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A MODIFIED UNSTRUCTURED MATHEMATHICAL MODEL FOR THE PENICILLIN G FED-BATCH FERMENTATION

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Summary

In this paper, an updated unstructured mathematical model for the penicillin G fed-batch fermentation is proposed, in order to correct some physical and biochemical shortcomings in the model of Heijnen et al. (1979, *Biotechnol. Bioeng.*, **21**, 2175–2201) and the model of Bajpai and Reuß (1980, *J. Chem. Tech. Biotechnol.*, **30**, 332–344). Its main features are the consistency for all values of the variables, and the ability to adequately describe different metabolic conditions of the mould. The model presented here can be considered as the translation of the latest advances in the biochemical knowledge of the penicillin biosynthesis.

Introduction

Up to now, there exist at least two unstructured models for the penicillin G fed-batch fermentation that hold a promise for the optimization of the final amount of product with respect to the glucose feeding strategy : the model of Heijnen et al. (1979) and the model of Bajpai and Reuß (1980, 1981). Although both groups claim that their model might be well suited for control purposes, changing the glucose feeding strategy for a constant total amount of glucose results in a different behaviour.

Since the publication of both models, a lot of research has been done on the biochemistry of the penicillin synthesis. A detailed analysis of both models mentioned revealed some physical and biochemical shortcomings, mainly in the *metabolic assumptions* and in the *consistency* for some operational conditions (Nicolaï et al., 1990). It was also pointed out that under mild assumptions, both models have the same *general structure* based on balancing.

The aim of this paper is to present a modified unstructured model based on biochemical considerations, that *corrects the deficiencies* in both earlier models. We shall illustrate with some simulations that this updated model might serve as a basis for the design of real life experiments for *structure identification* and *parameter estimation* purposes. We also believe that this model can contribute both to a better understanding of the process itself and to the control of the fermentation, as will be reported elsewhere (Van Impe et al., 1991).

Deficiencies in the Current Models

As indicated above, both models reduce to the following general system of first order differential equations (S : substrate, X : biomass, P : product, V : volume, F : glucose input)

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

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

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

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

The structural differences between both models are due to structural differences in the specific rates μ, π and σ, which are interrelated by $\sigma = \mu/Y_{x/s} + m_s + \pi/Y_{p/s}$.

Heijnen et al. use a *Blackman*–type kinetics for $\pi(\mu)$, and a *Monod*–law for $\sigma(C_s)$, with an *endogenous* metabolism viewpoint. Bajpai and Reuß use a *Haldane* substrate inhibition kinetics for $\pi(C_s)$, a *Contois*–law for $\mu(C_s, C_x)$, and a *maintenance* metabolism concept.

An *endogenous* metabolism (Heijnen et al.) can be justified in low substrate concentration conditions. However, at high substrate concentrations, this assumption might not be valid, and should be replaced by a *maintenance* metabolism as in the Bajpai and Reuß model.

Further, the Bajpai and Reuß model is inconsistent in low substrate concentration conditions, because the maintenance metabolism concept can lead to (physically impossible) negative substrate concentrations, in cases where the glucose feeding rate becomes too small.

As for the *specific production kinetics*, Heijnen et al. used continuous culture data that may not have been extensive enough as to reveal substrate inhibition or more probably repression effects. Further, their model predicts π becoming zero if the net growth rate μ decreases to zero. This is in contradiction with some experiments of Pirt and Righelato (1967) for limited time ranges. For negative growth rates, the production rate kinetics is not defined, thus causing the model to become inconsistent in conditions of very low substrate levels. The Bajpai and Reuß substrate inhibition production kinetics is believed to reflect biochemical evidence better, although a reliable set of *parameters* is not available up to now (Nicolaï et al., 1990).

A Modified Model

The basic design requirements are :

- The updated model must have the same *general structure* as given above (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 additional *parameters* (in order to avoid unnecessary complications in parameter estimation studies).
- 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 Bajpai and Reuß has been chosen.
- The specific substrate to biomass conversion rate (*Monod*– or *Contois*–kinetics) 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 :

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

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

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

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

As we believe there is biochemical evidence that the occurrence of a particular metabolism is dictated *directly* by the limiting substrate C_s, rather than by the value of μ, we consider the endogenous fractions f_m and f_p of respectively maintenance requirements and production as functions of C_s only. Between a lot of possible expressions that match the desired behaviour, the following functions are chosen :

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

as an exponential function can be easily handled analytically, in particular in taking partial derivatives as is required for Optimal Control.
As a result of balancing, the specific glucose uptake rate is given by :

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

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

Observe that $(1 - f_i(C_s))$ in the above expression, with f_i as mentioned, is a *Tessier*–type kinetics. 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 : $(E_m = E_p) \to 0$

For very small 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 : $(E_m = E_p) \to +\infty$

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 :

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

$$\sigma = \mu_{substr}/Y_{x/s}$$

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

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.

Simulation Environment

All computations were done on a *VAX-VMS* system, using *NAG*-routines *E04ABF* for minimization and *D02EBF* for stiff systems integration. *Three main cases* are distinguished : (1) $E_m = E_p = 1.\ 10^{-9}$ g/L (simulating maintenance metabolism) (2) $E_m = E_p = 1.$ g/L (simulating a mixed maintenance-endogenous metabolism) (3) $E_m = E_p = 1.\ 10^{+12}$ g/L (simulating an endogenous metabolism). For μ_{substr} *Contois*-kinetics has been chosen, in order to make comparison with the original Bajpai and Reuß model possible. A set of 120 *reference data* for C_s, C_x and C_p has been generated, using the original Bajpai and Reuß model (Table 1), and the following *initial and operational conditions* : $S_0 = 0$ g, $X_0 = 10.5$ g, $P_0 = 0$ g, $V_0 = 7$ L, $s_F = 500$ g/L, $F = 0.025$ L/h and $t_f = 120$ h.

Results and Discussion

In a first simulation experiment with $S_0 = 0$ g, the value of μ_p is adjusted so as to minimize the Euclidian distance to the *reference data* in the three cases mentioned. The necessary adjustments of μ_p are all within reported measurement accuracies (Table 2). The other model parameters are as proposed by Bajpai and Reuß (1981). The resulting time profiles are shown in Figure 1a. Of course, the results for the *maintenance* model coincide with the reference data of the original Bajpai and Reuß model, as C_s never becomes zero for this particular feeding strategy. For the *mixed metabolism* model, the predicted values for S, X and P match the sample data almost exactly. For the *endogenous* model, the fit of X and P is still very good, although the S-profiles differ somewhat in the growth phase. These are very important results, as they indicate that it is virtually *impossible to make a distinction between different kinds of metabolic behaviour*, using data obtained with this particular feeding strategy.

In Figure 1b, the value of S_0 is increased, so the value of F must decrease in order to keep the total substrate amount added *fixed* to 1500 g. In the three cases shown, we have used the same model parameter combinations as in Figure 1a. The resulting profiles however (corresponding to $S_0 = 1376$ g) reveal large differences between the three main cases. The *maintenance* model predicts almost no product synthesis : the constant feeding rate is too small to prevent C_s from going to zero. As a consequence, the mould switches to endogenous metabolism (see Special Case 1); biomass X decreases, while $\pi(C_s)$ remains too small to start significant production.

Comparison of the simulation results of Figures 1a and 1b indicates that this model might be useful for *model structure characterization* purposes, when chosing an appropriate feeding strategy. The impact of more complex feeding strategies is discussed elsewhere (Van Impe et al., 1991).

Analogue phenomena occur during parameter estimation. Comparison of reported values indicates that e.g. the value of K_x is very uncertain. For the mixed metabolism model with $S_0 = 0$ g, we have adjusted μ_p to minimize the norm to the reference data specified above, for some realistic values of K_x (Table 3). In Figure 2a ($S_0 = 0$ g) the fitting is almost exact, thus indicating that it is virtually *impossible to estimate the correct value of* K_x, using data obtained with this particular feeding strategy.

In Figure 2b ($S_0 = 1376$ g), the same parameter combinations as in Figure 2a have been used. Again we observe large differences in final penicillin amount.

Comparison of the simulation results of Figures 2a and 2b indicates that this model might be useful also for *model parameter estimation* purposes, when chosing an appropriate feeding strategy.

Conclusions

We believe that the deficiencies in the earlier models are *corrected well* with this *modified model.* Only two additional parameters—with a clear physical interpretation—yield a more complete model representation, that can represent a lot of different fermentation conditions. Its advantages are twofold.

First of all, we have indicated with some simple examples that a proper choice of the input feeding rate to this model might give indications about the *metabolic nature* of the mould and about the *model parameters.* As a result, simulations obtained with this model using more complex feeding strategies might serve as a basis for the design of real life experiments for *model structure characterization* and *parameter estimation* purposes.

Secondly, this model is well suited for optimization studies, the results of which are being published elsewhere (Van Impe et al., 1991).

Substrate, biomass and penicillin profiles for the three main cases

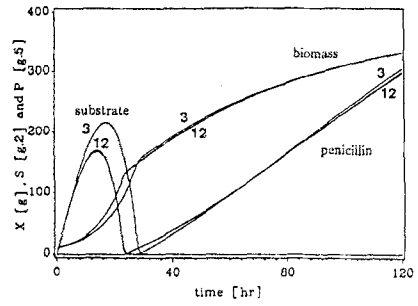

Figure 1a $S_0 = 0$ g

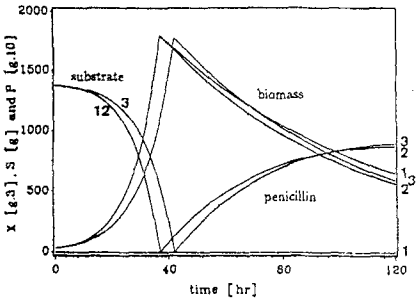

Figure 1b $S_0 = 1376$ g

Substrate, biomass and penicillin profiles for the mixed metabolism model for some K_x and μ_p combinations

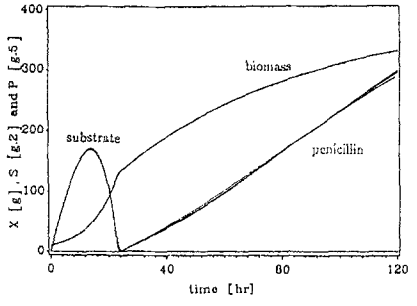

Figure 2a $S_0 = 0$ g

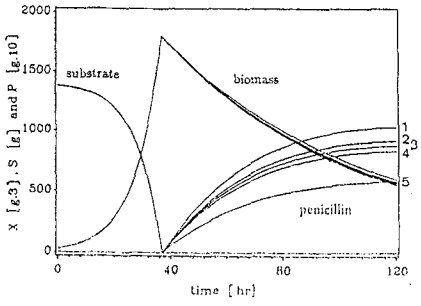

Figure 2b $S_0 = 1376$ g

Table 1

model parameters					
μ_x	[hr^{-1}]	0.11	$Y_{x/s}$	[g DM/g]	0.47
K_x	[g/g DM]	0.006	$Y_{p/s}$	[g/g]	1.2
K_p	[g/L]	0.0001	k_h	[hr^{-1}]	0.01
K_i	[g/L]	0.1	m_s	[g/g DM hr]	0.029

Table 2

Simulation	E_m [g/L]	E_p [g/L]	μ_p [g/g DM hr]	$P(t_f, S_0 = 0\ g)$ [g]	$P(t_f, S_0 = 1376\ g)$ [g]
1	$1.\ 10^{-9}$	$1.\ 10^{-9}$	0.00400	59.651	< 0.15
2	1.	1.	0.00506	59.239	87.141
3	$1.\ 10^{12}$	$1.\ 10^{12}$	0.00529	60.865	89.538

Table 3

Simulation	K_x [g/g DM]	μ_p [g/g DM hr]	$P(t_f, S_0 = 0\ g)$ [g]	$P(t_f, S_0 = 1376\ g)$ [g]
1	0.010	0.00606	59.800	102.649
2	0.007	0.00531	59.357	91.187
3	0.006	0.00506	59.239	87.141
4	0.005	0.00480	59.020	82.760
5	0.001	0.00377	58.009	57.827

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Nomenclature

t	: time (h)
S	: amount of substrate in broth (g)
X	: amount of cell mass in broth (g)
P	: amount of product in broth (g)
V	: fermentor volume (L)
F	: 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^{-1})
$Y_{x/s}$	: cell mass on substrate yield (g DM/g)
$Y_{p/s}$	: product on substrate yield (g/g)
σ	: specific substrate consumption rate (g/g DM hr)
μ	: specific growth rate (hr^{-1})
μ_{substr}	: specific substrate to biomass conversion rate (hr^{-1})
μ_x	: maximum specific substrate to biomass conversion rate (hr^{-1})
π	: specific production rate (g/g DM hr)
μ_p	: specific production constant (g/g DM hr)

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