^+ x^+ \qquad x^+(0) = x_0^+ \tag{1}$$

$$\frac{ds}{dt} = -\frac{\mu_1^+ x^+}{Y_1} \qquad s(0) = s_0 \tag{2}$$

$$\frac{dp}{dt} = \varepsilon_1(\alpha\mu_1^+ x^+ + \beta x^+) - k_d p; \qquad p(0) = 0 \tag{3}$$

where

$$\mu_1^+ = \frac{\mu_{m1}^+ s}{K_{s1} + s} \tag{4}$$

The mass balance equations for the production phase (after IAA addition) with a constant segregation coefficient, $t_s < t < t_f$, are

$$\frac{dx^+}{dt} = \mu_2^+ x^+(1 - a) \tag{5}$$

$$\frac{dx^-}{dt} = \mu^- x^- + a\mu_2^+ x^+ \tag{6}$$

$$\frac{ds}{dt} = -\frac{1}{Y_2}(\mu_2^+ x^+ + \mu^- x^-) \tag{7}$$

$$\frac{dp}{dt} = \epsilon_2(\alpha\mu_2^+ x^+(1 - a) + \beta x^+) - k_d p \tag{8}$$

where

$$\mu_2^+ = \mu_{m2}^+\left(\frac{s}{K_{s2} + s}\right) \tag{9}$$

$$\mu^- = \mu_m^-\left(\frac{s}{K_{s2} + s}\right) \tag{10}$$

When IAA is added to induce the expression of the cloned gene, the plasmid-free cells are generated at a rate of $\mu_2^+ x^+ a$ (where a is the segregation coefficient) due to the overproduction of proteins causing plasmid instability. Plasmid-free cells have to be considered in formulating the mass balance equations for this phase. It is interesting to note that ϵ_1 and ϵ_2 represent the transcriptional efficiencies at phases 1 and 2, respectively.[4] The growth yield (Y) and the saturation constant, K_s, are assumed identical for both cell types. Available experimental data[5-8] suggest that this is the case.

The following performance index (PI) is considered in this study,

PI = Product Price − Separation Cost − Maintenance Cost

with the assumptions that (1) the product is intracellular; (2) the volume of buffer solution, in which the harvested cells are suspended, is directly proportional to the total amount of cell, and the separation cost is also proportional to the total cell mass (or the total volume of buffer suspension); (3) cell breakup and removal costs are much smaller than the rest of separation costs; (4) the purification costs are inversely proportional to the product concentration in the buffer solution; and (5) maintenance cost is proportional to the total operation time. The performance index can be based on either unit batch (PI1) or unit time (PI2). PI1 is useful when the demand for the product is limited while PI2 is proper when the demand for the product is large.

The performance index 1 based on unit batch can be expressed by eq. (11):

$$\text{PI1} = \$_{p/g} vp - \frac{k_1 v}{\left(\frac{p}{x^+ + x^-}\right)}(x^+ + x^-) - k_2 t_f \tag{11}$$

The above equation is divided by $\$_{p/g}v$, to obtain the following equation

$$\text{PI} = p - \frac{k_1}{\$_{p/g}}\frac{x^+ + x^-}{\left(\frac{p}{x^+ + x^-}\right)} - \frac{k_2}{\$_{p/g}v} t_f \tag{12}$$

$$= p - K_1 \frac{x^+ + x^-}{\left(\frac{p}{x^+ + x^-}\right)} - K_2 t_f$$

$$= p'x^+ - K_1(1 + x^-/x^+)^2 x^+/p' - K_2 t_f \quad (13)$$

where $p'(=p/x^+)$ is the intracellular concentration. Thus, the performance index depends upon the intracellular product concentrations, the operation time, the plasmid-containing cell concentration and the ratio of plasmid-free to plasmid-containing cells x^-/x^+. The time-based performance index is obtained by dividing the batch based performance index by the total operation time, t_f:

$$\text{PI2} = \frac{\text{PI1}}{t_f} \quad (14)$$

or

$$\text{P2} = \frac{\text{P1}}{t_f} \quad (15)$$

These performance indices defined above will be maximized through manipulating the switching time and the total operation time.

Continuous Operations

Separation of the growth stage from the production stage during fermentation can be achieved by employing a two-stage continuous-flow stirred tank reactor (CSTR) system. The first reactor is operated to maximize the cell growth and plasmid stability while the second reactor is manipulated for the maximum product formation from the cloned genes. This concept of a two-stage culture system has been demonstrated experimentally by Siegel and Ryu[1] for recombinant *E. coli* cells harboring a temperature sensitive promoter.

The segregational instability of plasmids causes the fermentation system to be dynamic with respect to the number of plasmid-containing cells in the reactor and a single CSTR is not expected to attain steady states without applying a selective pressure favoring the plasmid-harboring population.[4] However, in two CSTRs in series, the plasmids are stably maintained in the first reactor; by repressing the cloned gene expression, the two-stage continuous culture may be operated at a desired steady state.

The feed is distributed to both tanks to obtain better control of substrate concentrations. Parameter F_1 is the feed rate to and from the first tank. Parameter F_2 is the feed rate to the second tank. Thus, the outlet flow rate of the second tank is $F_1 + F_2$.

The mass balance equations for growth stage (first tank) are

$$\frac{dx_1^+}{dt} = (\mu_1^+ - D_1)x_1^+ \quad (16)$$

$$\frac{ds_1}{dt} = (s_0 - s_1)D_1 - \frac{1}{Y_1}\mu_1^+ x_1^+ \quad (17)$$

and

$$\frac{dp_1}{dt} = \epsilon_1(\alpha\mu_1^+ x_1^+ + \beta x_1^+) - p_1 D_1 - k_d p_1 \quad (18)$$

At steady state the left-hand sides of eqs. (16)–(18) vanish to yield

$$s_1 = \frac{D_1 K_{s1}^+}{\mu_{m1}^+ - D_1} \quad (19)$$

$$x_1^+ = Y_1\left(s_0 - \frac{D_1 K_{s1}^+}{\mu_{m1}^+ - D_1}\right) \quad (20)$$

and

$$p_1 = \epsilon_1(\alpha D_1 + \beta)\left(\frac{Y_1}{D_1 + k_d}\right)\left(s_0 - \frac{D_1 K_{s1}^+}{\mu_{m1}^+ - D_1}\right) \quad (21)$$

The mass balance equations for production stage (second tank) are

$$\frac{dx_2^+}{dt} = [x_1^+ - (1 + R)x_2^+]\frac{D_2}{1 + R} + \mu_2^+ x_2^+(1 - a) \quad (22)$$

$$\frac{dx_2^-}{dt} = \mu_2^+ x_2^+ a + (\mu_2^- - D_2)x_2^- \quad (23)$$

$$\frac{ds_2}{dt} = [s_1 + s_0 R - s_2(R + 1)]\frac{D_2}{1 + R} - \frac{1}{Y_2}(\mu_2^+ x_2^+ + \mu_2^- x_2^-) \quad (24)$$

and

$$\frac{dp_2}{dt} = [p_1 - (1 + R)p_2]\frac{D_2}{1 + R} - k_d p_2 + \epsilon_2[\alpha\mu_2^+ x_2^+(1 - a) + \beta x_2^+] \quad (25)$$

where

$$R = \frac{F_2}{F_1} \quad \text{and} \quad D_2 = \frac{F_1 + F_2}{v_2} \quad (26)$$

Steady-state equations are obtained by setting to zero the left-hand sides of eqs. (22)–(25),

$$x_2^+ = \frac{D_2 x_1^+}{[D_2 - \mu_2^+(1 - a)](1 + R)} \quad (27)$$

where

$$\mu_2^+ = \frac{\mu_{m2}^+ s_2}{K_{s2} + s_2} \quad (28)$$

$$x_2^- = \frac{\mu_2^+ a D_2 x_1^+}{(D_2 - \mu_2^-)(D_2 - \mu_2^+(1 - a))(1 + R)} \quad (29)$$

where

$$\mu_2^- = \frac{\mu_{m2}^- s_2}{K_{s2} + s_2} \quad (30)$$

and

$$p_2 = \frac{1}{(k_d + D_2)} \left\{ \frac{p_1 D_2}{1 + R} + \epsilon_2 [\alpha \mu_2^+ x_2^+ (1 - a) + \beta x_2^+] \right\} \quad (31)$$

The substitution of eqs. (27) and (29) into the steady-state version of eq. (24) ($ds_2/dt = 0$) and rearrangement give the following cubic equation which can be solved for the substrate concentration in the second reactor, s_2:

$$(\overline{A} + \overline{C} + \overline{E})s_2^3 + (\overline{B} + \overline{C}K_{s2} + \overline{D} + \overline{F} + 2K_{s2}\overline{E})s_2^2 + (\overline{D}K_{s2} + 2K_{s2}\overline{F} + \overline{E}K_{s2}^2)s_2 + \overline{F}K_{s2}^2 = 0 \quad (32)$$

where

$$\overline{A} = -Y_2(1 + R)(1 - a)\mu_{m2}^+ \mu_{m2}^- \quad (33)$$

$$\overline{B} = (1 - a)\mu_{m2}^+ \mu_{m2}^- [x_1^+ + Y_2(s_1 + s_0 R)] \quad (34)$$

$$\overline{C} = D_2 Y_2 (R + 1)[(1 - a)\mu_{m2}^+ + \mu_{m2}^-] \quad (35)$$

$$\overline{D} = -D_2 x_1^+ \mu_{m2}^+ - D_2 Y_2 (s_1 + s_0 R)[(1 - a)\mu_{m2}^+ + \mu_{m2}^-] \quad (36)$$

$$\overline{E} = -D_2^2 Y_2 (R + 1) \quad (37)$$

and

$$\overline{F} = D_2^2 Y_2 (s_1 + s_0 R) \quad (38)$$

Thus the steady-state concentrations of the plasmid-containing cells (x_2^+), the plasmid-free cells (x_2^-), the substrate (s_2), and the product (p_2) in the second reactor are computed from eqs. (27)–(32), respectively.

The performance index can be expressed as in the case of batch operations.

$$\text{PI} = \$_{p/g} v_2 D_2 p_2 - \frac{k_1 v_2 D_2 (x_2^+ + x_2^-)}{\left(\frac{p_2}{x_2^+ + x_2^-}\right)} - k_2 \quad (39)$$

The above equation is divided by $\$_{p/g} v_2$ to obtain the following equation

$$\text{PIC} = D_2 p_2 - \frac{k_1}{\$_{p/g}} \frac{D_2 (x_2^+ + x_2^-)}{\left(\frac{p_2}{x_2^+ + x_2^-}\right)} - \frac{k_2}{\$_{p/g} v_2} \quad (40)$$

$$= D_2 p_2 - K_1 \frac{D_2 (x_2^+ + x_2^-)}{\left(\frac{p_2}{x_2^+ + x_2^-}\right)} - K_2 \quad (41)$$

RESULTS AND DISCUSSION

The values of key parameters used for calculation were determined from the previous work[3] on recombinant *E. coli* cells harboring a cloned *trp* operon. The specific growth rate of recombinant cells decreases to about one-half when IAA concentration is 40 μg/mL culture or higher. The specific growth rate of x^- cells was found to be approximately 70% of that of x^+ cells in VB minimal broth.[3] The addition of IAA to the growth medium enhances the rate of cloned gene expression and also renders plasmids unstable. A constant segregation coefficient ($a = 0.027$) was used to account for plasmid instability for the production stage. As noted earlier, Y and K_s of x^+ cells are not significantly different from those of x^- cells. The parameters β and k_d which are thought to yield insignificant effect on the optimization characteristics, are assumed to be negligible. The nominal parameter values used for computational work are $\mu_{m1}^+ = 0.5$; $\mu_{m2}^+ = 0.27$; $\mu_m^- = 0.19$; $K_{s1} = 0.005$; $K_{s2} = 0.005$; $Y_1 = 0.5$; $Y_2 = 0.5$; $\epsilon_1 = 0.1$; $\epsilon_2 = 1$; $\alpha = 0.05$; $x_0^+ = 0.1$; and $s_0 = 10$. Three different performance indices, P1, P2, and PIC, are used depending upon the mode of operation.

Batch and Fed Batch Operations

Coefficients K_1 and K_2 in the performance indices are determined when $p = 0.221$, $x^+ + x^- = 5.09$, and $t_f = 12$ by forcing the separation and maintenance costs to be 30% and 10%, respectively, of the product price. Calculated K_1 and K_2 values are 0.000566 and 0.00184, respectively. These values are used for calculations of the performance indices for the proposed batch and the fed-batch operations.

Figures 1 and 2 show the above two performance indices as a function of switching time at various fixed total operation times. These clearly show the existence of an optimal switching time (t_s) for a given value of operation time, t_f. In the case of the batch-based performance index, P1, a longer t_f gives a better performance; however, there is an optimal t_f for the case of time based performance index, P2.

The relationship between the optimal switching time(t_s) and the total operation time(t_f) is represented in Figure 3. The optimal t_s increases almost linearly with t_f until $t_f =$ 10 h and then decreases almost linearly with the operating time, t_f. For operating times less than 10 h, the optimal switching time is almost equal to the total operation time less 4.68 h. This implies that the optimal strategy is to keep the production phase to be 4.68 h. But, for operation times greater than 10 h, $t_f > 10$ h, the optimal switching time, t_s, decreases with the total operation time, t_f. A longer t_s implies a larger amount of substrate consumption so that if t_s is long, more substrate is consumed for growth and very little is left for product formation. Therefore, when t_f is long, one must save the substrate for the late part of the production phase by reducing the switching time (derepressing earlier).

The maximum performance indices obtained with the corresponding optimal switching times, are shown as a function of the total operation times in Figures 4 and 5. The maximum performance index 1 (per batch basis) increases with the increasing total operation times up to the range (14 h) studied. The dependence of the time-based performance index on the total operation times is rather

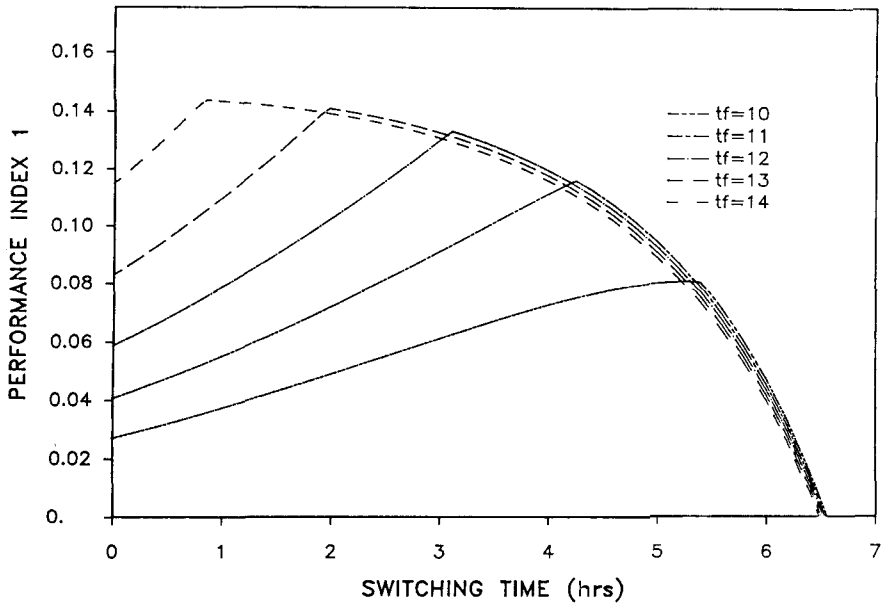

Figure 1. Effect of the switching time on the performance index 1.

different from that of the batch-based performance. The maximum performance index 2 (per unit time basis) increases first and then decreases due to substrate limitation, showing a peak at the operating time of 12 h. Effects of medium substrate concentration on the productivity will be discussed shortly in more detail. It is interesting to mention that an optimal operation time for the maximum performance index 1 (per batch basis) is also found when the maintenance cost fraction becomes significant with respect to the product price. The results obtained with $K_2 = 0.0092$ (five times as much as that in Fig. 4) are shown in Figure 6.

As discussed before, the productivity was significantly improved by keeping the limiting substrate concentration high. However, it should be noted that high glucose concentrations are known[9] to be responsible for acetic acid production by the host, *E. coli*, which at a moderate concentration is known to slow down the growth rate and the expression rate of the cloned gene. Hence, it is necessary to keep low the residual glucose concentration to avoid the accumulation of acetic acid in the broth. The substrate concentration can be kept constant without a significant volume change by adding a highly concentrated feed solution. When the total operating and switching times are 12.9 and 8.3 h, respectively, and for a feed glucose concentration of 50%, the total volume change in fed-batch culture is 8.8% while the productivity with volume change is 0.055 g/L/h as compared to 0.059 g/L/h when the volume change is ignored. Thus, the difference is only 6.8%,

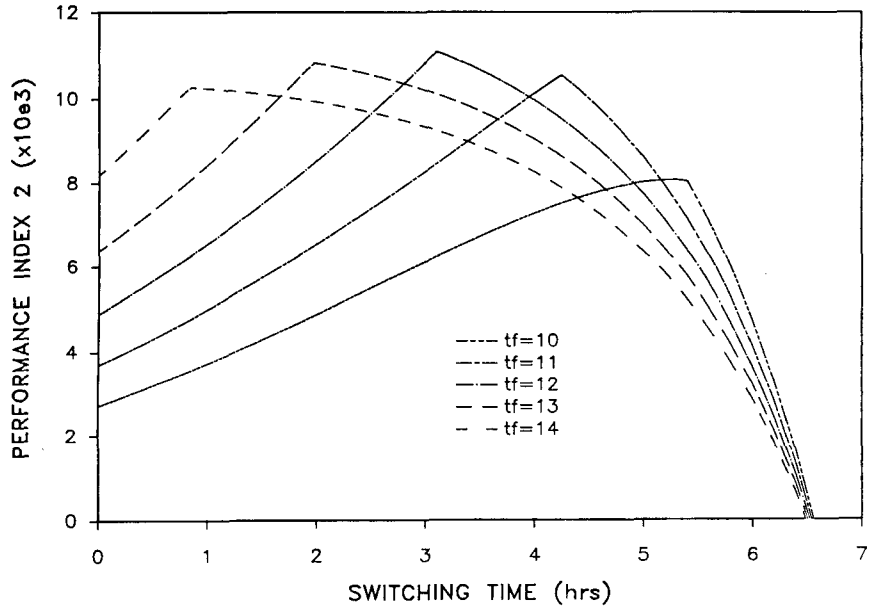

Figure 2. Effect of the switching time on the performance index 2.

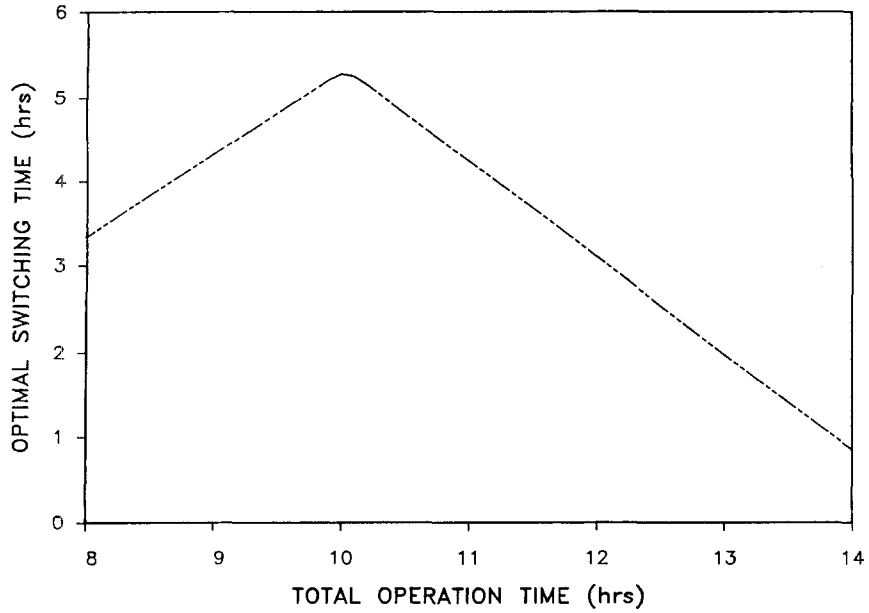

Figure 3. Optimal switching time vs. total operation time.

and for the comparison purpose with continuous bioreactors this difference is insignificant as shown in Table II. Figures 7 and 8 show the performance indices that are achieved with the optimal switchings (t_s) at various operation times (t_f) as a function of the substrate concentration that is kept constant throughout the fermentation. Figures 7 and 8 indicate that 1 g/L substrate concentration is high enough to give approximately the maximum performance index. With the substrate concentration kept at 2 g/L, the performance indices at various t_f are shown as a function of t_s in Figures 9 and 10. As in the case of the increasing region of Figure 3, the optimal t_s is approximately 4.68 h less than t_f regardless of t_f. Unlike the case treated in Figure 3, a decreasing region does not occur in this case because the substrate is not limited. Figures 9 and 10 depict that the longer t_f is, the higher performance indices are for this model. Practically, however, the productivity would be limited by the maximum packing density of the cell, product degradation, other nutrients or waste metabolites.

Continuous Operation

As in the case of batch operations, coefficients K_1 and K_2 were determined when $v_1 = v_2$, $p_2 = 0.117$, $x_2^+ + x_2^- = 4.89$, and $D_2 = 0.6$, by forcing the separation and maintenance costs to be 30% and 10%, respectively, of the

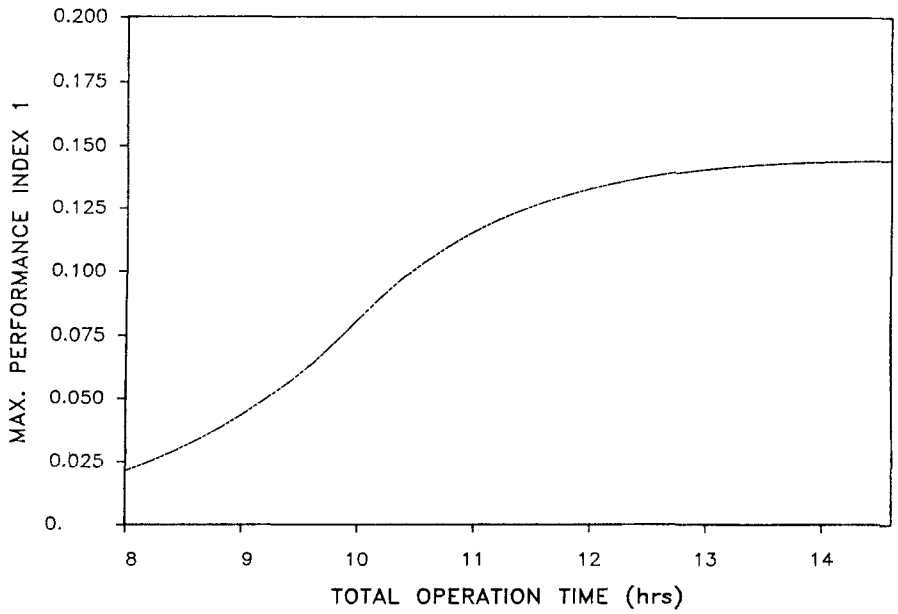

Figure 4. Effect of the total operation time on the maximum performance index 1.

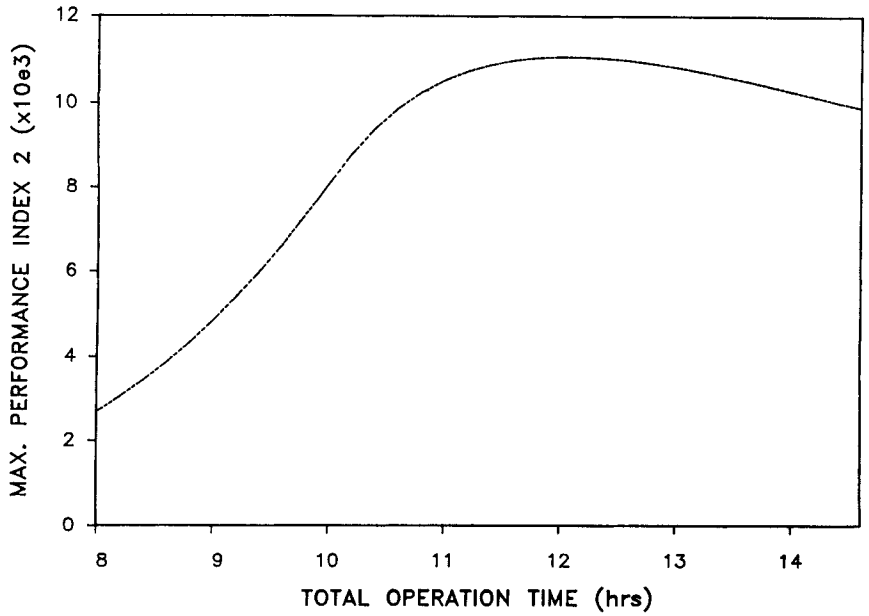

Figure 5. Effect of the total operation time on the maximum performance index 2.

product price. Calculated K_1 and K_2 are 0.000172 and 0.007, respectively. These values are used to evaluate the continuous operations.

The effects of dilution rate on the performance index defined by eq. (41) are shown in Figures 11 and 12. Figure 11 is for the case of equal reactor volume, $v_1 = v_2$ and Figure 12 is for the case of the second reactor being twice that of the first, $v_2 = 2v_1$. Both figures 11 and 12 indicate that for each D_2 there exists an optimal D_1 and for each D_1 there exists an optimal D_2. Figures 11 and 12 also show that what is important is not the specific values of D_1 and D_2 but a combination of D_1 and D_2. Although there are many combinations of D_1 and D_2 which result in the performance close to the maximum value, there is a unique optimal combination which yields the maximum performance index.

Table I lists the optimal combinations of dilution rates for the maximum performance index for different ratios of v_2/v_1. For v_2/v_1 ratios as high as 5, the optimal dilution rate of the second bioreactor is almost constant at 0.343/h and the optimal dilution rate of the first bioreactor is proportional to v_2/v_1. This implies that the outlet flow rate from the first tank is invariant if v_2 is held constant. The same outlet flow rate of the first tank implies the same

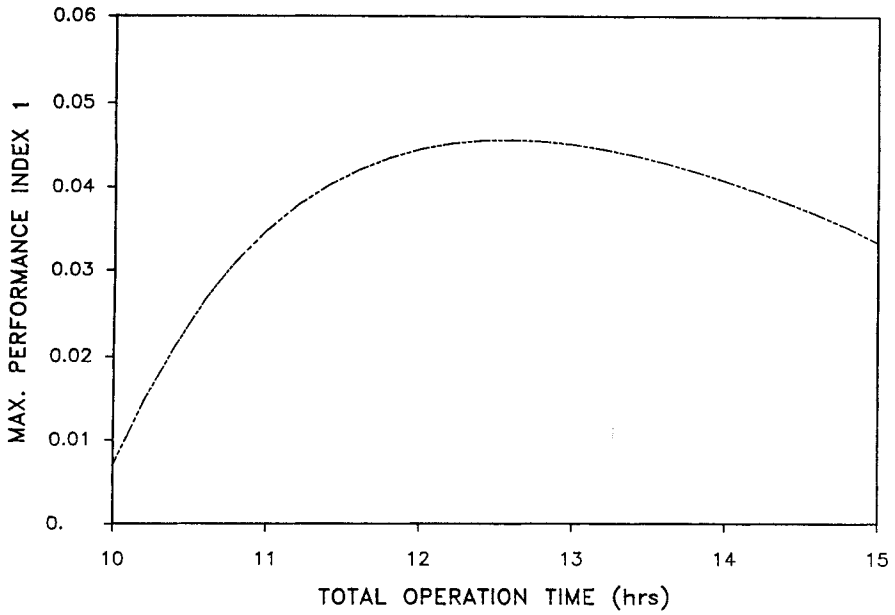

Figure 6. Effect of the total operation time on the maximum performance index 1 (in the case of high maintenance cost).

Table II. Comparison of bioreactor performances.

Mode	Productivity	Conditions
Fed Batch	0.0590	$t_f = 12.9$; $t_s = 8.3$
Continuous	0.0237	$v_1/v_2 = 1$; $D_1 = 0.0806$; $D_2 = 0.343$

biomass inlet rate to the second tank, because the dilution rate of the first tank is not critical to the cell concentration of the first tank.

For large ratios of v_2/v_1 the optimal D_1 remains equal to 0.489, while the optimal D_2 decreases. The dilution rate (D_{1m}) of the first tank at which the biomass output rate (D_1x_1) reaches a maximum,[10] is calculated from the following equation:

$$D_{1m} = \mu_m\left[1 - \left(\frac{K_s}{s_0 + K_s}\right)^{0.5}\right] = 0.489 \qquad (42)$$

In this region D_1 is equal to D_{1m}. This implies that one must keep this value of D_1 to supply the maximum cell mass to the second tank. Therefore this represents a cell mass (F_1x_1) control region.

In summary, the combination of dilution rates in the two reactors rather than the volume ratio is critical for high performance. This is the case when substrate price is insignificant as compared to separation and maintenance costs.

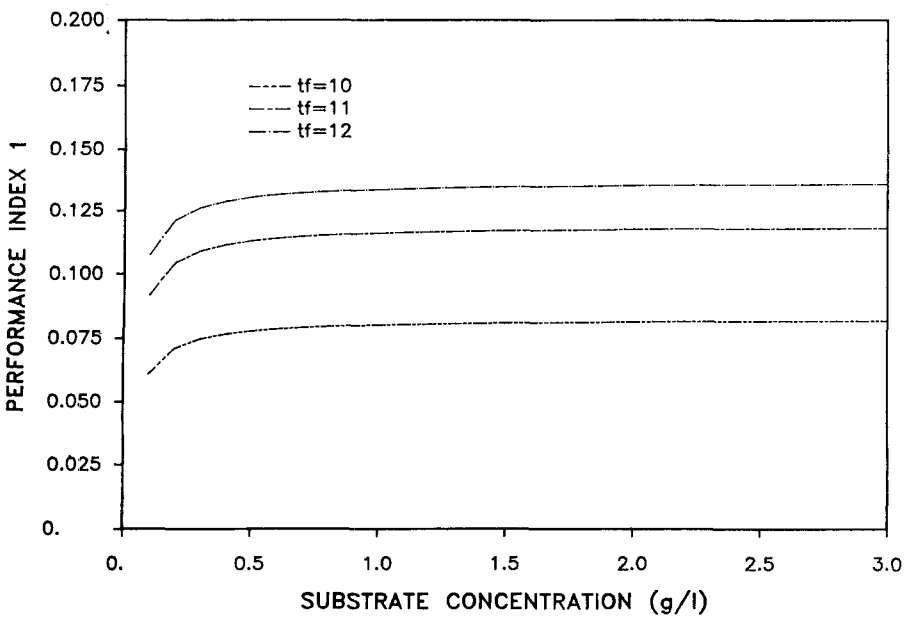

Figure 7. Effect of the substrate concentration on the performance index 1.

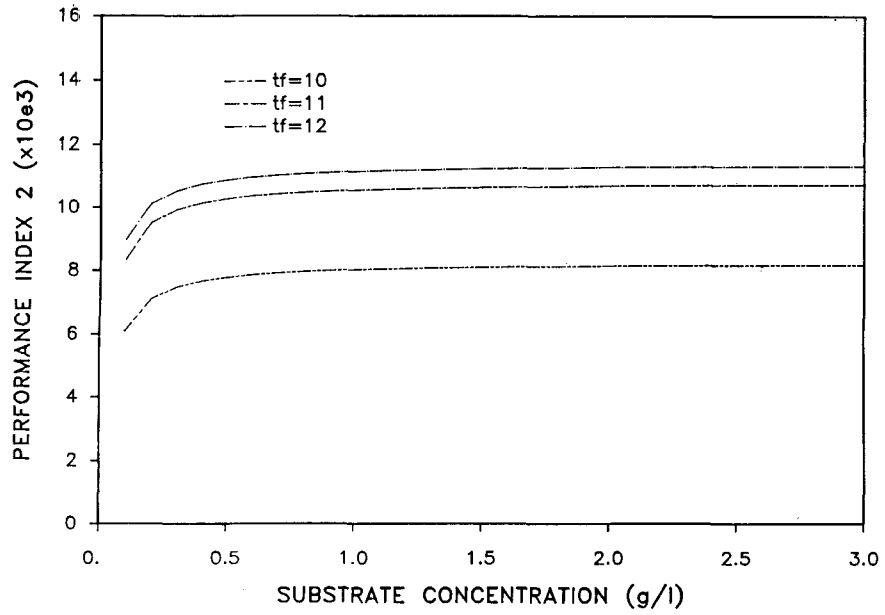

Figure 8. Effect of the substrate concentration on the performance index 2.

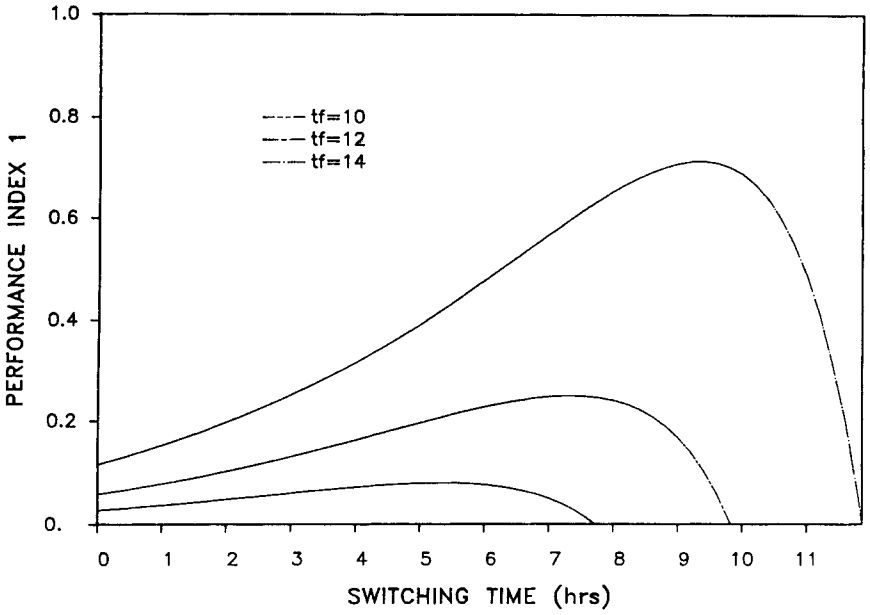

Figure 9. Effect of the switching time on the performance index 1 in the fed-batch operation.

However, if the substrate is expensive, one would pick a high v_2/v_1 ratio to minimize the first reactor volume v_1 at a fixed value of v_2 and consequently the substrate cost. These calculations were performed when v_2 was fixed.

Comparison of Performances of Fed-Batch and Continuous Cultures

Fed-batch and continuous operations can be compared if one chooses the productivity as the performance index. In the fed-batch operation, the productivity is defined as p/t_f (g/L/h). Performance index 2 (P2) defined by eqs. (15) and (12), is expressed by the following equation

$$\mathrm{P2} = \frac{p}{t_f} - K_1 \frac{x^+ + x^-}{\left(\frac{p}{x^+ + x^-}\right) t_f} - K_2 \tag{43}$$

The first term of the right-hand side of eq. (43) is the productivity, and the second and third terms represent the separation and maintenance costs, respectively.

In the continuous operation, the productivity can be expressed as,

$$pd = \frac{p_2(F_1 + F_2)}{v_1 + v_2} \tag{44}$$

$$= \frac{p_2 D_2}{1 + v_1/v_2} \tag{45}$$

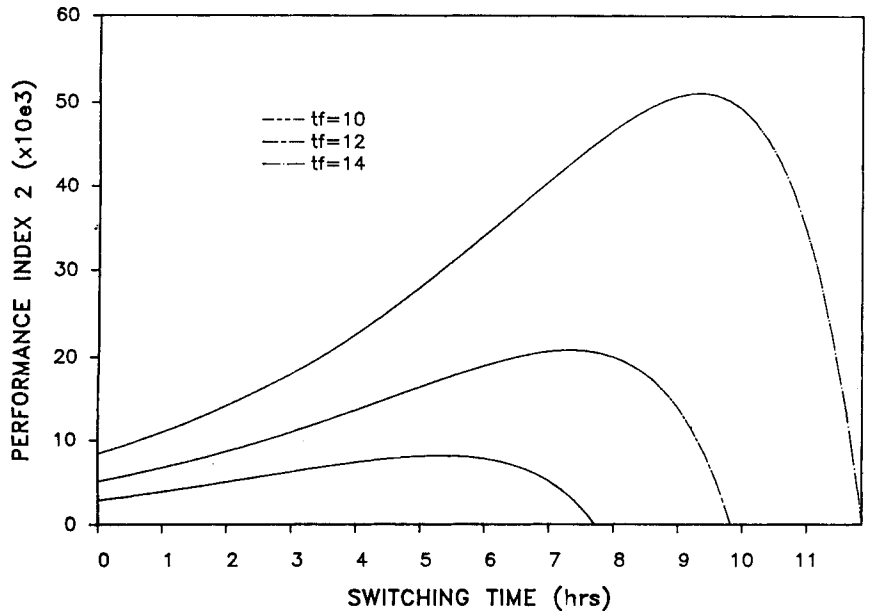

Figure 10. Effect of the switching time on the performance index 2 in the fed-batch operation.

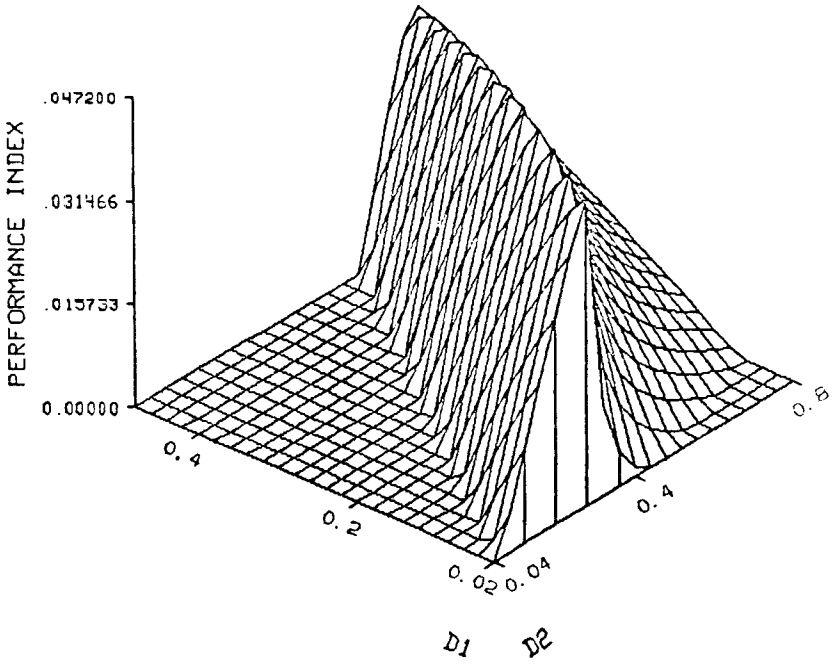

Figure 11. Effect of the dilution rates on the performance index ($v_1 = v_2$).

Eq. (41) is divided by $1 + v_1/v_2$ to obtain the following equation

$$\frac{\text{PIC}}{1 + \frac{v_1}{v_2}} = \frac{D_2 p_2}{1 + \frac{v_1}{v_2}} - K_1 \frac{D_2(x_2^+ + x_2^-)}{\left(\frac{p_2}{x_2^+ + x_2^-}\right)\left(1 + \frac{v_1}{v_2}\right)} - \frac{K_2}{\left(1 + \frac{v_1}{v_2}\right)} \tag{46}$$

The first term of the right-hand side of eq. (46) is the productivity of the continuous operation, and the second and third terms represent the separation and maintenance costs, respectively.

Equations (43) and (46) can be used to compare the various reactor performances as shown in Table II. Table II compares bioreactor productivities using a same amount of the substrate based on unit volume and time. The fed-batch mode is better. However, the down time between each batch is not considered in this model. If the down time is large, the two-stage continuous mode may give higher productivity. But the productivity of fed-batch is higher than twice of that of continuous mode when $v_1 = v_2$. Therefore, as long as the down time is less than the total operation time and the performance is not limited by other factors except the substrate concentration as discussed earlier, the fed-

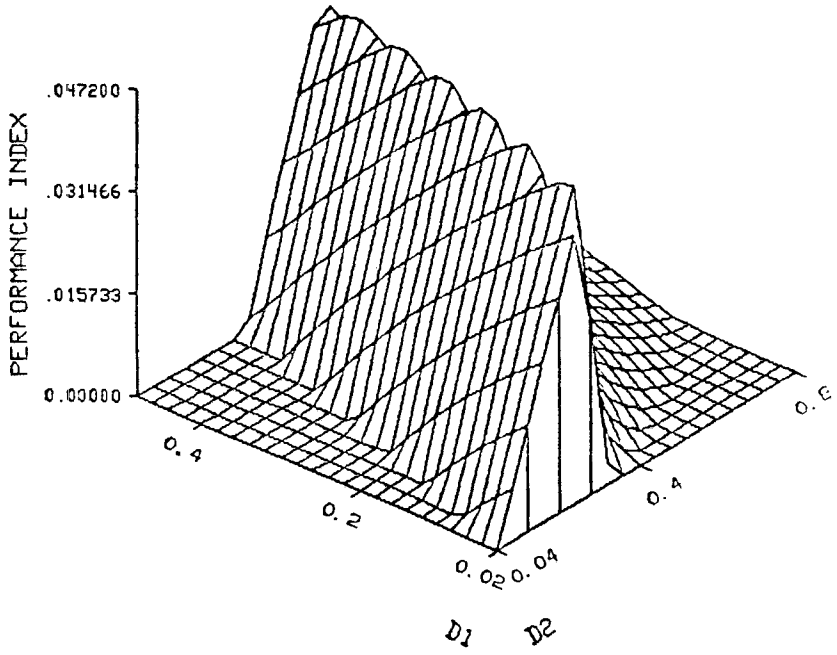

Figure 12. Effect of the dilution rates on the performance index ($v_2 = 2v_1$).

Table I. Performance of continuous bioreactor.

v_2/v_1	D_1	D_2	PIC
0.5	0.0403	0.343	4.73034×10^{-2}
1	0.0806	0.343	4.73034×10^{-2}
2	0.1613	0.343	4.73035×10^{-2}
5	0.4038	0.343	4.73035×10^{-2}
10	0.4890	0.309	4.70824×10^{-2}
50	0.4890	0.267	4.59263×10^{-2}

batch mode is superior. However, the segregational instability and transcriptional efficiency for a two-stage continuous system may be different from those of a fed-batch system. A further study is necessary to delineate the differences, if any, for a better comparison between these two modes of bioreactor operation.

CONCLUSIONS

For the recombinant *E. coli* system harboring a cloned *trp* promoter, the performance indices are calculated for various operating modes. The results indicate the existence of an optimal switching time from cell growth to product formation in a fed-batch mode, and of the best combination of dilution rates in a two-stage continuous fermentation system.

The study can provide a starting point for development of optimal operating scheme for novel recombinant DNA systems. The methodology used in this paper applies to other systems containing regulated promoters if functional relationships among environmental conditions, host-cell growth, plasmid stability, and cloned-gene expression are available.

Recent studies with recombinant *E. coli* suggest that acetic acids play a key inhibitory role in high cell density cultures. Experimental studies are in progress to characterize the formation and subsequent inhibition effects of metabolites on cell growth and product formation, induction kinetics of the cloned *trp* promoter by tryptophan analogs, and competition for the *trp* operator sites between tryptophan and inducers. Availability of such information would improve the present model by incorporating those effects into the model equations and hence establish a more sophisticated operating policy for improved productivity.

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NOMENCLATURE

a	segregation coefficient
$\overline{A}$	defined in eq. (33)
$\overline{B}$	defined in eq. (34)
$\overline{C}$	defined in eq. (35)
D	dilution rate (h^{-1})
$\overline{D}$	defined in eq. (36)
$\overline{E}$	defined in eq. (37)
F	flow rate, 1/hr
$\overline{F}$	defined in eq. (38)
k_d	decay constant of product (h^{-1})
k_1, k_2	coefficients in eq. (11)
K_s	saturation constant (g/L)
K_1, K_2	coefficients in eq. (13)
p	concentration of product (g/L)
p'	intracelluar concentration of product defined in eq. (13) [g/(L OD)]
PI	performance index of continuous operation defined in eq. (39) ($/h)
PIC	performance index of continuous operation defined in eq. (41) (g/L/h)
PI1	performance index 1 defined in eq. (11) ($)
PI2	performance index 2 defined in eq. (14) ($/h)
P1	performance index 1 defined in eq. (13) (g/L)
P2	performance index 2 defined in eq. (15) (g/L/h)
R	ratio of flow rates defined in eq. (26)
s	concentration of limiting substrate (g/L)
t	time (h)
v	working volume (L)
x	concentration of cells (OD)
Y	growth yield (OD L)/g
$\$_{p/g}$	product price per unit mass ($/g)

Greek letters

α	parameter of the Leudecking–Piret product formation model
β	parameter of the Leudecking–Piret product formation model
ϵ	transcriptional efficiency
μ	specific growth rate (h^{-1})
μ_m	maximum specific growth rate (h^{-1})

Subscripts

f	total operation
s	switching
0	feed
1	growth stage
2	production stage

Superscripts

$^+$	plasmid-containing cells
$^-$	plasmid-free cells

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