

# Optimal Control of the Penicillin G Fed-Batch Fermentation : An Analysis of a New Unstructured Model

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## Abstract

### Optimal Control of the Penicillin G Fed-Batch Fermentation : An Analysis of a New Unstructured Model

#### Summary

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 new unstructured mathematical model based on balancing methods. This new 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 a suboptimal heuristic 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.

**Key Words**      Optimal Control, non-linear systems, biotechnological modeling, penicillin fed-batch fermentation, maintenance and endogenous metabolism

## 1 Introduction

Nowadays penicillin G is an almost 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 a part of the total process time seems to be the preferred fermentation technology. The optimization of product formation during fermentation as a part of total process control has gained a renewed attention<sup>1</sup>.

In recent years there has been a growing interest in the biochemistry and modeling of the penicillin biosynthesis. Nicolaï et al.<sup>2</sup> reconsidered the biochemical fundamentals and the consistency of two models that allow for the optimization of the final product amount with respect to the substrate feeding rate: the model of Heijnen et al.<sup>3</sup> and the model of Bajpai and Reu<sup>4,5</sup>. As a result, a new hypothetical model has been constructed which incorporates recent biochemical knowledge. 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 principal purpose of this paper is to investigate the possible effect of *model structure differences only* on feeding strategy optimization results. The paper is organized as follows. Section 2 summarizes the new unstructured mathematical model proposed by Nicolaï et al. In Section 3, we illustrate the use of Optimal Control theory in determining the optimal feed rate profile that maximizes the final amount of product, for a given amount of substrate. In Section 4, we derive a suboptimal strategy based on mathematical and microbial knowledge, that is found to be a useful alternative for the optimal profile and opens perspectives for more reliable model-independent control schemes. Some conclusions are formulated in Section 5.

## 2 The mathematical model of Nicolaï et al.

### 2.1 Mathematical description of the new model

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

$$\frac{dS}{dt} = -\sigma X + s_f u \quad (1)$$

$$\frac{dX}{dt} = \mu X \quad (2)$$

$$\frac{dP}{dt} = \pi X - k_h P \quad (3)$$

$$\frac{dV}{dt} = u \quad (4)$$

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

In studying the optimal glucose feed rate profile that maximizes the final product amount, a lot of work has been done on both the model of Heijnen et al. and the model of Bajpai and Reuß<sup>6-10</sup>. A detailed analysis of both models revealed some physical and biochemical shortcomings. Based on recent advances in the biochemical knowledge of the penicillin biosynthesis, Nicolai et al.<sup>2</sup> have presented a new unstructured mathematical model. The basic design requirements were :

- The *general structure* of the new model must be the same as used by both Heijnen et al. and Bajpai and Reuß (equations (1)-(4)). In particular, material balances have to be satisfied.
- 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 as possible (in order to avoid unnecessary complications in parameter estimation studies) additional *parameters*.
- The biochemical evidence presented in another paper<sup>2</sup> 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<sup>11</sup> and also used by Bajpai and Reuß was chosen.
- The specific substrate to biomass conversion rate (*Contois-* or *Monod-*kinetics<sup>11</sup>) is not fixed *a priori*, as both are acceptable from the biochemical point of view.
- The new model must be *consistent* as to allow physically acceptable values for all variables involved, under different fermentation conditions.
- 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 equations (1)–(4), using the following specific rates :

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

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

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

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

The functions  $f_m(C_s)$  and  $f_p(C_s)$ , a measure for the *endogenous fraction* of respectively maintenance requirements and production, are chosen as follows :

$$f_m(C_s) = \exp(-C_s/E_m) \quad f_p(C_s) = \exp(-C_s/E_p) \quad (8)$$

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

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

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

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*.

Figure 1 Behaviour of  $\exp(-C_s/E)$  as a function of  $C_s$  for different values of  $E$

- *Special Case 1* :  $(E_m = E_p) \rightarrow 0$

For very low values of  $E_i$ , the endogenous fraction approximates zero for all values of  $C_s > 0$  as in the original Bajpai and Reuß model. The specific rates reduce to :

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

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

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.

- *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 :

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

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

- *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 :

$$\begin{aligned}\mu &= \mu_{substr} - Y_{x/s}m_s \\ \sigma &= \mu_{substr}/Y_{x/s} + \pi/Y_{p/s}\end{aligned}$$

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 respectively maintenance requirements and production.

Remark that functions  $f_i(C_s)$  of the following form :

$$f_i(C_s) = 1 - \frac{C_s}{K_i + C_s} \quad (11)$$

also meet all model design requirements mentioned above. Recently, Beeftink et al.<sup>12,13</sup> proposed a model for a simple microbial growth process without product formation, with a *transition*-function between maintenance and endogenous metabolism of this form ( $K_i$  was set equal to the Monod saturation constant of the specific substrate to biomass conversion rate). However, this Monod-type transition-function does not approach zero as fast as the exponential function which was used here (with  $K_i$  set equal to  $E_i$ ). Further, there is in fact no reason for fixing  $K_i$  a priori, so the number of additional parameters is the same. Finally,

it is more difficult to handle a Monod-type transition-function analytically, in particular in taking partial derivatives as is required for Optimal Control (see Section 3).

## 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<sub>X</sub>*-routine *MAXLIKE* for parameter estimation. For all simulations mentioned in this paper, we have made the following assumptions. We always set  $E_m$  equal to  $E_p$  (further on simply denoted with  $E$ ), as there is no *a priori* reason for not doing so. For  $\mu_{substr}$  *Contois*-kinetics has been chosen, to make the comparison with the original Bajpai and Reuß model possible. The *nominal parameter set* (due to Bajpai and Reuß<sup>5</sup>) and the *initial conditions* are summarized in Table 1. The total amount of substrate available for fermentation is equal to  $\alpha = 1500$  g. A set of 120 *reference data* for  $S$ ,  $X$  and  $P$  has been generated, using the original Bajpai and Reuß model with a zero initial amount of substrate and a constant feed rate strategy during 120 hrs.

Table 1 *Parameters and initial conditions used in simulations*

We have tried to fit the model to these reference data in four cases : (1)  $E = 1. \cdot 10^{-9}$  g/L (simulating a maintenance metabolism) (2)  $E = 4.5622 \cdot 10^{-3}$  g/L (simulating a mixed maintenance-endogenous metabolism with an endogenous fraction of 50 percent at  $C_s = \sqrt{K_p K_i}$ , which maximizes  $\pi$ ) (3)  $E = 1.$  g/L (simulating a mixed maintenance-endogenous metabolism) (4)  $E = 1. \cdot 10^{+12}$  g/L (simulating an endogenous metabolism).

The value of  $\pi_m$  is adjusted—since its value seems not very reliable<sup>2</sup>—so as to minimize the Euclidian distance to the *reference data*. Some numerical values are summarized in Table 2. The corresponding time profiles have been plotted in Figure 2. Of course, the results for the maintenance model coincide with the reference data, as  $C_s$  never becomes negative for this particular feeding strategy. For  $1. \cdot 10^{-9} < E < 1.$  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.

We believe that 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 shall be illustrated further on that the metabolic assumptions might be very important for feeding strategy optimization.

Table 2 *Estimation of  $\pi_m$  and corresponding final state ( $t_f = 120$  hrs), for some values of  $E$*

**Figure 2** *Substrate, biomass and penicillin profiles during constant feed rate fed-batch fermentation as predicted by the new model —  $S_0 = 0$  g*

### 2.3 Statement of the optimization problem

An obvious choice for a state space vector is given by (the superscript “ $\mathbf{T}$ ” denotes the transpose of a vector) :

$$\mathbf{x} = \begin{pmatrix} x_1 & x_2 & x_3 & x_4 \end{pmatrix}^{\mathbf{T}} \triangleq \begin{pmatrix} S & X & P & V \end{pmatrix}^{\mathbf{T}} \quad (12)$$

and with the definition of :

$$\mathbf{f} = \begin{pmatrix} f_1 & f_2 & f_3 & f_4 \end{pmatrix}^{\mathbf{T}} \triangleq \begin{pmatrix} -\sigma X & \mu X & \pi X - k_h P & 0 \end{pmatrix}^{\mathbf{T}} \quad (13)$$

$$\mathbf{b} = \begin{pmatrix} b_1 & b_2 & b_3 & b_4 \end{pmatrix}^{\mathbf{T}} \triangleq \begin{pmatrix} s_F & 0 & 0 & 1 \end{pmatrix}^{\mathbf{T}} \quad (14)$$

we obtain the following state space model linear in the control  $u$  :

$$\frac{d\mathbf{x}}{dt} = \mathbf{f}(\mathbf{x}) + \mathbf{b}u \quad (15)$$

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

$$x_{4,0} = V_* + x_{1,0}/s_F \quad (16)$$

Note that glucose is added as a solution with concentration  $s_F$ .

The optimization problem we consider in this paper is to determine for the given set of differential equations (1)–(4) the optimal feed rate profile that minimizes the performance index :

$$J(u) = g(\mathbf{x}(t_f)) \triangleq -P(t_f) \quad (17)$$

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

- $t_0 = 0, t_f = \text{free}$
- all variables have to be kept positive, i. e. for all  $t$  in  $[0, t_f]$  :

$$x_i(t) \geq 0, \text{ for } i = 1, \dots, 4 \quad u(t) \geq 0 \quad (18)$$



- the initial amount of substrate is *free*; the initial conditions  $x_{1,0}$  and  $x_{4,0}$  are only constrained by equation (16). In other words, some initial conditions can be manipulated to minimize the performance measure, so (17) should be replaced by :

$$J \equiv J(u, x_0) = -x_3(t_f) \quad (19)$$

- the total amount of feed is fixed, i. e. :

$$x_{1,0} + s_F \int_{t_0}^{t_f} u(t) dt = \alpha \quad (20)$$

Notice that the last *isoperimetric* constraint on the input is equivalent to a *physical* constraint of the form—due to differential equation (4)—:

$$x_{4,f} \equiv V(t_f) = V_f, V_f \text{ fixed} \quad (21)$$

### 3 Optimal Control on the new model

#### 3.1 Statement of the two point boundary value problem

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

$$H = \lambda^T (f(x) + bu) \triangleq \phi + \psi u \quad (22)$$

$$\phi = \lambda_1 f_1 + \lambda_2 f_2 + \lambda_3 f_3 \quad \psi = s_F \lambda_1 + \lambda_4 \quad (23)$$

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

$$\frac{d\lambda}{dt} = -\frac{\partial H}{\partial x} = -\frac{\partial f^T}{\partial x} \lambda \quad (24)$$

Together with the state equations (15), we obtain a system of  $2 \times n$  first order differential equations—where  $n$  denotes the dimension of the state vector  $x$ , 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 equation (16)
- $x_{4,f}$  is given due to equation (21)
- $\lambda_{i,f}$ ,  $i = 1, \dots, 3$  are given by :

$$\lambda_{i,f} = \frac{\partial g}{\partial x_i}(t_f) \quad \text{or} \quad \left( \lambda_{1,f} \quad \lambda_{2,f} \quad \lambda_{3,f} \right)^T = \left( 0 \quad 0 \quad -1 \right)^T \quad (25)$$

It should be clear that we need still another boundary condition, as  $x_{1,0}$  and  $x_{4,0}$  are not given explicitly. It can be shown that the missing condition is given by<sup>8</sup> :

$$\psi(0) \equiv s_F \lambda_{1,0} + \lambda_{4,0} = 0 \quad (26)$$

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

$$\min_{\text{all admiss } u} H(\mathbf{x}^*, \lambda^*, u) = H(\mathbf{x}^*, \lambda^*, u^*) \quad (27)$$

which is Pontryagin's Minimum Principle<sup>14</sup> for this case.

Since the state equations (15) and the cost index (19) are time-invariant,  $H$  remains constant along an optimal trajectory. As the final time  $t_f$  is free, we know that  $H = 0$ .

### 3.2 Computational algorithm

#### *Optimal Control with bounded input and fixed initial state*

As a first step in the solution, we will solve the given problem subject to an *additional* constraint on the input ( $0 \leq u(t) \leq U_{max}$ ,  $U_{max}$  given), and with the *complete* initial state being given (say  $x_{1,0} = 0$ , so  $x_{4,0} = V_*$ , and condition (26) is superfluous).

As the Hamiltonian  $H$  is *linear* in the control  $u$ , we know by Pontryagin's Minimum Principle<sup>14</sup> that our problem has become a *Bang-Singular-Bang* problem, i. e. :

$$u(t) = \begin{cases} U_{max} & \text{if } \psi < 0 \\ u_{sing} & \text{if } \psi = 0 \quad t_i \leq t \leq t_{i+1} \\ 0 & \text{if } \psi > 0 \end{cases} \quad (28)$$

On any *singular* interval  $[t_i, t_{i+1}]$ , the *singular* control is obtained by repeatedly differentiating the function  $\psi$  until  $u$  appears explicitly. We obtain successively :

$$\frac{d\psi}{dt} = \dot{\lambda}^T \mathbf{b} = -\lambda^T \frac{\partial \mathbf{f}}{\partial \mathbf{x}} \mathbf{b} \triangleq \lambda^T \mathbf{d} = 0 \quad (29)$$

$$\frac{d^2\psi}{dt^2} = \dot{\lambda}^T \mathbf{d} + \lambda^T \frac{\partial \mathbf{d}}{\partial \mathbf{x}} \dot{\mathbf{x}} = -\lambda^T \frac{\partial \mathbf{f}}{\partial \mathbf{x}} \mathbf{d} + \lambda^T \frac{\partial \mathbf{d}}{\partial \mathbf{x}} (\mathbf{f} + \mathbf{b}u) = 0 \quad (30)$$

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

$$u_{sing}(t) = \frac{\lambda^T ((\partial \mathbf{f} / \partial \mathbf{x}) \mathbf{d} - (\partial \mathbf{d} / \partial \mathbf{x}) \mathbf{f})}{\lambda^T (\partial \mathbf{d} / \partial \mathbf{x}) \mathbf{b}} \quad (31)$$

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  $\lambda$ . 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* :

$$\psi \equiv \lambda^T \mathbf{b} = 0 \quad \frac{d\psi}{dt} \equiv \lambda^T \mathbf{d} = 0 \quad \phi \equiv \lambda^T \mathbf{f} = 0 \quad (32)$$

We conclude 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 the following *model-independent* conjecture (based on reported fermentation data) :

**Conjecture 1** *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.<sup>15</sup> However, simulations have indicated that our scheme is numerically more reliable, as it does not use any costate variable at all. For the most general case of low initial values for  $S$  and  $X$ , it can be summarized as follows :

- Make a guess of  $t_1$ , or equivalently, determine the amount of substrate reserved to the growth-phase. Integrate the state equations (15) from  $t = 0$  to  $t = t_1$  with  $u(t) = U_{max}$ .
- 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*.
- Integrate the state equations using the above determined singular control (31) until condition (21) is met at  $t = t_3$ .
- 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  $J(u, \mathbf{x}_0)$  (19). This completes the *production-phase*.
- For the same guess of  $t_1$ , refine the value of  $t_2$  by considering  $t_{2,new} = t_{2,old} \pm \delta t$ , with  $\delta t$  as small as required. Save the time  $t_2$  for which  $J(u, \mathbf{x}_0)$  reaches its minimum.
- Restart the procedure with a new guess of  $t_1$  in order to minimize  $J(u, \mathbf{x}_0)$ . For the problem at hand, a linear search method for  $t_1$  has been chosen.

A detailed mathematical justification for this algorithm has been given in an other paper<sup>8</sup>.

### *Optimal Control with unbounded input and some initial states free*

In Reference 8, it is shown how to solve the *original* problem considering the limit  $U_{max} \rightarrow \infty$ . As a consequence,  $t_1 \rightarrow 0$ . It was concluded there that omitting the upper bound on the input leads to the injection of all substrate reserved to growth at the beginning of the fermentation, the growth-phase becoming a *batch*-phase ( $u(t) = 0$ ). So in the above computational algorithm, the only modification is to replace the time  $t_1$  by the *equivalent* initial condition  $x_{1,0}$ .

### 3.3 Simulation results

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

## 4 A heuristic control strategy

### 4.1 Derivation of suboptimal profiles

In this section, we propose a *heuristic* control based on mathematical and microbial knowledge. The construction of a suboptimal profile is based principally on the concept of a *biphasic* fermentation (see Conjecture 1). Some of the ideas concerning heuristic  $C_s$ -control are reported elsewhere<sup>6,7</sup>.

For the control during *growth*, we 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  $\mu$  for all  $t$  during growth (equation (6)).

During *production*, we focus on the specific rate  $\pi$ . Equation (5) indicates that  $\pi$  reaches its maximum at  $C_s \triangleq C_{s,crit} = \sqrt{K_p K_i}$ . The maximum value is  $\pi_{max} = \pi_m / (1 + 2\sqrt{K_p/K_i})$ . So during production, we shall keep  $C_s$  at  $C_{s,crit}$ . The control needed can be obtained from the differential equations (1) and (4) :

$$u_{production} = \frac{\sigma X}{s_F - C_s} \quad (33)$$

As a consequence, the *conjunction* point  $t_2$  of growth and production is simply dictated by the condition  $C_s = C_{s,crit}$ . 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  $C_s = C_{s,crit}$ . As in the case of Optimal Control, a *two-dimensional optimization of  $S_0$  and  $t_2$*  is obtained.

Remark that for  $\mu_{substr}$  modeled with *Monod*-kinetics, the control (33) also keeps  $\mu$  constant during production. An equivalent *heuristic  $\mu$ -control* for *Contois*-kinetics follows from the condition  $d\mu/dt = 0$  :

$$u_{production} = \frac{\mu_{max} K_x C_x (C_s \mu + C_x \sigma) / (K_x C_x + C_s)^2 + F(E_m, E_p) \sigma X / V}{\mu_{max} K_x C_x s_F / V (K_x C_x + C_s)^2 + F(E_m, E_p) (s_F - C_s) / V} \quad (34)$$

where

$$F(E_m, E_p) \triangleq \exp(-C_s/E_m) m_s Y_{x/s} / E_m + \exp(-C_s/E_p) \frac{Y_{x/s}}{Y_{p/s}} \left( \frac{\pi}{E_p} - \frac{d\pi}{dC_s} \right) \quad (35)$$

Before giving some simulation results, some advantages of these suboptimal profiles are mentioned. It is well-known that putting an *optimal control* into practice may be hampered by a lot of problems. If the control law is not obtained in complete closed-loop form—as is the case here—, it cannot compensate for unmodeled disturbances, parameter variations, . . . Further on, as Optimal Control is a very model-sensitive technique, a *feed-forward* shall 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 in the process.

On the other hand, the *suboptimal* profiles we present here are the translation of a more realistic control objective, namely *setpoint* control, for which even adaptive control algorithms can be developed. As suggested by e.g. Dochain et al.<sup>16</sup>, one could keep  $\mu$  constant *without* the knowledge of an exact analytic expression for it, so the algorithm becomes really model-independent. Further on, there would not be a need for a complete measurement of the state, a problem which has not been solved completely up to now.

## 4.2 Simulation results

Some numerical results and corresponding time profiles obtained with the above algorithms are summarized in Table 3 and Figures 3 to 5. As for the heuristic controls in these Figures,

the value of  $C_x$ , respectively  $\mu$  on the switching time  $t_2$  has been optimized. In order to compare the performance of the different feeding policies, we have introduced a *gain*  $G$ , defined as :

$$G \triangleq 100 \times \frac{J(u^*, x_0^*) - J(u_{ref}, x_{0,ref})}{J(u_{ref}, x_{0,ref})} \% \quad (36)$$

where the superscript “\*” denotes the optimal or heuristic strategy, and the subscript “*ref*” denotes a *reference* strategy : a *constant* strategy with zero initial substrate amount, in the same time as the optimal strategy.

**Table 3** *Numerical results for optimal and heuristic control, for some values of  $E$*

**Figure 3** *Substrate, biomass, penicillin,  $\mu$ ,  $\pi$  and  $u$  profiles for heuristic and optimal control*

$$E_m = E_p = 1. 10^{-9}$$

**Figure 4** *Substrate, biomass, penicillin,  $\mu$ ,  $\pi$  and  $u$  profiles for heuristic and optimal control*

$$E_m = E_p = 4.5622 10^{-3}$$

**Figure 5** *Substrate, biomass, penicillin,  $\mu$ ,  $\pi$  and  $u$  profiles for heuristic and optimal control*

$$E_m = E_p = 1. 10^{+12}$$

From these we can make the following conclusions :

- there is a maximum in realizable gain, as a function of the endogenous fraction
- the performances of the heuristic strategies are excellent, independent of the metabolic assumptions
- for the maintenance model, the control profiles  $u(t)$  almost coincide; for mixed and endogenous metabolism, the differences in  $u(t)$  become significant, although the differences in final amount of product remain small
- for the mixed and endogenous models, the rapid decay of  $C_x$  during production (in other words the negative value of  $\mu$ ) is somewhat unexpected. However, there might be some indications that penicillin production does not necessarily stop at  $\mu \leq 0^2$ . A detailed analysis of the parameters of an endogenous metabolism is the subject of an ongoing study.

## 5 Conclusions

We believe that the assumptions about the fundamental *nature of 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, a new unstructured mathematical model proposed by Nicolai 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 with zero initial substrate amount.

We presented also a *heuristic control* strategy based on mathematical and microbial insight, that proved to be a successful alternative for the optimal control, for both maintenance and endogenous metabolism. 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 suggested that feeding strategy optimization studies can be a valuable tool in the design of real life experiments for structure identification purposes of biotechnological processes.

## Nomenclature

$t$	: time (hr)
$S$	: amount of substrate in broth (g) (glucose)
$X$	: amount of cell mass in broth (g DM) (biomass)
$P$	: amount of product in broth (g) (penicillin)
$V$	: fermentor volume (L)
$u$	: input substrate feed rate (L/hr)
$C_s$	: $\triangleq S/V$ substrate concentration in broth (g/L)
$C_x$	: $\triangleq X/V$ cell mass concentration in broth (g/L)
$C_p$	: $\triangleq P/V$ 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 (g/L)
$E_p$	: parameter related to the endogenous fraction of production (g/L)
$K_x$	: Contois saturation constant for substrate limitation of biomass production (g/g DM)
$K_s$	: 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 DM hr)
$k_h$	: penicillin hydrolysis or degradation constant (hr <sup>-1</sup> )
$Y_{x/s}$	: cell mass on substrate yield (g DM/g)
$Y_{p/s}$	: product on substrate yield (g/g)
$G$	: gain due to substrate feeding rate optimization (%)
$\alpha$	: total amount of glucose available for fermentation (g)
$\sigma$	: specific substrate consumption rate (g/g DM hr)
$\mu$	: specific growth rate (hr <sup>-1</sup> )
$\mu_{substr}$	: specific substrate to biomass conversion rate (hr <sup>-1</sup> )
$\mu_C$	: maximum specific growth rate for Contois kinetics (hr <sup>-1</sup> )
$\mu_M$	: maximum specific growth rate for Monod kinetics (hr <sup>-1</sup> )
$\pi$	: specific production rate (g/g DM hr)
$\pi_m$	: specific production constant (g/g DM hr)



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**Table 2** *Estimation of  $\pi_{in}$  and corresponding final state ( $t_f = 120$  hrs), for some values of  $E$*

**Table 3** *Numerical results for optimal and heuristic control, for some values of  $E$*

<i>parameters</i>			
$\mu_C$	0.11	$K_x$	0.006
$\pi_m$	0.004	$k_h$	0.01
$K_p$	0.0001	$K_i$	0.1
$Y_{x/s}$	0.47	$Y_{p/s}$	1.2
$m_s$	0.029	$s_F$	500
<i>initial conditions</i>			
$X_0$	10.5	$S_0$	<i>to be specified</i>
$P_0$	0	$V_0$	$7 + S_0/s_F$
$t_0$	0	$\alpha$	1500

Table 1 *Parameters and initial conditions used in simulations*

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

Table 2 *Estimation of  $\pi_m$  and corresponding final state ( $t_f = 120$  hrs), for some values of  $E$*

$E$ (g/L)	$1 \cdot 10^{-9}$	$4.5622 \cdot 10^{-3}$	1.	$1 \cdot 10^{+12}$
<i>Optimal Control</i>				
$S_0$ (g)	528	880	1409	1411
$t_2$ (hr)	28.271	32.749	37.016	42.106
$t_f$ (hr)	132.033	125.200	117.602	122.493
$P_f$ (g)	63.846	70.652	87.448	89.709
$G$ (%)	3.961	16.822	49.064	45.909
<i>Heuristic <math>C_s</math>-Control <math>C_s(t_2) = \sqrt{K_p K_i}</math></i>				
$S_0$ (g)	533	940	1401	1404
$t_2$ (hr)	28.355	33.338	36.969	42.059
$t_f$ (hr)	131.323	124.427	116.906	121.974
$P_f$ (g)	63.597	68.436	87.164	89.430
$G$ (%)	3.555	13.160	48.580	45.455
<i>Heuristic <math>C_s</math>-Control <math>C_s(t_2)</math> optimized</i>				
$S_0$ (g)	551	1124	1417	1418
$t_2$ (hr)	28.644	34.926	37.075	42.165
$t_f$ (hr)	131.372	122.252	117.250	122.189
$P_f$ (g)	63.724	69.263	87.258	89.511
$G$ (%)	3.762	14.527	48.740	45.587
<i>Heuristic <math>\mu</math>-Control <math>\mu(t_2)</math> optimized</i>				
$S_0$ (g)	551	1113	1406	1407
$t_2$ (hr)	28.644	34.838	36.990	42.069
$t_f$ (hr)	131.321	122.727	117.511	122.445
$P_f$ (g)	63.736	69.439	87.428	89.686
$G$ (%)	3.782	14.817	49.030	45.870

Table 3 Numerical results for optimal and heuristic control, for some values of  $E$

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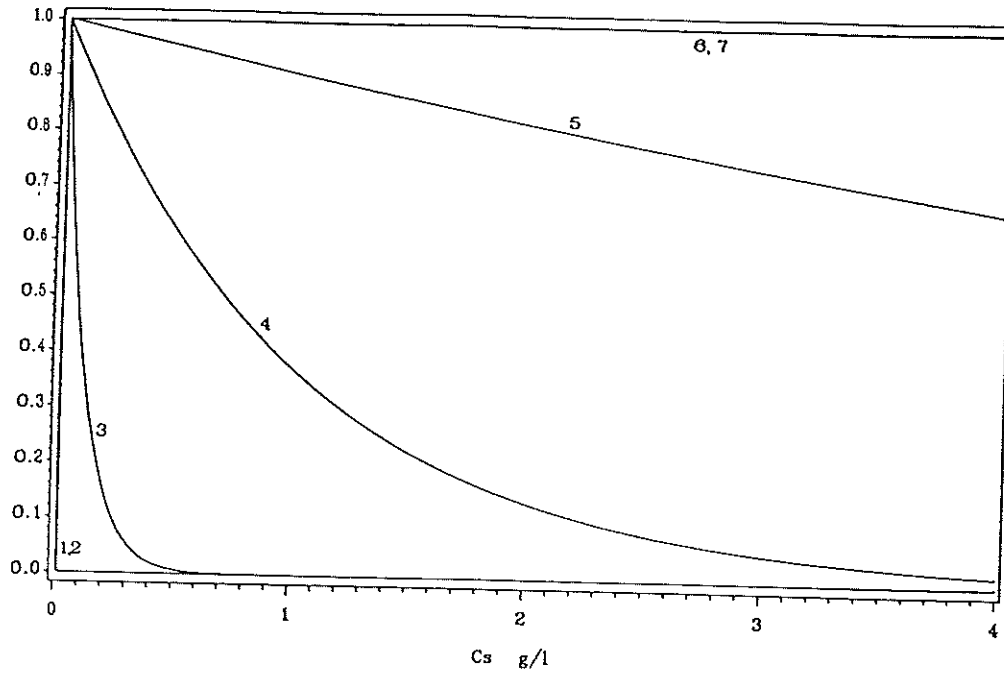
**Figure 1** *Behaviour of  $\exp(-C_s/E)$  as a function of  $C_s$  for different values of  $E$*

**Figure 2** *Substrate, biomass and penicillin profiles during constant feed rate fed-batch fermentation as predicted by the new model —  $S_0 = 0$  g*

**Figure 3** *Substrate, biomass, penicillin,  $\mu$ ,  $\pi$  and  $u$  profiles for heuristic and optimal control*  
 $E_m = E_p = 1. 10^{-9}$

**Figure 4** *Substrate, biomass, penicillin,  $\mu$ ,  $\pi$  and  $u$  profiles for heuristic and optimal control*  
 $E_m = E_p = 4.5622 10^{-3}$

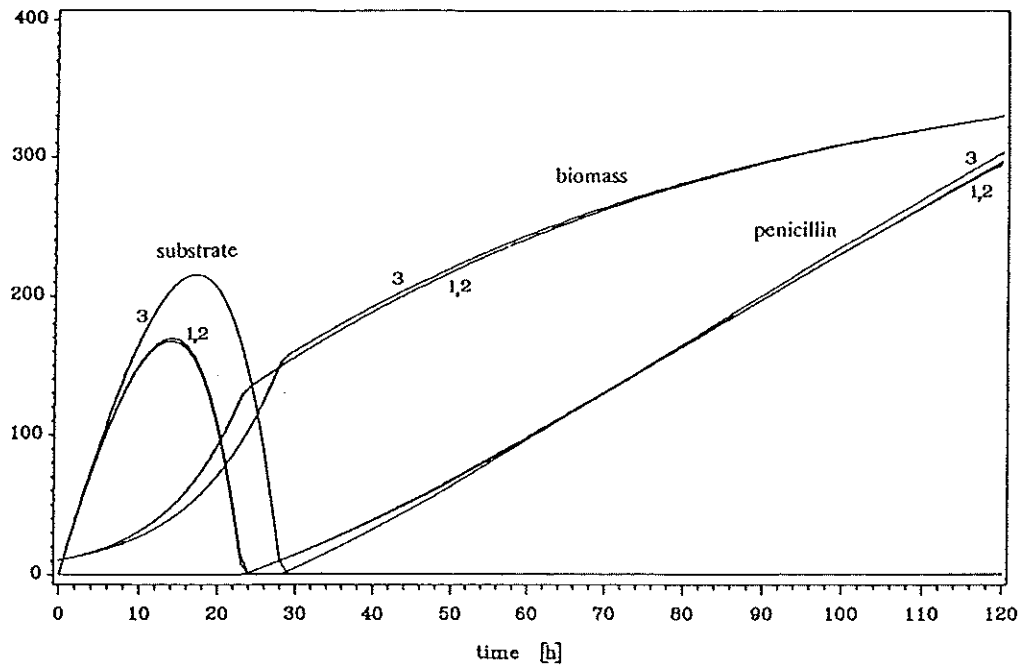
**Figure 5** *Substrate, biomass, penicillin,  $\mu$ ,  $\pi$  and  $u$  profiles for heuristic and optimal control*  
 $E_m = E_p = 1. 10^{+12}$



**Legend :**

- (1)  $E = 1. 10^{-9} \text{ g/L}$
- (2)  $E = 1. 10^{-3} \text{ g/L}$
- (3)  $E = 1. 10^{-1} \text{ g/L}$
- (4)  $E = 1. \text{ g/L}$
- (5)  $E = 1. 10^{+1} \text{ g/L}$
- (6)  $E = 1. 10^{+3} \text{ g/L}$
- (7)  $E = 1. 10^{+12} \text{ g/L}$

Figure 1 Behaviour of  $\exp(-C_s/E)$  as a function of  $C_s$  for different values of  $E$



**Legend :**

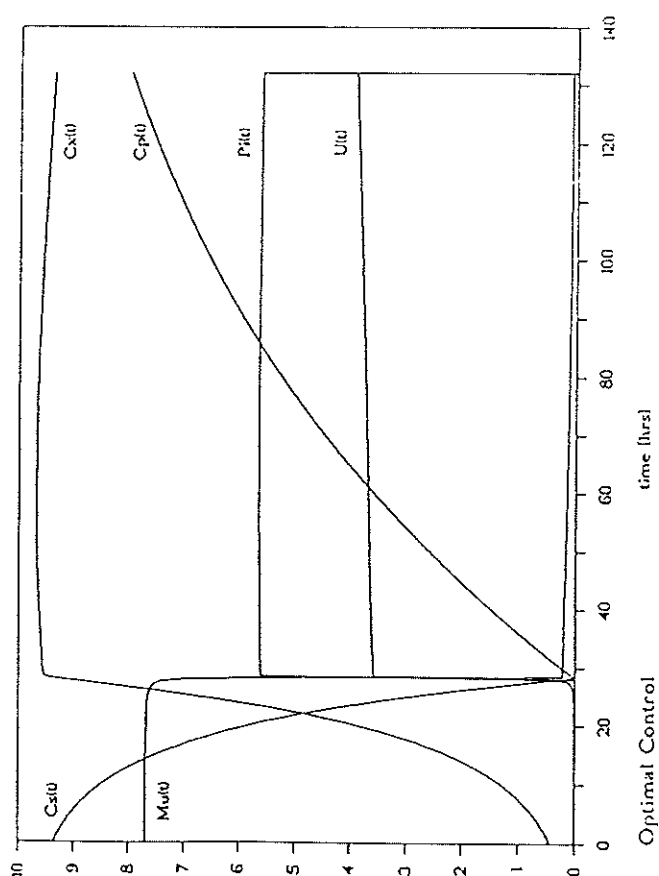
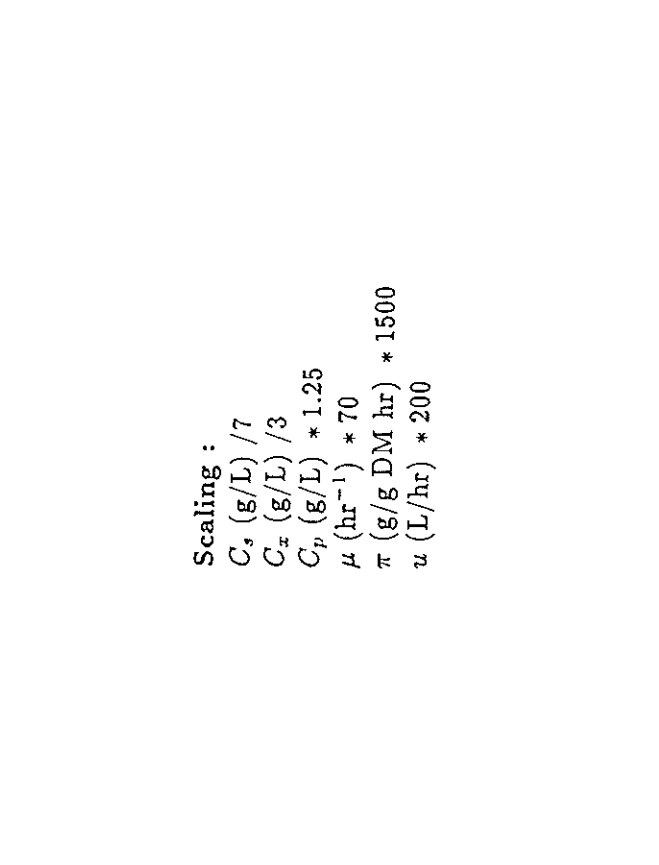
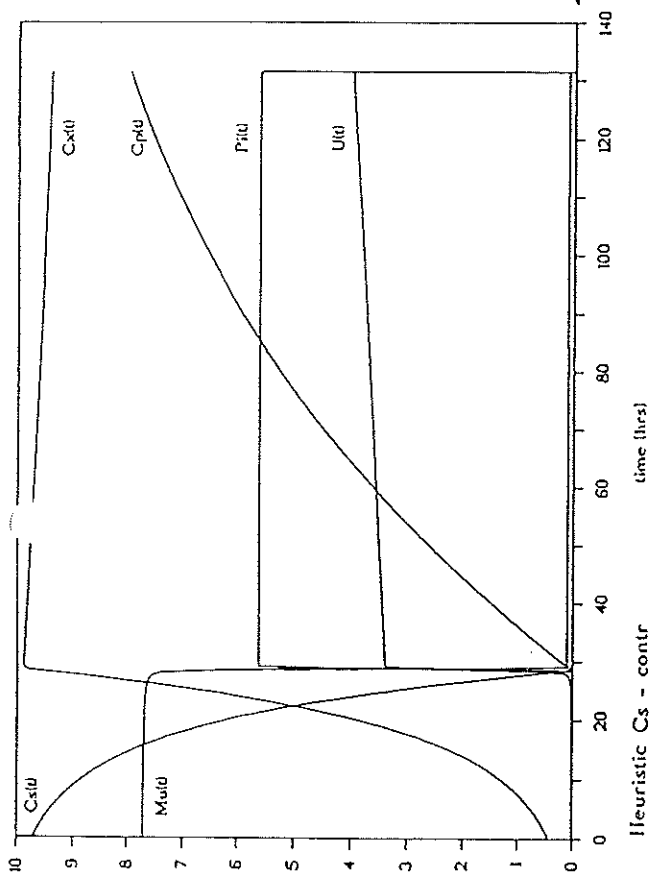
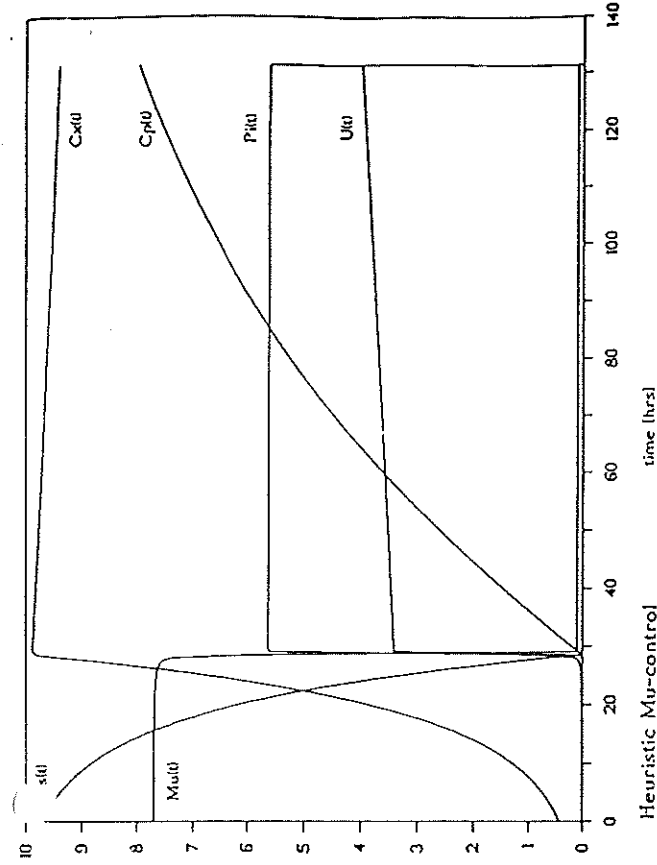
(1)  $E_m = E_p = 1. 10^{-9}$  g/L

(2)  $E_m = E_p = 1.$  g/L

(3)  $E_m = E_p = 1. 10^{+12}$  g/L

Figure 2 Substrate, biomass and penicillin profiles during constant feed rate fed-batch fermentation as predicted by the new model —  $S_0 = 0$  g

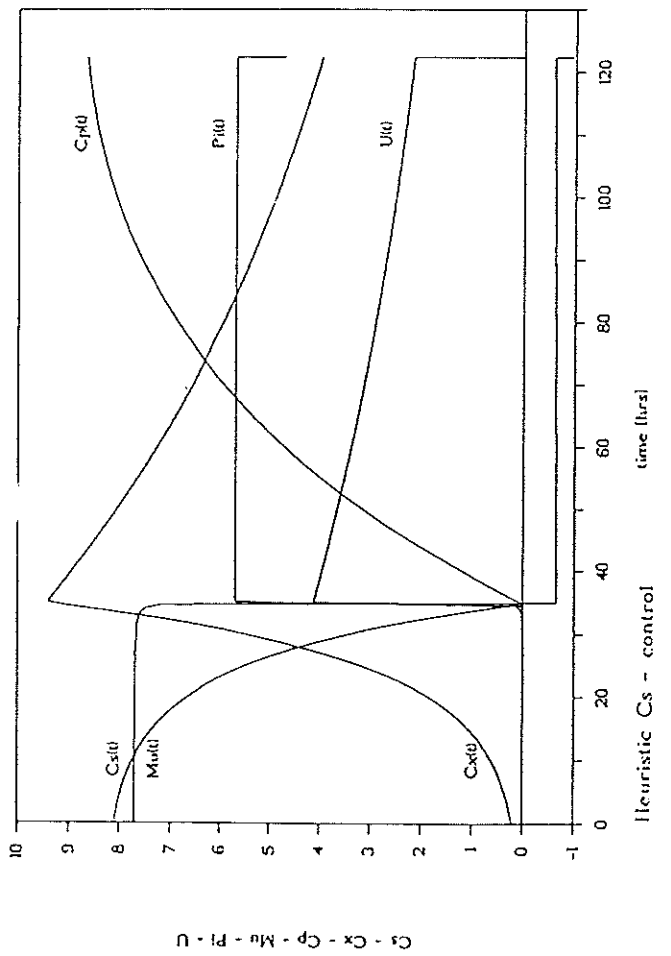
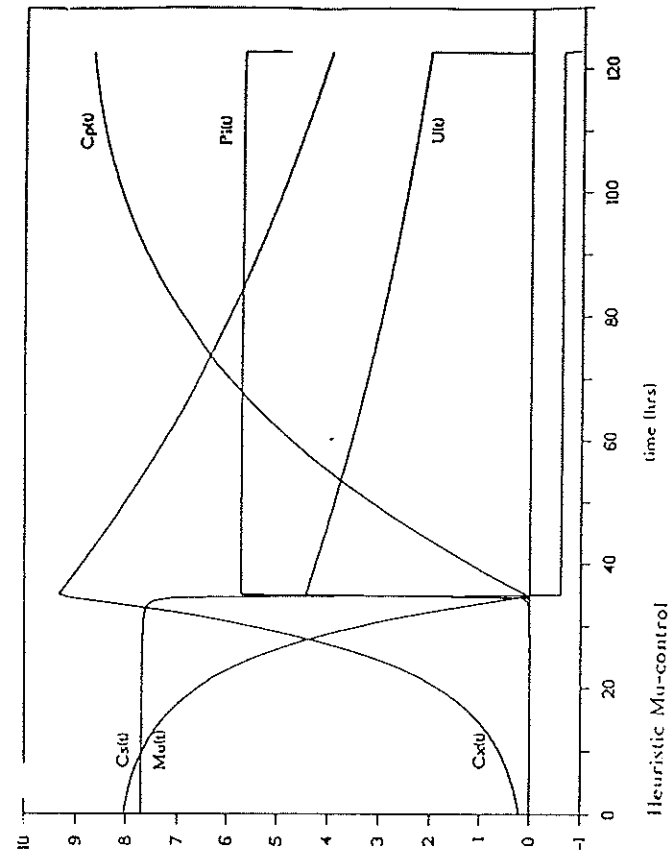




Scaling :  
 $C_s$  (g/L) / 7  
 $C_x$  (g/L) / 3  
 $C_p$  (g/L) \* 1.25  
 $\mu$  (hr<sup>-1</sup>) \* 70  
 $\pi$  (g/g DM hr) \* 1500  
 $u$  (L/hr) \* 200

Figure 3 Substrate, biomass, penicillin,  $\mu$ ,  $\pi$  and  $u$  profiles for heuristic and optimal control

$$E_m = E_p = 1.10^{-9}$$



Scaling :

- $C_s$  (g/L) / 15
- $C_x$  (g/L) / 5.5
- $C_p$  (g/L) \* 1.25
- $\mu$  ( $\text{hr}^{-1}$ ) \* 70
- $\pi$  (g/g DM hr) \* 1500
- $u$  (L/hr) \* 350

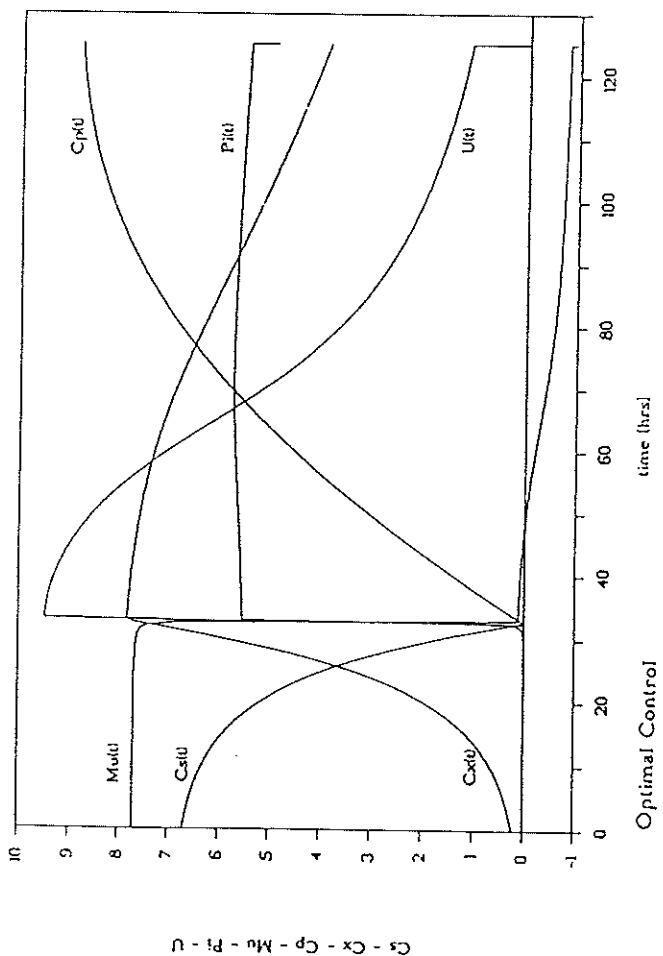
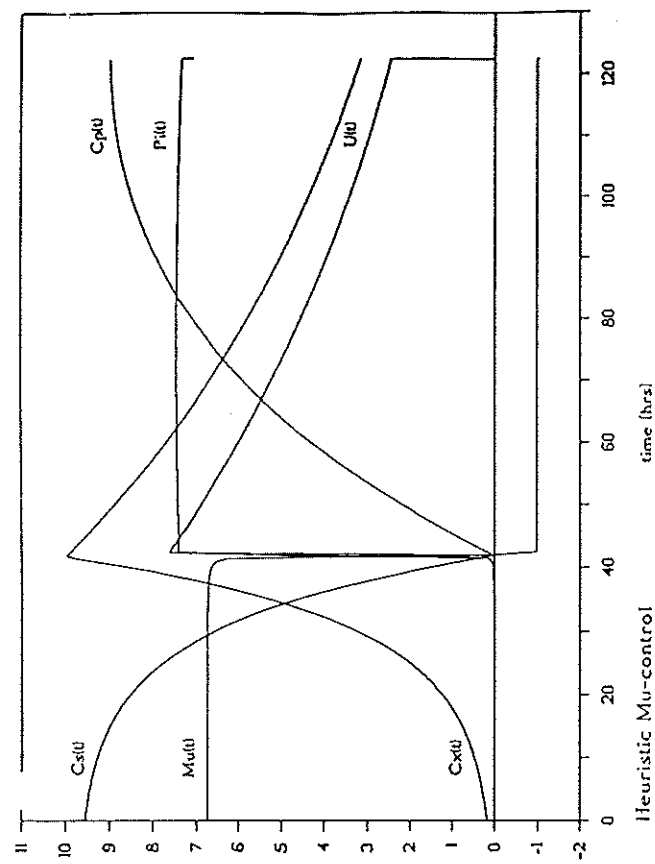


Figure 4 Substrate, biomass, penicillin,  $\mu$ ,  $\pi$  and  $u$  profiles for heuristic and optimal control

$$E_m = E_p = 4.5622 \cdot 10^{-3}$$



Scaling :  
 $C_s$  (g/L) / 15  
 $C_x$  (g/L) / 6  
 $C_p$  (g/L) \* 1  
 $\mu$  ( $\text{hr}^{-1}$ ) \* 70  
 $\pi$  (g/g DM hr) \* 1500  
 $u$  (L/hr) \* 2000

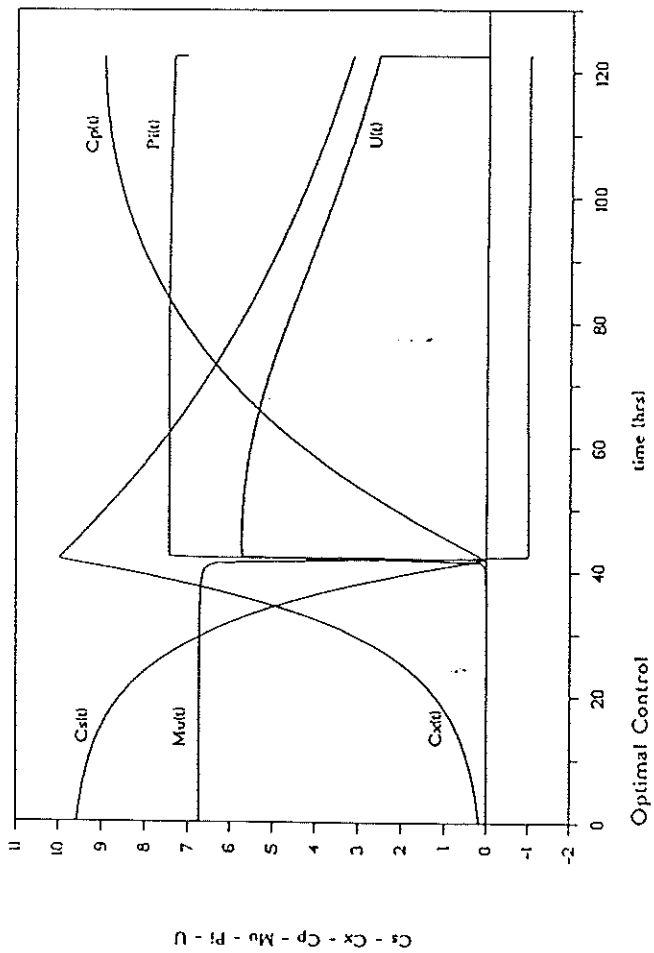
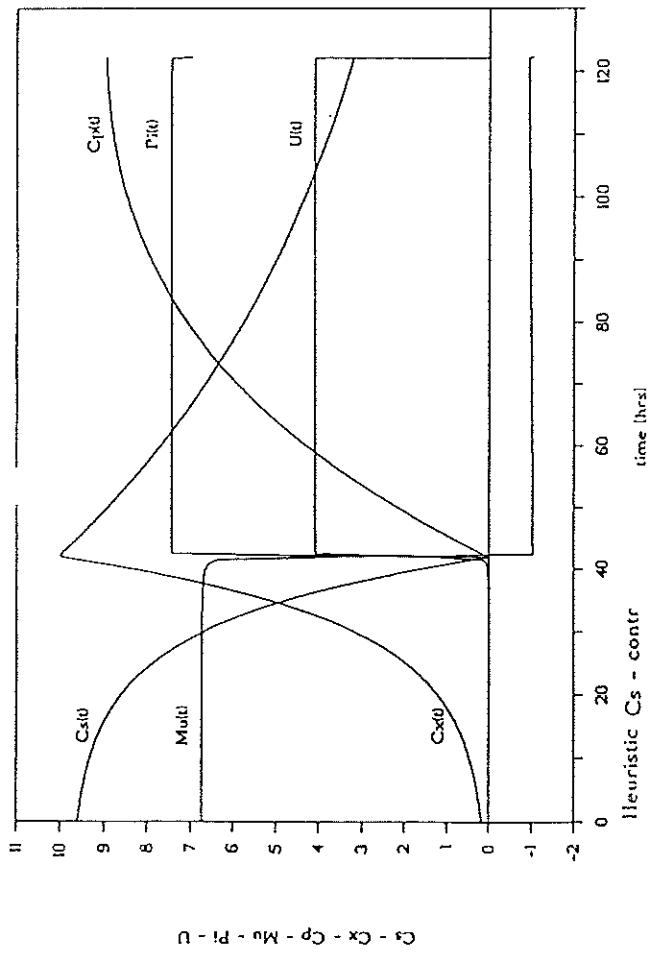


Figure 5 Substrate, biomass, penicillin,  $\mu$ ,  $\pi$  and  $u$  profiles for heuristic and optimal control

$$E_m = E_p = 1.10^{+12}$$

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