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Jan F. Van Impe [a]; Bart M. Nicolaï [b]; Peter A. Vanrolleghem [c]; Jan A. Spriet [d]; Jan A. Spriet [e]; Bart De Moor [e]; Joos Vandewalle [e]
[a] ESA T—Department of Electrical Engineering, Katholieke Universiteit Leuven, Leuven, Belgium [b] Department of Agricultural Engineering, Katholieke Universiteit Leuven, Leuven, Belgium [c] Department of Agricultural Engineering, Rijksuniversiteit Gent, Cent, Belgium [d] Department of Agricultural Engineering, Katholieke Universiteit Leuven, [e] ESAT—Department of Electrical Engineering, Katholieke Universiteit Leuven,

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OPTIMAL CONTROL OF THE PENICILLIN G FED-BATCH FERMENTATION: AN ANALYSIS OF A MODIFIED UNSTRUCTURED MODEL

JAN F. VAN IMPE†

ESAT—Department of Electrical Engineering
Katholieke Universiteit Leuven
Kardinaal Mercierlaan 94 B-3001 Leuven Belgium

BART M. NICOLAÏ

Department of Agricultural Engineering
Katholieke Universiteit Leuven
Kardinaal Mercierlaan 92 B-3001 Leuven Belgium

PETER A. VANROLLEGHEM

Department of Agricultural Engineering
Rijksuniversiteit Gent
Coupure Links 653 B-9000 Gent Belgium

JAN A. SPRIET

Department of Agricultural Engineering
Katholieke Universiteit Leuven

BART DE MOOR‡, JOOS VANDEWALLE

ESAT—Department of Electrical Engineering
Katholieke Universiteit Leuven

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This paper presents the application of optimal control theory in determining the optimal glucose feed rate profile for the penicillin G fed-batch fermentation, using a modified unstructured mathematical model based on balancing methods. This updated model allows for a smooth transition between maintenance and endogenous metabolism, while all variables take on physically acceptable values under all conditions.

It is illustrated that the resulting computational algorithm is independent of the exact nature of the metabolism (maintenance or endogenous). In this scheme, the unusual optimization of some free initial states is included. However, as shown by simulations, the nature of the metabolism might be a key factor in concluding whether or not altering the substrate feeding strategy has an important influence on the final amount of product.

Mathematical and microbial insight leads to the construction of an alternative suboptimal heuristic

† Corresponding author.
‡ Research associate with the N.F.W.O. (Belgian National Fund for Scientific Research).

feeding strategy, with an excellent performance under all metabolic conditions. It can serve as a basis for the development of more practical and reliable control schemes.

It is indicated that feeding strategy optimization studies can be a tool in the design of real life experiments for model structure identification purposes.

KEYWORDS Optimal control Non-linear systems Biotechnological modeling Penicillin fed-batch fermentation Maintenance and endogenous metabolism.

1. INTRODUCTION

Penicillin G is a common antibiotic produced on a large scale. A fed-batch process design in which the rate limiting substrate (glucose) is fed continuously during at least part of the total process time seems to be the preferred fermentation technology. The optimization of product formation during fermentation as part of total process control has gained renewed attention (Spriet, 1987; San and Stephanopoulos, 1989).

The model of Heijnen *et al.* (1979) and the model of Bajpai and Reuß (1980, 1981), both claiming to be accurate descriptions of the fermentation process, allow for the optimization of the final amount of product with respect to the substrate feeding rate. However, feeding strategy optimization studies indicate that the realizable gain—i.e. the optimal product amount compared with the outcome of a constant feeding pattern in the same time with zero initial substrate amount—depends highly on the model used: the Heijnen *et al.* model predicts a gain in the region of one hundred percent, while the Bajpai and Reuß model predicts a gain ranging from only a few percents up to several hundred percent, depending on the parameter set used. In addition, the Heijnen *et al.* model is very sensitive towards different feeding policies, while the Bajpai and Reuß model is not. Both the conceptual differences between the two approaches and the corresponding parameters used have been shown to play an important role (Van Impe *et al.*, 1991a, 1992).

In recent years there has been a growing interest in the biochemistry of penicillin biosynthesis. Based on a critical review of the biochemical fundamentals and the consistency of the models mentioned, a modified unstructured model has been constructed in our group which incorporates recent biochemical knowledge (Nicolaï *et al.*, 1991). Its most striking features are the guarantee for physically acceptable values of all variables under all conditions, and a smooth transition between maintenance and endogenous metabolism as a function of the substrate concentration.

The contributions reported in this paper can be summarized as follows. The principal purpose is to investigate the possible effect of different metabolic assumptions—found back in model structure differences—on feeding strategy optimization results, using the modified model. In determining the (theoretical) optimal feed rate profile which maximizes the final amount of product for a given amount of substrate, the use of optimal control theory shall be illustrated. Further, a suboptimal strategy based on mathematical and microbial knowledge will be presented, that is found to be a useful alternative for the optimal profile, independent of the model structure. This heuristic strategy opens perspectives for more reliable model-independent control schemes. Some results in this context have been reported elsewhere (Van Impe *et al.*, 1991b).

2. A MODIFIED MATHEMATICAL MODEL

2.1 Mathematical Description of the Model

The usual form of an unstructured mathematical model for penicillin fermentation processes is given by the following set of mass balance equations:

$$\frac{dS}{dt} = -\sigma X + s_F u \tag{1}$$

$$\frac{dX}{dt} = \mu X \tag{2}$$

$$\frac{dP}{dt} = \pi X - k_h P \tag{3}$$

$$\frac{dV}{dt} = u \tag{4}$$

For an explanation of all symbols used, refer to the Nomenclature at the end of this paper.

A detailed analysis of both the model of Heijnen *et al.* and the model of Bajpai and Reuß revealed some physical and biochemical shortcomings. The main difference between the two models rests in the first place in assumptions about the nature of the metabolism for maintenance requirements of living biomass and product synthesis. In a maintenance metabolism concept (as adopted in the Bajpai and Reuß model), the energy supply for maintenance purposes and production is assumed to be provided by consumption of the external substrate. This can lead to (physically impossible) negative substrate concentrations, in cases where the feeding rate u becomes too small. In an endogenous metabolism concept (as adopted in the Heijnen *et al.* model), energy supply for maintenance purposes and production is assumed due to combustion of part of the biomass. This is not realistic in a situation where enough substrate is available. Based on recent advances in the biochemical knowledge of penicillin biosynthesis, Nicolaï *et al.* (1991) have presented a modified unstructured mathematical model. The basic design requirements were:

1. The general structure of the updated model must be the same as used by both Heijnen *et al.* and Bajpai and Reuß [Eqs. (1–4)]. In particular, material balances have to be satisfied.
2. There must be a smooth transition between maintenance and endogenous metabolism as a function of C_s: for C_s approaching zero endogenous metabolism is required; for high C_s values maintenance metabolism must be modeled. Further, it must be possible to adjust the endogenous fraction for a certain value of C_s, using as few additional parameters as possible (in order to avoid unnecessary complications in parameter estimation studies).
3. Recent biochemical evidence suggests that penicillin biosynthesis might be subjected to glucose repression. Although the exact mechanism (e.g. repression or inhibition) is not known yet, glucose inhibition kinetics as proposed by Haldane and also used by Bajpai and Reuß were chosen.

4. The specific substrate to biomass conversion rate function (Contois- or Monod-kinetics) is not fixed a priori, as both are acceptable from the biochemical point of view.
5. The modified model must be consistent as to allow physically acceptable values for all variables involved, under different fermentation conditions.
6. The right hand side of the resulting state equations must have continuous derivatives up to second order with respect to all state variables, in order to make the application of standard optimal control theory possible.

These requirements have been incorporated into the model Eqs.(1–4), using the following specific rates:

$$\pi = \pi_m \frac{C_s}{K_p + C_s + C_s^2/K_i} \tag{5}$$

$$\mu = \mu_{\text{substr}} - Y_{x/s}(f_m(C_s)m_s + f_p(C_s)\pi/Y_{p/s}) \tag{6}$$

where μ_{substr} is the specific substrate to biomass conversion rate, either modeled by Contois- or Monod-kinetics:

$$\mu_{\text{substr}} = \mu_C \frac{C_s}{K_x C_x + C_s} \quad \text{(Contois)} \quad \text{or} \quad \mu_{\text{substr}} = \mu_M \frac{C_s}{K_s + C_s} \quad \text{(Monod)} \tag{7}$$

The function $f_m(C_s)$ [resp. $f_p(C_s)$] is a measure for the endogenous fraction of the maintenance requirements of living biomass [resp. product synthesis]. They are chosen as follows:

$$f_m(C_s) = \exp(-C_s/E_m) \qquad f_p(C_s) = \exp(-C_s/E_p) \tag{8}$$

As a result of balancing, the specific glucose uptake rate is given by:

$$\sigma = \mu/Y_{x/s} + m_s + \pi/Y_{p/s} \tag{9}$$

$$= \mu_{\text{substr}}/Y_{x/s} + m_s(1 - f_m(C_s)) + \pi(1 - f_p(C_s))/Y_{p/s} \tag{10}$$

In Figure 1, the endogenous fraction $f(C_s)$ versus C_s is shown for some values of the parameter E. A physical interpretation can be assigned to the parameters E_m and E_p as follows: they represent the glucose concentration at which the respective endogenous fraction is equal to 36.8 percent.

Special Case 1: $(E_m = E_p) \rightarrow 0$
For very low values of E_i, the endogenous fraction approximates to zero for all values of $C_s > 0$ as in the original Bajpai and Reuß model. The specific rates reduce to:

$$\mu = \mu_{\text{substr}}$$

$$\sigma = \mu_{\text{substr}}/Y_{x/s} + m_s + \pi/Y_{p/s}$$

which represents a maintenance metabolism. However, for $C_s = 0$, $f_m = f_p = 1$. In other words, the metabolism becomes completely endogenous, thus preventing C_s from becoming negative.

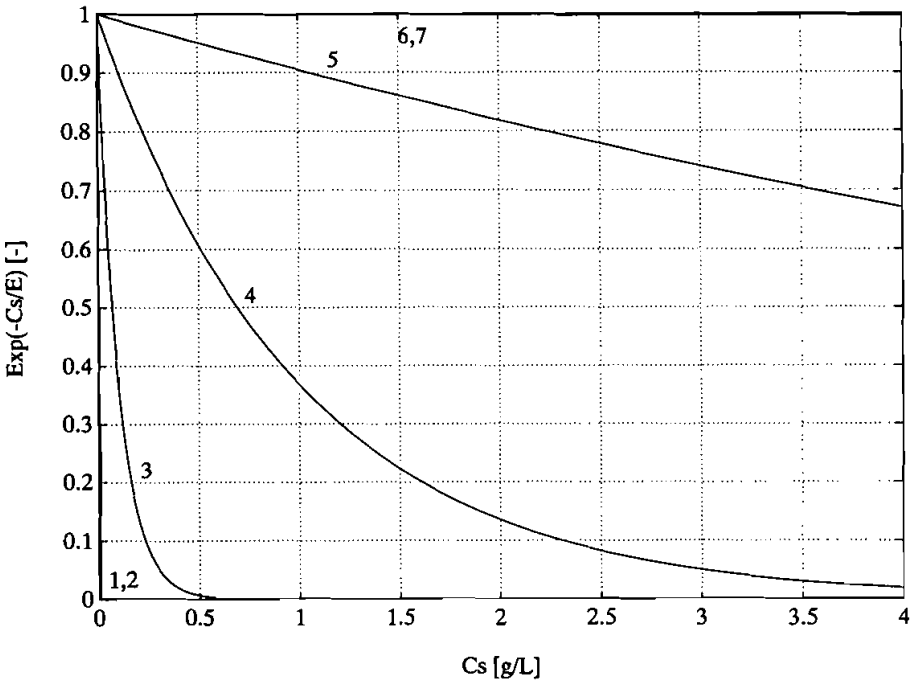

FIGURE 1 Behaviour of $\exp(-C_s/E)$ as a function of C_s for different values of E. Legend: (1) $E = 10^{-9}$ g/L, (2) $E = 10^{-3}$ g/L, (3) $E = 10^{-1}$ g/L, (4) $E = 1.$ g/L, (5) $E = 10^{+1}$ g/L, (6) $E = 10^{+3}$ g/L, (7) $E = 10^{+12}$ g/L.

Special Case 2: $(E_m = E_p) \rightarrow +\infty$

On the contrary, using very high values for E_i, the endogenous fraction approximates 100 percent for every value of C_s. The specific rates reduce to:

$$\mu = \mu_{\text{substr}} - Y_{x/s}(m_s + \pi/Y_{p/s})$$
$$\sigma = \mu_{\text{substr}}/Y_{x/s}$$

which represents an endogenous metabolism as used by Heijnen *et al.*

Although both special cases correspond to known metabolic concepts (maintenance and endogenous metabolism respectively), the physical interpretation of the parameters E_m and E_p as stated above has been lost.

Additional Special Cases

Combining Special Cases 1 and 2, two additional special cases can be constructed: a first one with $E_m \rightarrow 0$ and $E_p \rightarrow +\infty$, a second with $E_m \rightarrow +\infty$ and $E_p \rightarrow 0$. For instance, in the second case the specific rates reduce to:

$$\mu = \mu_{\text{substr}} - Y_{x/s}m_s$$
$$\sigma = \mu_{\text{substr}}/Y_{x/s} + \pi/Y_{p/s}$$

This models a process where production only occurs at the expense of substrate, while maintenance requirements of the mould are fulfilled by endogenous respiration.

A more general case: $0 < E_m < +\infty$, $0 < E_p < +\infty$
For intermediate values of E_m and E_p, there is a smooth transition between maintenance and endogenous metabolism as a function of C_s. Note that the ability to choose different values for E_m and E_p makes it possible to simulate different endogenous fractions of maintenance requirements and production respectively.

2.2 Some Simulation Results

All computations were done on a VAX–VMS system, using the NAG-routines D02EHF and D02EBF for stiff systems integration, and the MATRIX$_X$-routine MAXLIKE for parameter estimation. For all simulations mentioned in this paper, the following assumptions were made. Values of E_m were always set equal to E_p (further on simply denoted with E), as there is no a priori reason for not doing so. For μ_{substr} Contois-kinetics were chosen, to make the comparison with the original Bajpai and Reuß model possible. The nominal parameter set (due to Bajpai and Reuß, 1981) and the initial conditions are summarized in Table I. The total amount of substrate available for fermentation is equal to $\alpha = 1500$ g. A set of 120 reference data for S, X and P were generated, using the original Bajpai and Reuß model with a zero initial amount of substrate and a constant feed rate strategy during 120 hrs.

The model is fitted to these reference data for values of E between $E = 10^{-9}$ g/L (simulating a maintenance metabolism) and $E = 10^{+12}$ g/L (simulating an endogenous metabolism). The value of π_m is adjusted—since its value seems not very reliable—so as to minimize the Euclidian distance to the reference data. Some numerical values are summarized in Table II. Note that $E = 4.5622\,10^{-3}$ g/L represents a mixed maintenance-endogenous metabolism with an endogenous fraction of 50 percent at $C_s = \sqrt{K_p K_i}$, which maximizes π. For all values of E, π_m remains within the range of reported values. The corresponding time profiles have been plotted in Figure 2.

Of course, the results of the maintenance model coincide with the reference data, as C_s never becomes negative for this particular feeding strategy. For

TABLE I

Parameters and initial conditions used in simulations

Parameters			
μ_C	0.11 (h^{-1})	K_x	0.006 (g/g dry weight)
π_m	0.004 (g/g dry weight h)	k_h	0.01 (h^{-1})
K_p	0.0001 (g/L)	K_i	0.1 (g/L)
$Y_{x/s}$	0.47 (g dry weight/g)	$Y_{p/s}$	1.2 (g/g)
m_s	0.029 (g/g dry weight h)	s_F	500 (g/L)
Initial conditions			
X_0	10.5 (g dry weight)	S_0	to be specified (g)
P_0	0 (g)	V_0	$7 + S_0/s_F$ (L)
t_0	0 (h)	α	1500 (g)

TABLE II

Estimation of π_m and corresponding final state ($t_f = 120$ hrs), for some values of E

E (g/L)	π_m (g/g dry weight h)	$S(t_f)$ (g)	$X(t_f)$ (g)	$P(t_f)$ (g)
10^{-9}	$4.0000\ 10^{-3}$	$4.9761\ 10^{-2}$	330.28	59.651
$4.5622\ 10^{-3}$	$4.0750\ 10^{-3}$	$9.0286\ 10^{-2}$	330.28	59.683
1.	$5.0634\ 10^{-3}$	$3.6834\ 10^{-1}$	330.42	59.277
10^{+12}	$5.2861\ 10^{-3}$	$3.8212\ 10^{-1}$	330.97	60.822

$10^{-9} < E < 1.0$ g/L, the fit is almost exact. For the endogenous model, the fit of X and P is still very good, although the S-profiles differ somewhat in the growth phase.

This is a very important result, as it indicates that it is virtually impossible to make a distinction between different kinds of metabolic behaviour, using data from fermentations with this particular feeding strategy. However, it will be illustrated further on that the metabolic assumptions might be very important for feeding strategy optimization.

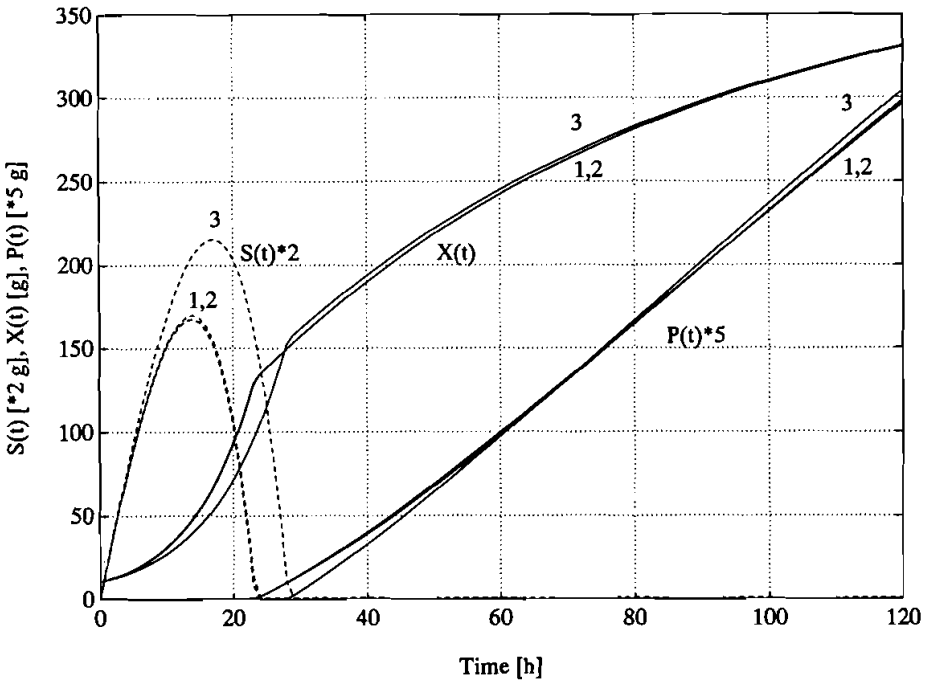

FIGURE 2 Substrate, biomass and penicillin profiles during constant feed rate fed-batch fermentation as predicted by the modified model–$S_0 = 0$ g. Legend: (1) $E_m = E_p = 10^{-9}$ g/L, (2) $E_m = E_p = 1.$ g/L, (3) $E_m = E_p = 10^{+12}$ g/L.

2.3 Statement of the Optimization Problem

An obvious choice for a state space vector is given by ("T" denotes the transpose):

$$\mathbf{x} = (x_1 \quad x_2 \quad x_3 \quad x_4)^T \triangleq (S \quad X \quad P \quad V)^T \tag{11}$$

and with the definition of:

$$\mathbf{f} = (f_1 \quad f_2 \quad f_3 \quad f_4)^T \triangleq (-\sigma X \quad \mu X \quad \pi X - k_h P \quad 0)^T \tag{12}$$

$$\mathbf{b} = (b_1 \quad b_2 \quad b_3 \quad b_4)^T \triangleq (s_F \quad 0 \quad 0 \quad 1)^T \tag{13}$$

the following state space model (which is linear in the control u) is obtained:

$$\frac{d\mathbf{x}}{dt} = \mathbf{f}(\mathbf{x}) + \mathbf{b}u \tag{14}$$

Numerical values for the initial conditions are given in Table I. $x_{2,0}$ and $x_{3,0}$ are given, $x_{1,0}$ and $x_{4,0}$ are related by (V_* denotes the given initial volume without substrate):

$$x_{4,0} = V_* + x_{1,0}/s_F \tag{15}$$

Note that glucose is added as a solution with concentration s_F. The optimization problem considered in this paper is to determine for the given set of differential Eqs. (1–4) the optimal feed rate profile which minimizes the performance index:

$$J(u) = g[\mathbf{x}(t_f)] \triangleq -P(t_f) \tag{16}$$

i.e. maximizes the final amount of product, subject to the following constraints:

—$t_0 = 0$, t_f = free

—all variables have to be kept positive, i.e. $\forall t$ in $[0, t_f]$: $x_i(t) \geq 0$, for $i = 1, \ldots, 4$; $u(t) \geq 0$

—the initial amount of substrate is free; the initial conditions $x_{1,0}$ and $x_{4,0}$ are only constrained by Eq. (15). In other words, some initial conditions can be manipulated to minimize the performance measure, so (16) should be replaced by:

$$J \equiv J(u, \mathbf{x}_0) = -x_3(t_f) \tag{17}$$

—the total amount is feed is fixed, i.e.:

$$x_{1,0} + s_F \int_{t_0}^{t_f} u(t)\, dt = \alpha$$

The last isoperimetric constraint on the input is equivalent to the physical constraint:

$$x_{4,f} \equiv V(t_f) = V_f, \quad V_f \text{ fixed} \tag{18}$$

3. OPTIMAL CONTROL ON THE MODIFIED MODEL

3.1 Statement of the Two Point Boundary Value Problem (TPBVP)

The Hamiltonian H for this problem is given by ($\boldsymbol{\lambda}$ is the vector of adjoint variables):

$$H = \boldsymbol{\lambda}^T(\mathbf{f}(\mathbf{x}) + \mathbf{b}u) \triangleq \phi + \psi u \tag{19}$$

$$\phi = \lambda_1 f_1 + \lambda_2 f_2 + \lambda_3 f_3 \qquad \psi = s_F \lambda_1 + \lambda_4 \tag{20}$$

The adjoint vector $\boldsymbol{\lambda}$ satisfies the following system of differential equations:

$$\frac{d\boldsymbol{\lambda}}{dt} = -\frac{\partial H}{\partial \mathbf{x}} = -\frac{\partial \mathbf{f}^{\mathrm{T}}}{\partial \mathbf{x}}\boldsymbol{\lambda} \tag{21}$$

Together with the state Eqs. (14), a system of $2 \times n$ first order differential equations is obtained—where n denotes the dimension of the state vector $\mathbf{x}$, here $n = 4$—which needs of course $2 \times n$ boundary conditions. These can be specified as follows:

—$x_{2,0}$ and $x_{3,0}$ are given
—$x_{1,0}$ and $x_{4,0}$ are interrelated by Eq. (15)
—$x_{4,f}$ is given due to Eq. (18)
—$\lambda_{i,f}$, $i = 1, \ldots, 3$ are given by:

$$\lambda_{i,f} = \frac{\partial g}{\partial x_i}(t_f) \quad \text{or} \quad (\lambda_{1,f} \quad \lambda_{2,f} \quad \lambda_{3,f})^{\mathrm{T}} = (0 \quad 0 \quad -1)^{\mathrm{T}} \tag{22}$$

It should be clear that another boundary condition is still needed, as $x_{1,0}$ and $x_{4,0}$ are not given explicitly. It can be shown that the missing condition is given by (Van Impe, 1992):

$$\psi(0) \equiv s_F\lambda_{1,0} + \lambda_{4,0} = 0 \tag{23}$$

An extremal control $u^*(t)$ follows from the minimization of the Hamiltonian H over all admissible control functions:

$$\min_{\text{all admiss } u} H(\mathbf{x}^*, \boldsymbol{\lambda}^*, u) = H(\mathbf{x}^*, \boldsymbol{\lambda}^*, u^*) \tag{24}$$

which is Pontryagin's Minimum Principle (1962) for this case. Since the state Eqs. (14) and the cost index (17) are time-invariant, H remains constant along an optimal trajectory. As the final time t_f is free, it follows that $H = 0$.

3.2 Computational Algorithm for the Optimal Control with Bounded Input and Fixed Initial State

As a first step in the solution, the given problem will be solved subject to an additional constraint on the input ($0 \le u(t) \le U_{\max}$, $U_{\max}$ given), and with the complete initial state being given (say $x_{1,0} = 0$, so $x_{4,0} = V_*$, and condition (23) is superfluous).

As the Hamiltonian H is linear in the control u, application of Pontryagin's Minimum Principle leads to the following Bang–Singular–Bang control:

$$u(t) = \begin{cases} U_{\max} & \text{if} \quad \psi < 0 \\ u_{\text{sing}} & \text{if} \quad \psi = 0,\ t_i \le t \le t_{i+1} \\ 0 & \text{if} \quad \psi > 0 \end{cases} \tag{25}$$

On any singular interval $[t_i, t_{i+1}]$, the singular control is obtained by repeatedly

differentiating the function ψ until u appears explicitly:

$$\frac{d\psi}{dt} = \dot{\boldsymbol{\lambda}}^T\mathbf{b} = -\boldsymbol{\lambda}^T\frac{\partial \mathbf{f}}{\partial \mathbf{x}}\mathbf{b} \triangleq \boldsymbol{\lambda}^T\mathbf{d} = 0 \tag{26}$$

$$\frac{d^2\psi}{dt^2} = \dot{\boldsymbol{\lambda}}^T\mathbf{d} + \boldsymbol{\lambda}^T\frac{\partial \mathbf{d}}{\partial \mathbf{x}}\dot{\mathbf{x}} = -\boldsymbol{\lambda}^T\frac{\partial \mathbf{f}}{\partial \mathbf{x}}\mathbf{d} + \boldsymbol{\lambda}^T\frac{\partial \mathbf{d}}{\partial \mathbf{x}}(\mathbf{f} + \mathbf{b}u) = 0 \tag{27}$$

The last equation can be solved for u_{sing}:

$$u_{\text{sing}}(t) = \frac{\boldsymbol{\lambda}^T((\partial \mathbf{f}/\partial \mathbf{x})\mathbf{d} - (\partial \mathbf{d}/\partial \mathbf{x})\mathbf{f})}{\boldsymbol{\lambda}^T(\partial \mathbf{d}/\partial \mathbf{x})\mathbf{b}} \tag{28}$$

Remark that in this case the denominator of the above expression is indeed different from zero. Obviously, this problem is a singular problem of order 2.

Note that both the numerator and the denominator are linear in the costate $\boldsymbol{\lambda}$. On any singular interval, the optimal control is a nonlinear feedback law of the state-variables only, as there are three linear homogeneous equations in the costate-variables:

$$\psi \equiv \boldsymbol{\lambda}^T\mathbf{b} = 0 \qquad \frac{d\psi}{dt} \equiv \boldsymbol{\lambda}^T\mathbf{d} = 0 \qquad \phi \equiv \boldsymbol{\lambda}^T\mathbf{f} = 0 \tag{29}$$

It follows that the TPBVP has been reduced to the determination of the optimal sequence and the corresponding switching times. The solution to this problem starts from constraints imposed by the following model-independent conjecture (based on common industrial practice):

Conjecture. *The feed rate profile must be determined so that during the growth phase the cells grow as fast as possible, while during the production phase the cells are forced to produce the desired product as much as possible.*

The resulting straightforward algorithm can be seen as a modification of the one proposed by Lim *et al.* (1986). However, simulations have indicated that our scheme is numerically more reliable, as it does not use any costate variable at all. Note that the above conjecture is not really required since the Eqs. (25)–(28) in principle fully specify the optimal control profile. However, a detailed mathematical justification for the following computational algorithm shows that the feed rate profile determined this way satisfies all necessary optimality conditions and is indeed optimal (Van Impe, 1992). For the most general case of low initial values for S and X, it can be summarized as follows (see also Figure 3):

ALGORITHM

1. Make a guess of t_1, or equivalently, determine the amount of substrate reserved to the growth-phase. Integrate the state Eqs. (14) from $t = 0$ to $t = t_1$ with $u(t) = U_{\max}$.
2. Make a guess of t_2. Integrate the state equations from $t = t_1$ to $t = t_2$ with $u(t) = 0$. This completes the growth-phase.

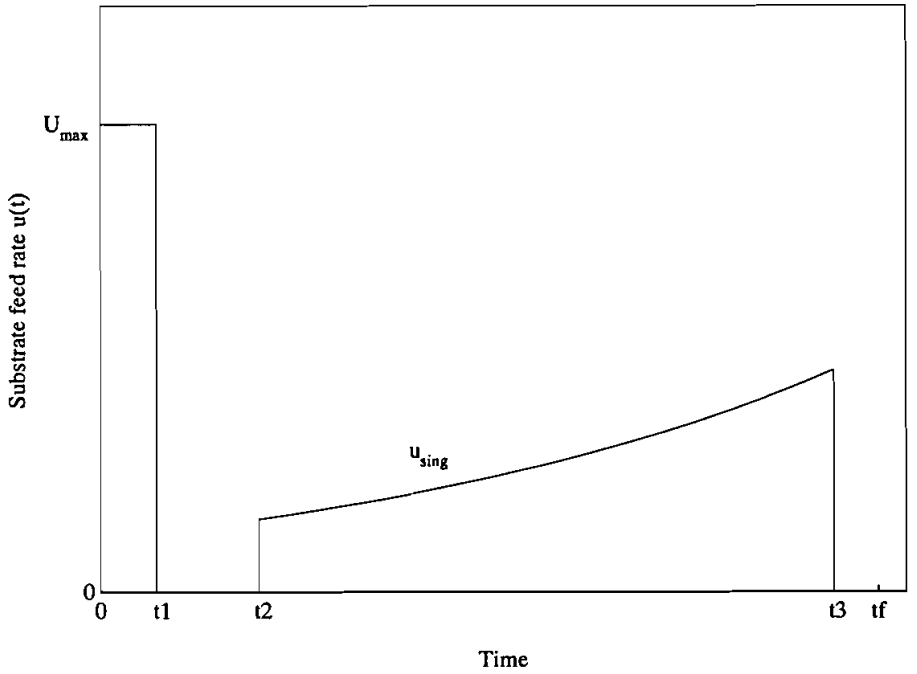

FIGURE 3 Optimal volumetric feed rate profile for bounded input and low initial values of S and X.

3. Integrate the state equations using the above determined singular control (28) until condition (18) is met at $t = t_3$.
4. Complete the integration with $u(t) = 0$ until dP/dt becomes 0 at $t = t_f$ (as $H(t_f) \equiv \phi(t_f) \equiv -f_3(t_f) = 0$), and store the value of the cost index $J(u, \mathbf{x}_0)$ (17). This completes the production-phase.
5. For the same value of t_1, search for the time t_2 for which $J(u, \mathbf{x}_0)$ reaches its minimum by repeating Steps 2–4.
6. Go to Step 1 with a new guess of t_1 in order to minimize $J(u, \mathbf{x}_0)$ as a function of t_1.

3.3 Computational Algorithm for the Optimal Control with Unbounded Input and Some Initial States Free

The original problem can be solved by considering the limit $U_{max} \to \infty$ (Van Impe, 1992). As a consequence, $t_1 \to 0$. Therefore, omitting the upper bound on the input leads to the injection of all substrate used for growth at time $t = 0$, the growth-phase becoming a batch-phase $[u(t) = 0]$. So in the above algorithm, the only modification is to replace the time t_1 by the equivalent initial condition $\mathbf{x}_{1,0}$.

Observe that the numerical value of the switching times t_i and of the singular control is determined by the value of the parameter E. However, the optimal control sequence as given in the algorithm (e.g. batch-singular-batch in the case of an unbounded input) is independent of the metabolism involved.

Simulation results will be given after the introduction of some suboptimal heuristic strategies, in order to make the comparison between the performance of different strategies more easy.

4. A HEURISTIC CONTROL STRATEGY

In this section, a heuristic control based on mathematical and microbial knowledge is proposed. The construction of a suboptimal profile is based principally on the concept of a biphasic fermentation (described in the Conjecture). Some of the ideas concerning heuristic C_s-control are reported elsewhere (Van Impe, 1992).

For the control during growth, refer to the previous section: the substrate consumed for growth is added all at once at $t = 0$ in order to obtain the highest possible value of μ for all t during growth (Eq. (6)).

During production, we focus on the specific rate π. Equation (5) indicates that π reaches its maximum at $C_s \triangleq C_{s,\text{crit}} = \sqrt{K_p K_i}$. The maximum value is $\pi_{\max} = \pi_m/(1 + 2\sqrt{K_p/K_i})$. So during production, keep C_s at $C_{s,\text{crit}}$, using the following control [from (1) and (4)]:

$$u_{\text{production}} = \frac{\sigma X}{s_F - C_s} \tag{30}$$

As a consequence, the conjunction point t_2 of growth and production is simply dictated by the condition $C_s = C_{s,\text{crit}}$. The control is stopped at $t = t_3$ when all substrate is used. As in the optimal case, the concluding batch-phase is stopped when $dP/dt = 0$.

Note that the complete suboptimal control (called heuristic C_s-control) is obtained in closed-loop for a given S_0. As a result, the optimization problem is reduced to the one-dimensional optimization of S_0.

A further refinement of this strategy exists in optimizing the switching time t_2. In other words, during production C_s is kept constant, but not necessarily at $C_s = C_{s,\text{crit}}$. As in the case of optimal control, a two-dimensional optimization of S_0 and t_2 is obtained.

Remark that for μ_{substr} modeled with Monod-kinetics, the control (30) also keeps μ constant during production. An equivalent heuristic μ-control for Contois-kinetics follows from the condition $d\mu/dt = 0$:

$$u_{\text{production}} = \frac{\mu_{\max} K_x C_x (C_s \mu + C_x \sigma)/(K_x C_x + C_s)^2 + F(E_m, E_p)\sigma X/V}{\mu_{\max} K_x C_x s_F / V (K_x C_x + C_s)^2 + F(E_m, E_p)(s_F - C_s)/V} \tag{31}$$

where

$$F(E_m, E_p) \triangleq \exp(-C_s/E_m) m_s Y_{x/s}/E_m + \exp(-C_s/E_p) \frac{Y_{x/s}}{Y_{p/s}} \left(\frac{\pi}{E_p} - \frac{d\pi}{dC_s} \right) \tag{32}$$

Before giving some simulation results, some advantages of these suboptimal profiles are mentioned. It is well-known that implementing an optimal control in practice may be hampered by many problems. In general, the optimal control is not obtained in complete closed-loop. So, as optimal control is a very model-

sensitive technique, a feed-forward will not generate the predicted results. As long as a sufficiently accurate model for the penicillin fermentation is not available, the determined profiles can be used only to obtain a greater qualitative insight into the process.

On the other hand, the suboptimal profiles presented here are the translation of a more realistic control objective, namely setpoint control, for which even adaptive control algorithms can be developed (Bastin and Dochain, 1990; Van Impe *et al.*, 1991c). One could keep e.g. μ constant without the knowledge of an exact analytic expression for it, so the algorithm becomes really model-independent. Furthermore, there would not be a need for a complete measurement of the state, a problem which has not been solved completely until now. Finally, observe that the heuristic control sequence is also independent of the metabolism involved.

5 SIMULATION RESULTS

Some numerical results obtained with the above algorithms are summarized in Table III. In order to compare the performance of the different feeding policies, define a gain G as:

$$G \triangleq 100 \times \frac{J(u, \mathbf{x}_0) - J(u_{\text{ref}}, \mathbf{x}_{0,\text{ref}})}{J(u_{\text{ref}}, \mathbf{x}_{0,\text{ref}})} \% \tag{33}$$

TABLE III

Numerical results for optimal and heuristic control, for some values of E

E (g/L)	S_0 (g)	t_2 (h)	t_f (h)	P_f (g)	G (%)
Optimal control					
10^{-9}	528	28.271	132.033	63.846	3.961
$4.5622\,10^{-3}$	880	32.749	125.200	70.652	16.822
1.	1409	37.016	117.602	87.448	49.064
10^{+12}	1411	42.106	122.493	89.709	45.909
Heuristic C_s-Control		$C_s(t_2) = \sqrt{K_p K_i}$			
10^{-9}	533	28.355	131.323	63.597	3.692
$4.5622\,10^{-3}$	940	33.338	124.427	68.436	13.160
1.	1401	36.969	116.906	87.164	48.580
10^{+12}	1404	42.059	121.974	89.420	45.455
Heuristic C_s-Control		$C_s(t_2)$ optimized			
10^{-9}	551	28.644	131.372	63.724	3.762
$4.5622\,10^{-3}$	1124	34.926	122.252	69.263	14.527
1.	1417	37.075	117.250	87.258	48.740
10^{+12}	1418	42.165	122.189	89.511	45.587
Heuristic μ-Control		$\mu(t_2)$ optimized			
10^{-9}	551	28.644	131.321	63.736	3.782
$4.5622\,10^{-3}$	1113	34.838	122.727	69.439	14.817
1.	1406	36.990	117.511	87.428	49.030
10^{+12}	1407	42.069	122.445	89.686	45.870

where the subscript "ref" denotes a reference strategy: a constant strategy with zero initial substrate amount, during the same time as the strategy under consideration.

From Table III, two important remarks can be made. The performance of the heuristic strategies is excellent, independent of the metabolic assumptions. Further, the results indicate that there exists a maximum realizible gain, as a function of the endogenous fraction E. In order to obtain a more detailed picture, the heuristic C_s-control (with $C_s(t_2) = \sqrt{K_p K_i}$) has been calculated for a large number of values of E. The results are shown in Figure 4. The gain G reaches its maximum value $G_{max} = 50.390\%$ for $\log(E) = 1.250$, while the minimum value is $G_{min} = 3.692\%$ for $\log(E) \rightarrow -\infty$ (which represents the model of Bajpai and Reuß). Observe that $P(t_f)$ has a (very smooth) maximum value $P_{f,max} = 89.68$ g, for $\log(E) = 1.875$. The optimal value of $S(t_0)$ increases uniformly with E: for a maintenance metabolism ($E \rightarrow 0$), 35.53% of the available substrate amount is needed at the beginning of the fermentation, while for an endogenous metabolism ($E \rightarrow \infty$) as much as 93.6% is needed. This result can be explained as follows. When E moves from $E = 0$ to $E \rightarrow \infty$, the endogenous fraction of energy supply for maintenance requirements of living biomass and product synthesis increases uniformly. As a result, an increasing amount of biomass must be built up during the growth phase (requiring an increasing amount of substrate), which is then partially combusted during the production phase.

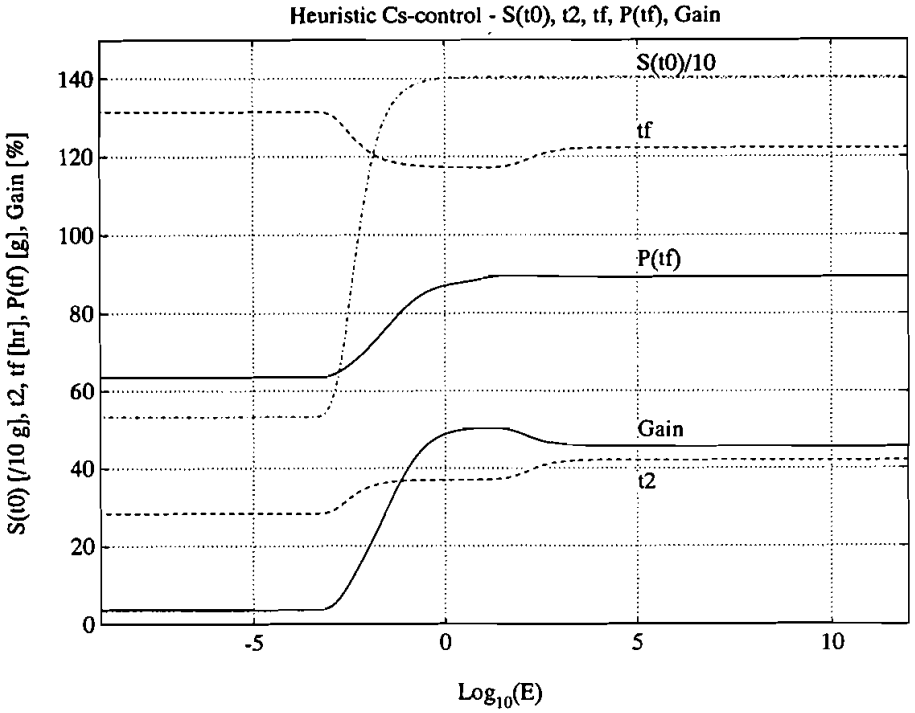

FIGURE 4 Heuristic C_s-control: $S(t_0)$, t_2, t_f, $P(t_f)$ and G as functions of E.

These simulations sufficiently illustrate that the metabolic condition of the mould might be a key factor in concluding whether or not changing the glucose feeding strategy for a given amount of substrate has an effect on the final amount of product. However, in order to identify the parameter E, the glucose feeding strategy must be designed carefully: remember that it is impossible to extract E from data resulting from a constant glucose input rate. We believe that future research should concentrate in the first place on the design of a sufficiently exciting input pattern, for which an adaptive implementation of the heuristic controllers presented in this paper could serve as a basis.

6. CONCLUSIONS

It is indicated that the assumptions about the fundamental nature of the metabolism might have a great influence on feeding strategy optimization results of the penicillin G fed-batch fermentation, based on mathematical modeling.

In order to test the importance of the metabolic nature of the mould, the modified unstructured mathematical model proposed by Nicolaï *et al.* has been used. The optimal control for a well-defined optimization problem has been derived, resulting in a numerically reliable, straightforward computational algorithm, that is independent of the metabolism involved. It has been shown that an endogenous metabolism allows for a greater gain in final product amount than a maintenance metabolism, as compared with the outcome of a constant feeding strategy in the same time with zero initial substrate amount.

A heuristic control strategy has also been presented, based on mathematical and microbial insight, that proved to be a successful alternative for optimal control, independent of the metabolic conditions. It was illustrated that these suboptimal controls can be calculated using essentially the same algorithm as for the optimal profiles. This heuristic methodology is in fact the translation of a more realistic control objective, namely setpoint control. It can serve as a basis for the development of more practical and reliable control schemes.

It is illustrated that feeding strategy optimization studies can be a valuable tool in designing experiments for structure identification purposes of biotechnological processes.

NOMENCLATURE

t	time (h)
S	amount of substrate in broth (g) (glucose)
S_0	initial amount of substrate in broth (g) (glucose)
X	amount of cell mass in broth (g dry weight) (biomass)
P	amount of product in broth (g) (penicillin)
V	fermentor volume (L)
u	input substrate feed rate (L/h)

C_s	S/V substrate concentration in broth (g/L)
C_x	X/V cell mass concentration in broth (g dry weight/L)
C_p	P/V product concentration in broth (g/L)
s_F	substrate concentration in feed stream (g/L)
E_m	parameter related to the endogenous fraction of maintenance requirements for biomass (g/L)
E_p	parameter related to the endogenous fraction of product synthesis (g/L)
K_x	Contois saturation constant for substrate limitation of biomass production (g/g dry weight)
K_s	Monod saturation constant for substrate limitation of biomass production (g/L)
K_p	saturation constant for substrate limitation of product formation (g/L)
K_i	substrate inhibition constant for product formation (g/L)
m_s	maintenance constant (g/g dry weight h)
k_h	penicillin hydrolysis or degradation constant (h^{-1})
$Y_{x/s}$	cell mass on substrate yield (g dry weight/g)
$Y_{p/s}$	product on substrate yield (g/g)
G	gain due to substrate feeding rate optimization (%)
α	total amount of glucose available for fermentation (g)
σ	specific substrate consumption rate (g/g dry weight h)
μ	specific growth rate (h^{-1})
μ_{substr}	specific substrate to biomass conversion rate (h^{-1})
μ_C	maximum specific growth rate for Contois kinetics (h^{-1})
μ_M	maximum specific growth rate for Monod kinetics (h^{-1})
π	specific production rate (g/g dry weight h)
π_m	specific production constant (g/g dry weight h)

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REFERENCES

Bajpai, R.K., and Reuß, M., 'A mechanistic Model for Penicillin Production', *J. Chem. Tech. Biotechnol.*, **30**, 332–344 (1980).

Bajpai, R.K., and Reuß, M., 'Evaluation of Feeding Strategies in Carbon-Regulated Secondary Metabolite Production through Mathematical Modelling', *Biotechnol. Bioeng.*, **23**, 717–738 (1981).

Bastin, G., and Dochain, D., *On-line Estimation and Adaptive Control of Bioreactors,* Elsevier Science Publishers B.V. (1990).

Heijnen, J.J., Roels, J.A., and Stouthamer, A.H., 'Applications of Balancing Methods in Modeling the Penicillin Fermentation', *Biotechnol. Bioeng.*, **21,** 2175–2201 (1979).

Lim, H.C., Tayeb, Y.J., Modak, J.M., and Bonte, P., 'Computational algorithms for Optimal Feed Rates for a Class of Fed-Batch Fermentation: Numerical Results for Penicillin and Cell Mass Production', *Biotechnol. Bioeng.*, **28,** 1408–1420 (1986).

Nicolaï, B.M., Van Impe, J.F., Vanrolleghem, P.A., and Vandewalle, J., 'A Modified Unstructured Mathematical Model for the Penicillin G Fed-Batch Fermentation', *Biotechnology Letters,* **13**(7), 489–494 (1991).

Pontryagin, L.S., Boltyanskii, V.G., Gamkrelidze, R.V., and Mishchenko, E.F., *The Mathematical Theory of Optimal Processes,* Wiley-Interscience, New York (1962).

San, K.Y., and Stephanopoulos, G., 'Optimization of Fed-Batch Penicillin Fermentation: A Case of Singular Optimal Control with State Constraints', *Biotechnol. Bioeng.*, **34,** 72–78 (1989).

Spriet, J.A., 'The Control of Fermentation Processes', Proceedings of the Forum for Applied Biotechnology, Gent, Belgium, 1987, 1371–1381 (1987).

Van Impe, J.F., Nicolaï, B.M., Spriet, J.A., De Moor, B., and Vandewalle, J., 'Optimal Control of the Penicillin G Fed-Batch Fermentation: An Analysis of the Model of Bajpai and Reuß', Proceedings of the European Simulation Multiconference, Copenhagen, Denmark, June 17–19, 1991, 867–872 (1991a).

Van Impe, J.F., Nicolaï, B.M., Vanrolleghem, P.A., Spriet, J.A., De Moor, B., and Vandewalle, J., 'Optimal Control of the Penicillin G Fed-Batch Fermentation: An Analysis of a New Unstructured Model', Proceedings of the European Control Conference, Grenoble, France, July 2–5, 1991, 1624–1629 (1991b).

Van Impe, J.F., Nicolaï, B.M., De Moor, B., and Vandewalle, J., 'Evaluation of On-line Conventional and Adaptive Controllers for the Penicillin G Fed-Batch Fermentation based on Simulations using a New Mathematical Model', Proceedings of the European Simulation Multiconference, Copenhagen, Denmark, June 17–19 1991, 873–878 (1991c).

Van Impe, J.F., 'Modeling and Optimal Adaptive Control of Biotechnological Processes', PhD thesis, Department of Electrical Engineering, K.U. Leuven (1992).