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DEVELOPMENT OF A COMPUTER ALGORITHM BASED ON A CONJUGATE GRADIENT APPROACH FOR OPTIMIZATION OF FED-BATCH FERMENTATIONS

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Abstract—The problem of optimization of fed-batch fermentations using the substrate feed rate as the control variable is singular in nature. Previous approaches, including the boundary condition iteration method and transformation to a nonsingular problem using a different control variable, do not work well for solving optimization of systems governed by more than four differential equations. The applicability of a first-order conjugate gradient algorithm for optimizing fed-batch fermentations was tested for systems of varing complexity. This approach does not need any variable transformation or *a priori* knowledge of the control arc sequence. Constraints on the feed rate are handled in a simple and direct manner. The algorithm worked very well for three, four, and five-dimensional singular systems. The correctness of the optimal profile was judged by observing the variation in the sign of the gradient of the Hamiltonian. The gradient was found to be zero during the singular period and had the appropriate sign on the boundary arcs. The optimization method based on conjugated gradient approach can be complementary to the boundary condition iteration method for determination of the exact optimum profile.

INTRODUCTION

Fed-batch mode of operation is particularly well suited for fermentations in which cell growth rate, product formation rate and/or product selectivity are significantly sensitive to the limiting substrate concentration. In such cases, a control over medium feed rate results in an improved control over substrate concentration inside the bioreactor, resulting in substantial improvement in reactor productivity. Consequently, in recent years a growing number of studies [1-10] have been devoted towards investigation of optimal control problems in fed-batch fermentations.

Medium feed rate is often used as the control variable in these investigations. Since feed rate appears linearly in the resulting Hamiltonian, the problem is singular in nature. Modak et al. [4, 5] developed a computational algorithm for solving singular optimal control problems of dimensions less than five. They argued that in some cases a physical insight into the problem can reveal the optimal sequence of maximum, minimum and singular arcs. The problem of determining the optimal feed rate was then reduced to that of an iteration over four variables, *viz.* the durations of the first two control arcs (maximum-minimum or minimum-maximum) and the values of two adjoint variables at the start of the singular period. This technique was successfully applied to a variety of biological systems. A major limitation of this method, however, is that it is applicable only for systems, which can be described by less than five differential equations. For higher dimensional systems, the number of possible switches in the control profile increases. This increases the possible permutations of maximum, minimum and singular arcs, thus making it very difficult, if not impossible, to *a priori* guess the sequence of control arcs. Furthermore, it increases the number of variables to be guessed.

In an effort to overcome these problems Modak and Lim [7] proposed a transformation approach, in which the original singular problem was converted to a nonsingular one by a proper choice of state and control variables. The culture volume was used as the control variable instead of the medium feed rate. This new

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problem was then solved by the steepest descent method. In principle, this approach can be used to optimize systems of any dimension. However, for systems with dimension greater than three, no results have been reported. Also, the new problem contains an inequality constraint on the time derivative of the control variable. The optimal control theory does not provide a satisfactory way of dealing with this constraint. If this constraint is ignored, the resulting converged control trajectory may not be optimal.

A conjugate gradient method for functional optimization was proposed by Lasden et al. [11]. It was proved that the directions in function space generated by the conjugate gradient method are such that the objective function is decreased at each step. Pagurek and Woodside [12] extended this technique to handle directly saturation constraints on the control variables. Stutts [13] tested the usefulness of this method to solve singular fed-batch optimization problems. The method worked really well with a three dimensional singular problem. The optimal feed rate computed for four or higher dimensional problems did not contain regions of maximum or minimum feed rates. This paper is concerned with investigating the applicability of a simple conjugate gradient approach for determining the optimal feed rate profiles for complex biological systems. Pagurek and Woodside's [12] first order method was tested for biological systems of varying complexity.

PROBLEM FORMULATION

1. Necessary optimality conditions

The problem of determining the best feed rate in a typical fed-batch fermentation can be stated as a problem in the calculus of variations [2].

$$\min_{F(t)} \quad \Pi = \pi(x(t_f)) \tag{1}$$

Here Π represents a suitably chosen performance index (sometimes refered to as the objective function), $x(t_f)$ is the value of the state vector x at the fixed final time t_f (when the fermentation is over). The objective is to minimize this performance index by a proper choice of the medium feed rate profile, F(t). Normally the substrate feed rate is constrained as,

$$0 = F_{min} \leq F \leq F_{max} \tag{2}$$

where F_{max} and F_{min} represent the maximum and minimum allowed feed rates, respectively. In addition, the state variables satisfy the following differential equations.

$$\frac{dx}{dt} = \mathbf{a}(x) + \mathbf{b}(x)F, \quad x(0) = x_0 \tag{3}$$

where **a** and **b** are vector functions of the state vector x, x_0 represents the vector of specified initial conditions.

Pontryagin's minimum principle [14] states that the above minimization problem is equivalent to the minimization of the Hamiltonian defined as,

$$H = \lambda^T[\mathbf{a}(x) + \mathbf{b}(x)F], \tag{4}$$

where the adjoint vector, λ(t), is defined by

$$\frac{d\lambda}{dt} = -\frac{\partial H}{\partial x}, \tag{5}$$

with the specified terminal conditions given by

$$\lambda^T(t_f) = \frac{\partial \Pi}{\partial x(t_f)} \tag{6}$$

The problem, as stated above, is a singular control problem, because the control variable (the substrate feed rate in this case) appears linearly in the Hamiltonian, so that the minimum principle does not provide an explicit solution for the optimal control profile. The necessary conditions for optimality have been discussed by Bryson and Ho [15].

The linear dependence of the Hamiltonian on feed rate enables one to determine the optimal feed rate by examining the coefficient of F, $\lambda^T\mathbf{b} = \phi$. If φ is identically zero over a finite time interval, the interval is called a singular interval and the corresponding feed rate, singular feed rate, F_s. It can be deduced [4] that the optimal feed rate F*(t) is given by,

$$F^*(t) = \begin{cases} F_{max} < 0 \\ F_s(t) = 0 \\ F_{min} > 0 \end{cases} \tag{7}$$

The singular feed rate can be determined [4] as a function of the state and adjoint variables as

$$F_s = \frac{\lambda^T(\mathbf{a}_x\mathbf{c} - \mathbf{c}_x\mathbf{a})}{\lambda^T\mathbf{c}_x\mathbf{b}} \tag{8}$$

Here $\mathbf{c} = \mathbf{a}_x\mathbf{b}$ and the subscript x refers to the Jacobian with respect to **x**. The singular control theory just tells us that the optimal control arcs can either be on the boundary or singular. It is not possible to determine the sequence and duration of these control arcs. Also, the singular feed rate depends on the adjoint variables, which are unknown. Numerical methods are hence necessary to determine the form of the optimal feed rate.

2. Computational method

Although Pagurek and Woodside [12] obtained better results using a second order conjugate gradient method for some systems, this method required determination of a lot of complex derivatives. Moreover, the memory requirements are considerably higher compared to the simple first order conjugate gradient method. The latter method was used exclusively for numerical calculations.

The hard constraint on the final volume, normally imposed during fed-batch optimization [4], was converted to a soft constraint by modifying the objective function by adding a penalty function.

$$\min_{F(t)} \quad \Pi = \pi(\mathbf{x}(t_f)) + K(V(t_f) - V_f)^2 \tag{9}$$

Here $V(t_f)$ is the final volume obtained by simulation, while V_f is the desired final volume. By choosing a proper value for the constant K, the difference $|V(t_f) - V_f|$ can be made arbitrarily small. No penalty functions are needed for control variable constraints of the type given by Eq. (2). The technique used in this study takes care of these constraints in a simple and direct manner. The algorithm consists of the following steps.

(1) The procedure begins with an initial guess for the feed rate profile, $F_0(t)$. This profile may contain boundary arcs. At the same time a function w is initialized.

$$w_0(t) = \begin{cases} 0 & \text{for } t \in \Omega \\ 1 & \text{elsewhere} \end{cases} \tag{10}$$

Here Ω represents the control boundary region.

(2) The state differential Eq. (3) are integrated using the guessed feed rate profile. The adjoint Eq. (5) are integrated using Eq. (6) as final conditions.

(3) The first iteration is a steepest descent step with the initial gradient direction calculated using the following equation.

$$s_0 = g_0 = \frac{\partial H}{\partial F} \tag{11}$$

(4) The control profile for the next iteration is computed as

$$F_1 = F_0 - \alpha_0 s_0 w_0 \tag{12}$$

where α_0 is chosen using a one dimensional search to minimize Π. A simple quadratic interpolation was used to implement this minimization. However, before Π is computed in each trial of the α-search, F_1 is truncated at the upper and lower bounds of the feed rate [Eq. (2)]. When the α_0 is determined, the function $w_1(t)$ is calculated using the procedure shown in Eq. (10).

(5) The state and adjoint equations are integrated again as before using the improved feed rate profile.

(6) The following quantities are evaluated.

$$I_i = \int_0^{t_f} w_i g_i^2(t)dt \tag{13}$$

$$\beta_i = \frac{I_i}{I_{i-1}} \tag{14}$$

Here i refers to the iteration number. If β_i turns out to be negative, a steepest descent step is taken by setting it to zero.

(7) The conjugate gradient direction, s_i, is determined using the following equation.

$$s_i = g_i + \beta_i s_{i-1} \tag{15}$$

Here g_i refers to the steepest descent direction.

(8) The control profile is modified by an α-search as outlined in step (4).

$$F_{i+1} = F_i - \alpha_i s_i w_i \tag{16}$$

(9) Steps 5-8 are repeated till the improvement in the performance index is negligible and the gradient of the Hamiltonian shows expected trends.

Some comments are in order here. The algorithm is somewhat sensitive to the initial control profile chosen. Some initial profiles cause the procedure to diverge. An important consideration is that the initial guessed feed rate profile should satisfy the final condition on volume. This assures that the penalty function term in Eq. (9) is zero at the beginning. If this term is very large compared to $\pi[\mathbf{x}(t_f)]$, the algorithm concentrates more on decreasing the penalty function than on decreasing π.

It was found that after every few iterations, the conjugate gradient technique tends to stagnate. Under these circumstances the same control profile gets repeated after two or three iterations. Reinitialization, by setting β_i to zero (a steepest descent step), was found to cure this problem, hence was incorporated in the algorithm. The optimal number of iterations, before reinitialization, were found to be between 15 and 30, depending on the system.

An important feature of this method is that it does not need any variable transformation to convert the singular problem into a nonsingular one. Thus the difficulties associated with differential constraints on control variables, present in the transformation approach, can be completely avoided. The convergence of this method near the optimum is somewhat slow, but the only measure needed, to ensure a decrease in the

Table 1. Parameters used for optimization of Modak's three-dimensional model

Case	A	B	C
$(XV)_0$, g	1.0	1.0	1.0
$(SV)_0$, g	0.0	0.0	1.0
V_0, L	1.0	1.0	1.0
V_f, L	5.0	5.0	5.0
S_f, g/l	10.0	10.0	10.0
F_{max}, L/hr	4.0	4.0	4.0
t_f, hr	3.8	3.8	3.8
K	10	1000	10

performance index at each iteration, is a smaller step size.

RESULTS

1. Optimization of a three-dimensional model

To test the effectiveness of the algorithm described above, an optimization of a simple three-dimensional model for fed-batch fermentations was attempted. The governing equations for this model can be written as,

$$\frac{d}{dt}(XV) = \mu XV \tag{17}$$

$$\frac{d}{dt}(SV) = FS_F - \frac{\mu XV}{Y} \tag{18}$$

$$\frac{dV}{dt} = F \tag{19}$$

Here X, S, V, F, μ, Y, S_F and t refer to the cell mass concentration, limiting substrate concentration, culture volume, substrate feed rate, specific cell growth rate, cell yield, substrate concentration in the feed and time, respectively. The cell growth is inhibited at high substrate concentrations, the maximum specific growth rate occurring at a concentration 0.24 g/L, and the cell yield is assumed to be constant.

$$\mu(S) = \frac{S}{0.03 + S + 0.5S^2}, \quad Y = 0.5 \tag{20}$$

The objective is to optimize the amount of cell mass obtained after a fixed final time t_f.

$$\min_{F(t)} \quad \Pi = -(XV)_f + K(V(t_f) - V_f)^2 \tag{21}$$

This problem has been solved by a control variable transformation approach [7]. It is known that the optimal singular feed rate maintains the substrate concentration constant at 0.24 g/L, thus maintaining the specific growth rate at its maximum value.

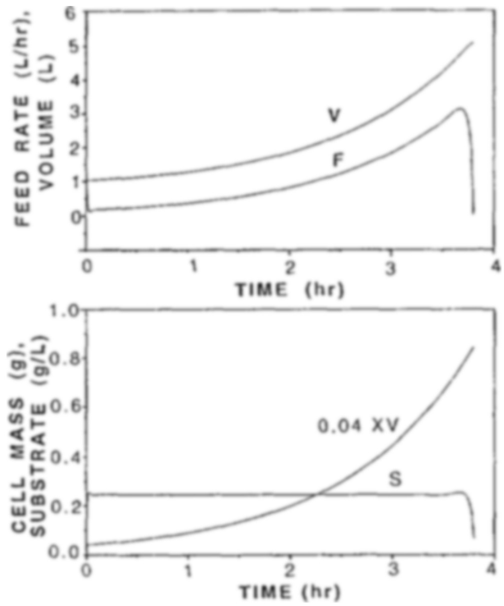

Fig. 1. Three-dimensional model (Case A): Optimal trajectories of state and control variables (V: reactor volume, F: feed rate, X: cell mass concentration, and S: substrate concentration).

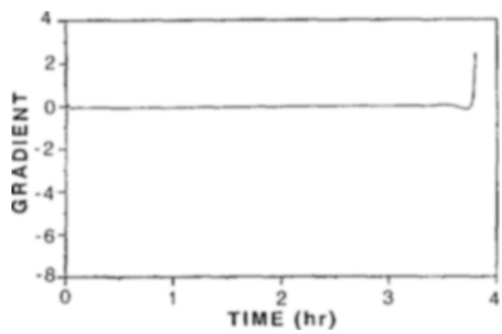

Fig. 2. Three-dimensional model (Case A): Gradient of the Hamiltonian ($\partial H/\partial F$).

In this study three different cases were considered (Table 1). In Case A, the initial substrate concentration was set to zero. According to singular control theory the optimum feed rate profile should consist of an initial period of maximum feed rate followed by singular and batch periods. This operating policy brings the substrate concentration to 0.24 g/L and maintains it there till the end of the fermentation. When the fermentor is full, there is a short batch period.

The final control and state variable profiles obtained using the conjugate gradient method are shown in Figure 1 and 2. The computed feed rate and substrate

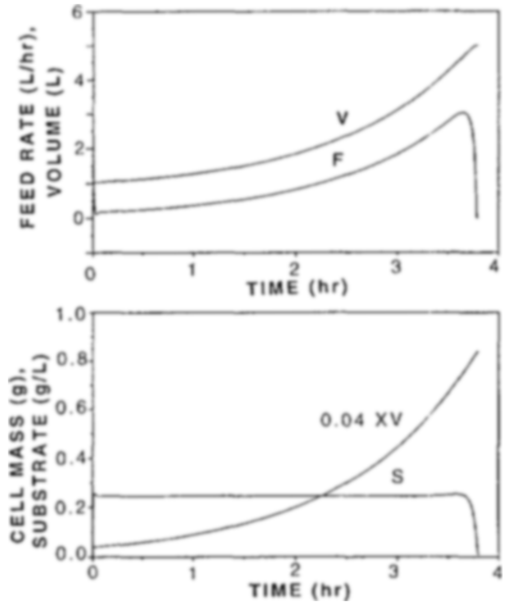

Fig. 3. Three-dimensional model (Case B): Optimal trajectories of state and control variables (V: reactor volume, F: feed rate, X: cell mass concentration, and S: substrate concentration).

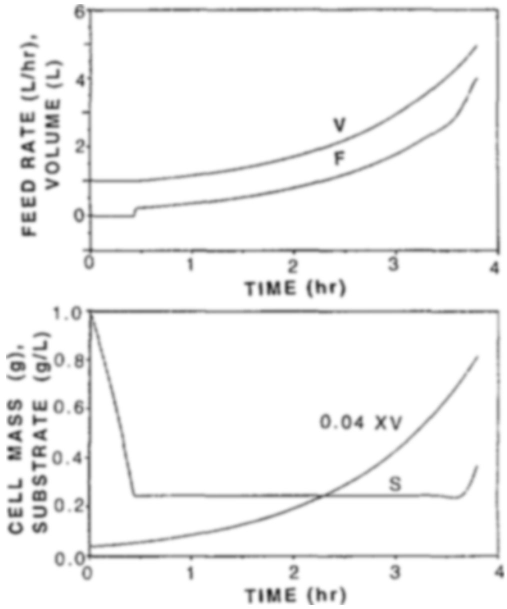

Fig. 5. Three-dimensional model (Case C): Optimal trajectories of state and control variables (V: reactor volume, F: feed rate, X: cell mass concentration, and S: substrate concentration).

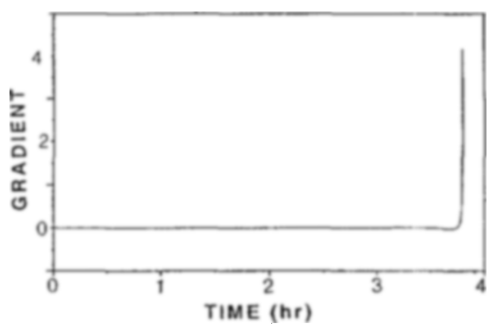

Fig. 4. Three-dimensional model (Case B): Gradient of the Hamiltonian ($\partial H/\partial F$).

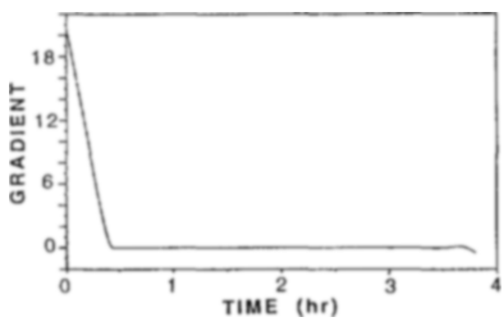

Fig. 6. Three-dimensional model (Case C): Gradient of the Hamiltonian ($\partial H/\partial F$).

concentration profiles agree very well with the predictions of singular control theory. The optimality of the converged feed profile can be established by looking at the gradient of Hamiltonian ($\partial H/\partial F$). The gradient is zero over the singular region, positive during the batch period, and negative during the initial period of maximum feed rate.

If a higher value of K is used, it is expected that the final volume would be closer to the desired value. This was tested using K=1000 (Case B). The final converged feed rate profile in Case A was chosen as the initial guess for Case B. The results are shown in Figure 3 and 4. The converged feed profile is very close to that obtained in Case A. The gradient of the Hamiltonian is a little smoother though. The final volume was indeed closer to the desired value (5.002 L in Case B compared to 5.056 L in Case A).

In Case C, a higher initial substrate concentration was chosen. The resulting feed rate profile (Figure 5) contains an initial batch period, which brings the substrate concentration down to 0.24 g/L. It is followed by a singular period, which maintains the glucose concentration constant. Near the end of the fermentation, the feed rate profile deviates a little from the

Table 2. Prameters used for optimization of the four-dimensional model

Case	A	B
X(0), OD	0.15	0.15
S(0), g/L	2.0	2.0
P(0), (units/ml OD)	0.1	0.1
V(0), L	0.6	0.6
V_f, L	1.2	1.2
S_f, g/L	10.0	10.0
F_{max}, L/hr	0.2	0.6
t_f, hr	10.0	10.0

optimum as indicated by the gradient of the Hamiltonian (Figure 6).

The conjugate gradient technique performed really well for this simple system. The algorithm was found to be robust and the choice of initial guess of feed profile did not affect convergence.

2. Optimization of a four-dimensional system

In the case of recombinant *Saccharomyces cerevisiae* with plasmid pRB58, which contains the SUC2 gene coding for the enzyme invertase, a four-dimensional model, written below, can describe the kinetics of cell growth and invertase production [16].

$$\frac{d}{dt}(XV)=\mu XV=(R_rY_{xr}+R_fY_{xf})XV \tag{22}$$

$$\frac{d}{dt}(SV)=FS_F-R_tXV \tag{23}$$

$$\frac{d}{dt}(PXV)=\left[\frac{k_pS}{K_p+S+K_sS^2}-k_dP\right]XV \tag{24}$$

$$\frac{dV}{dt}=F \tag{25}$$

The respiratory flux, R_r and fermentative flux, R_f depend on the total glucose flux R_t as follows.

$$R_t=\frac{k_tS}{K_t+S} \tag{26}$$

If $R_t>\frac{k_rS}{K_r+S}$, $R_r=\frac{k_rS}{K_r+S}$ and $R_f=R_t-R_r$

else $R_r=R_t$ and $R_f=0$

The objective of the optimization is to maximize the total invertase activity at the end of the fermentation. The final time t_f is assumed to be fixed.

$$\min_{F(t)} \quad \Pi=-(PXV)_f+K[V(t_f)-V_f]^2 \tag{27}$$

The parameters used for optimization are listed in Table 2. In Case A, the initial glucose concentration

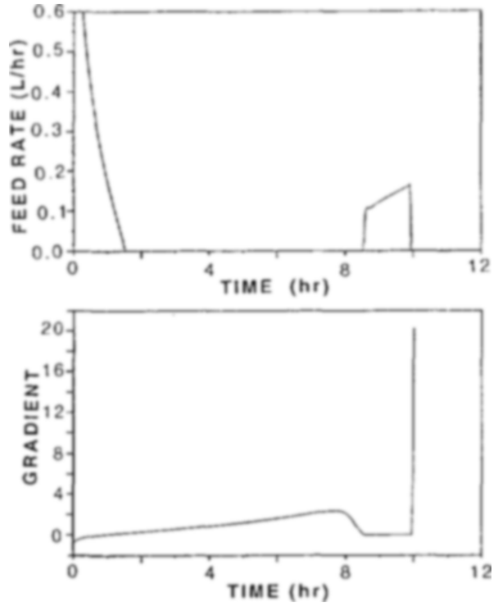

Fig. 7. Optimization of invertase production (Case A): Optimal flow rate and gradient of the Hamiltonian.

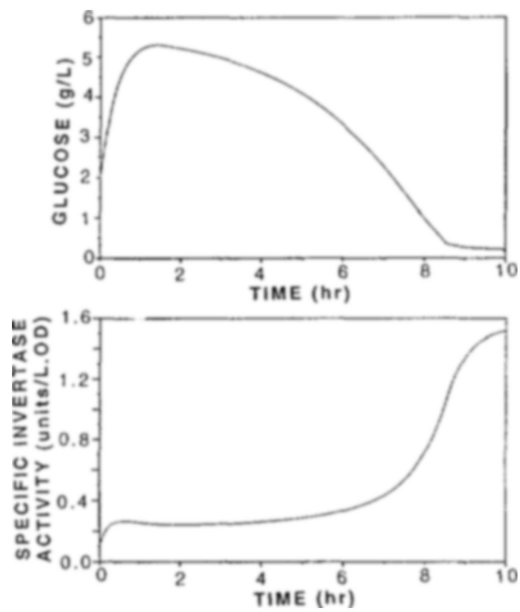

Fig. 8. Optimization of invertase production (Case A): Optimal glucose and invertase concentration profiles.

was chosen to be 2 g/L. The optimal feed rate (Figure 7 and 8) contains an initial maximum feed rate, which increases the glucose concentation to more than 5 g/L, thus increasing the specific cell growth rate. At these

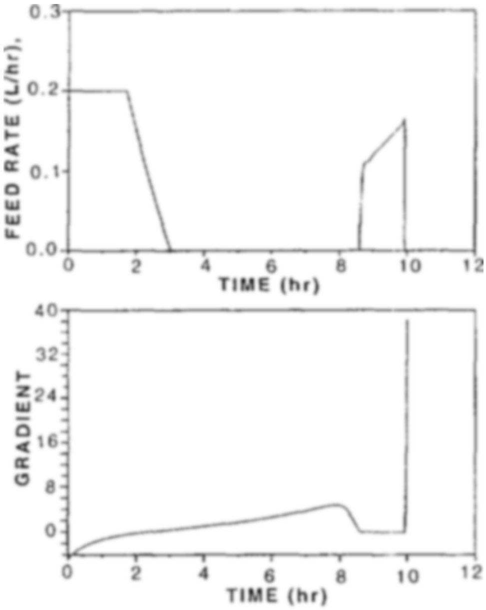

Fig. 9. Optimization of invertase production (Case B): Optimal flow rate and gradient of the Hamiltonian.

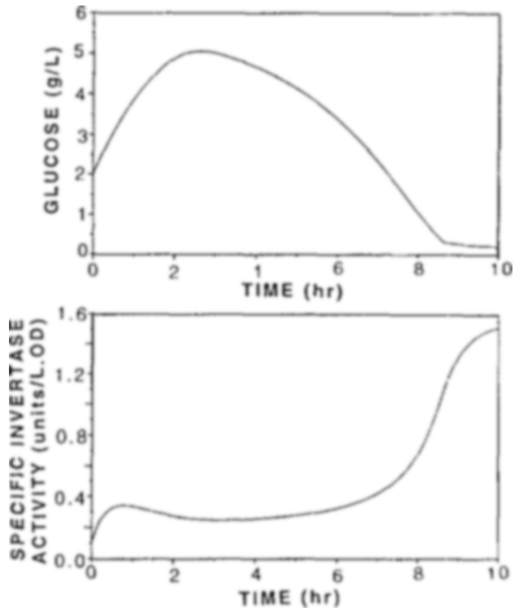

Fig. 10. Optimization of invertase production (Case B): Optimal glucose and invertase concentration profiles.

glucose levels, invertase production is repressed and the specific activity is almost constant. This period is followed by a batch period, in which the glucose concentration slowly drops to an optimum level for invertase expression, resulting in a gradual increase in the specific invertase activity. During the singular period, the glucose concentration stays almost constant around 0.225 g/L. Invertase production rate is very high during this singular phase. A small batch period follows, when the fermentor is full and the singular feed rate can no longer be implemented. Thus the optimal feed rate clearly results in an initial high cell growth phase followed by a high invertase production rate phase.

The effect of change in the maximum allowed feed rate was studied by decreasing its value to 0.2 L/hr (Case B, Figures 9 and 10). The only effect was a change in the duration of the maximum, batch and singular periods. The initial maximum feed rate period was longer to allow high glucose concentration, required to maximize cell growth at the beginning of the fermentation. Again the singular period maintains the glucose concentration at around 0.225 g/L.

In both cases, the resulting optimal feed rate profile clearly shows a period of maximum feed rate followed by batch and singular periods. The sign of the gradient of the Hamiltonian shows expected trends. At the transitions between different control arcs, the corners in the control profile are not sharp, as opposed to the predictions of the singular control theory. As optimum is approached, the performance index becomes very insensitive to small changes in the feed rate profile. Thus the algorithm converges to a profile, which has corners that are smoother than the exact optimal feed rate profile.

3. Optimization of a five-dimensional system

Five-dimensional systems are difficult to optimize with the two approaches previously developed by Modak et al. [5, 7]. A complex five-dimensional model was employed to verify the usefulness of conjugate gradient technique for solving optimization problems of high dimensionality. Sardonini and DiBiasio [17] developed a model to explain the growth kinetics of a plasmid-carrying strain of *S. cerevisiae*. It was found that under phosphate-limited growth conditions in a selective medium, the fraction of plasmid-free cell population was much larger than expected. This phenomenon was explained by assuming that the plasmid-free cells could grow in the selective medium by using a metabolite M, that is secreted into the medium by the plasmid-carrying strain, for growth. The model equations describing fed-batch fermentations are given below.

$$\frac{d}{dt}(X^{+}V)=(1-p)\mu^{+}X^{+}V \tag{28}$$

Table 3. Parameters used for optimization of DiBiasio's five-dimensional model

Model parameters	μ_{max}/hr	0.43
	K_s, (mg phosphate)/L	1.1
	K_m, (mg metabolite)/L	0.21
	p	0.14
	Y_s, OD per phosphate concentration	0.13
	Y_m, OD per metabolite concentration	0.03
	k, mg/(L. unit OD)	13.0
Optimization parameters	$(X^+V)_0$, OD.L	0.1
	$(X^-V)_0$, OD.L	0.01
	$(SV)_0$, mg	0.1
	$(MV)_0$, mg	0.05
	V_0, L	1.0
	V_f, L	5.0
	S_f, mg/L	6.8
	F_{max}, L/hr	0.5

$$\frac{d}{dt}(X^- V) = p\mu^+ X^+ V + \mu^- X^- V \tag{29}$$

$$\frac{d}{dt}(SV) = -\frac{\mu^+ X^+ V}{Y_s} - \frac{\mu^- X^- V}{Y_s} + FS_F \tag{30}$$

$$\frac{d}{dt}(MV) = k\mu^+ X^- V - \frac{\mu^- X^- V}{Y_M} \tag{31}$$

$$\frac{dV}{dt} = F \tag{32}$$

where $\mu^+ = \frac{\mu_{max} S}{K_S + S}$, $\mu^- = \frac{\mu^+ M}{K_M + M}$

In the above equations p represents the probability of plasmid loss upon cell division. Superscripts + and − represent plasmid-containing and plasmid free cells, respectively and Y_S, Y_M are yield coefficients.

For this system, a suitable objective may be the maximization of the amount of plasmid-containing cells at the end of the fermentation. This can be the case, for example, if plasmid-containing cells constitutively produce an intracellular product.

$$\min_{F(t)} \Pi = -(X^+ V)_f + K[V(t_f) - V_f]^2 \tag{33}$$

As the substrate concentration increases, the specific growth rate of plasmid-containing cells increases. However, this also results in an increase in the rate of production of the metabolite M, which increases the specific growth rate of plasmid-free cells.

The parameters used for optimization are listed in Table 3. The optimal feed rate consists of an initial batch period, followed by a period of maximum feed rate and a batch period at the end of the fermentation (Figures 11-13). The optimal control profile did not

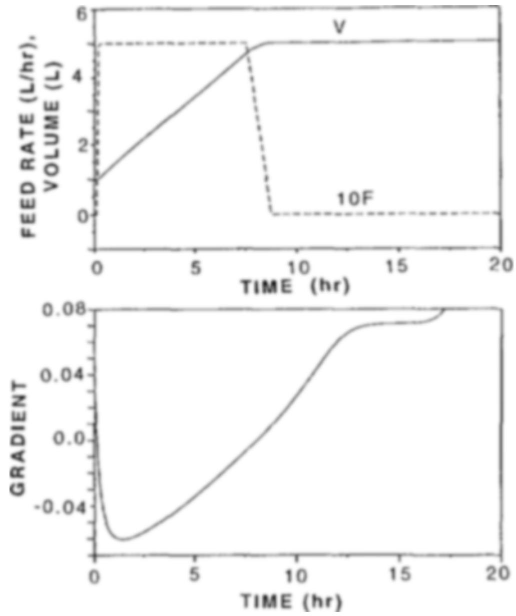

Fig. 11. Optimization of DiBiasio's model: Feed rate, volume and gradient of the Hamiltonian (V: reactor volume, and F: feed rate).

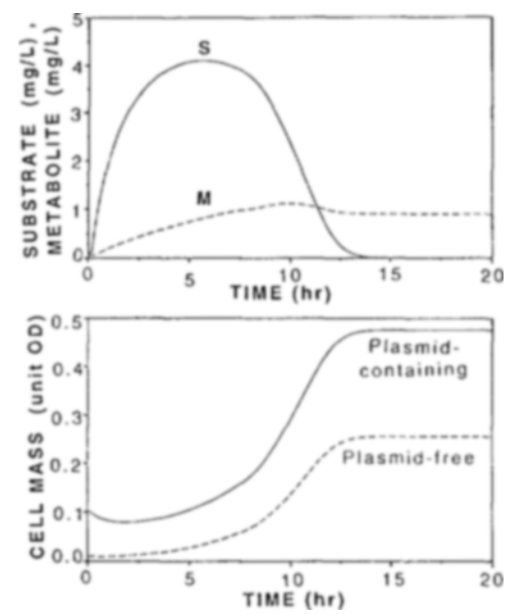

Fig. 12. Optimization of DiBiasio's model: Cell mass, substrate and metabolite concentration profiles (S: substrate concentration, and M: metabolite concentration).

contain any singular arcs. Although the final time was chosen to be 20 hours, the cells stop growing at around 15 hours. Thus, for any final time between 15 and 20 hours, the optimal feed rate profiles should be identical. The gradient of the Hamiltonian shows expected trends, which confirm the optimality of the converged feed rate profile.

CONCLUSIONS

A first-order conjugate gradient technique was demonstrated to be effective in solving a variety of optimization problems, ranging in difficulty from a simple three-dimensional model to a complex five-dimensional system. Although the convergence of this technique is somewhat slow, especially for the five-dimensional system, when the optimum is approached, the method converged to the correct optimal profile after a decrease in the step size. The correctness of the optimal profile can be judged by the variation in the gradient of the Hamiltonian. The gradient was found to be zero during singular periods, and had appropriate sign on boundary control arcs.

A boundary condition iteration method, previously developed by Modak et al. [5], was successful for systems of low dimensionality. However, this method fails for high dimensional systems because of two reasons. First, it becomes more and more difficult to guess the control arc sequence as the system dimension increases. Second, more adjoint variables need to be guessed at the junction points. The conjugate gradient method, on the other hand, does not need *a priori* guesses of control arc sequences. After an initial guess of the feed rate profile, which satisfies the constraint on final volume, the method proceeds towards the optimum in a smooth manner.

One of the attractive features of this method is its simplicity. The method does not require determination of many complex derivatives, which are needed for getting a functional form for the singular feed rate. It requires only a little more computation than the steepest descent approach. The presence of boundary arcs in the control profile actually results in a slightly reduced computation time per iteration.

The method proposed here does not need any variable transformation to convert the singular problem to a nonsingular one. This is an advantage, because the variable transformation result in constraints on the rate of change of the control variable. Appropriate theory to deal with such constraints is not currently available. Hence it is difficult to check the optimality of the converged control profiles obtained using the variable transformation method.

The conjugate gradient technique can be used to compute the optimal feed rates. Because this method does not use an explicit functional form for the singular feed rate, the converged feed rate profiles are not sharp at the corners of different control arcs. But in most cases, starting from an arbitrary initial guess of the feed rate, the method is able to get quite close to the optimal feed rate so fast. Thus within a few iterations, the general shape-the sequence of maximum, minimum and singular control arcs of the optimal profile-can be deduced without any *a priori* information. This sequence can then be used to calculate the optimal profile by Modak's [5] boundary condition iteration method. The conjugate gradient method can also provide good initial guesses for the switching times and adjoint variables to the boundary condition iteration method. Thus the approach developed here, and the boundary condition iteration approach developed by Modak, can be used in a complementary manner.

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NOMENCLATURE

F : fraction of plasmid-free cells, or feed rate L/hr
g : gradient of the Hamiltonian with respect to the control vector
H : Hamiltonian
I : a parameter defined in Eq. (13)
K, k : model parameters, or weight on the penalty function
k_d : first order inactivation constant [hr^{-1}]
p : probability of forming a plasmid-free cell upon cell division
R : fraction of glucose that is channeled through the fermentative pathway, or metabolic flux [(g glucose)/hr. OD]
S : substrate concentration [g/L]
s : conjugate gradient direction
t : time [hr]
V : volume [L]
w : a boundary function defined by Eq. (6), (12)
X : cell mass concentration [g/L or OD]
x : state vector
Y_{xr} : yield of respiratory pathway [OD/(g. glucose)]
Y_{xf} : yield of fermentative pathway [OD/(g. glucose)]

Greek Letters

α : a search parameter in the conjugate gradient method

β : a search parameter in the conjugate gradient method

λ : adjoint vector, or costate vector

μ : specific cell growth rate [hr]

Π : performance index, or objective function

π : invertase formation rate [KU/hr]

φ : gradient of Hamiltonian in singular problems

Superscripts

\+ : plasmid-containing cells

− : plasmid-free cells

* : optimal

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