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Evaluation of two unstructured mathematical models for the penicillin G fed-batch fermentation

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Abstract

The mathematical model for the penicillin G fed-batch fermentation proposed by Heijnen et al. (1979) is compared with the model of Bajpai & Reuß (1980). Although the general structure of these models is similar, the difference in metabolic assumptions and specific growth and production kinetics results in a completely different behaviour towards product optimization. A detailed analysis of both models reveals some physical and biochemical shortcomings. It is shown that it is impossible to make a reliable estimation of the model parameters, only using experimental data of simple constant glucose feed rate fermentations with low initial substrate amount. However, it is demonstrated that some model parameters might be key factors in concluding whether or not altering the substrate feeding strategy has an important influence on the final amount of product.

It is illustrated that feeding strategy optimization studies can be a tool in designing experiments for parameter estimation purposes.

Introduction

Penicillin G is one of the most important antibiotics and is 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.

This paper deals with models that allow for the optimization of the final amount of product with respect to the substrate feeding rate. The model of Heijnen et al. (1979) and the model of Bajpai & Reuß (1980, 1981), both claiming to be accurate descriptions of the penicillin fermentation process, are widely used for control purposes (Lim et al. 1986; Bonte et al. 1989; Van Impe et al. 1990, 1991)

However, when changing the glucose feeding strategy for a constant total amount of glucose, both models exhibit a different behaviour. Heijnen et al. stated that the final penicillin amount, as predicted by their model, depends heavily on the substrate feeding rate. The model of Bajpai & Reuß on the contrary proved to be rather insensitive towards different feeding policies. This has been confirmed recently by the application of optimal control theory to both models (Lim et al. 1986; Van Impe et al. 1990, 1991). Obviously, the model-based optimization may lead to feeding strategies which imply fermentation conditions (e.g. high substrate concentration, very low growth rates) which differ widely from the experimental fermentation conditions for which the model was developed. It is very well possible that in these conditions the model is not valid anymore so that the optimization results are completely erroneous. The aim of this paper is to investigate the biochemical and physical consistency of both models and the reliability of the reported model parameters, with special emphasis on conditions outside the region where the models were developed for.

Using a simple one-dimensional optimization scheme, it shall be shown that some parameters which are difficult to estimate, might have a great influence upon feeding strategy optimization results.

Review of two unstructured mathematical models for the penicillin G fed-batch fermentation

Based on a combination of balancing methods and kinetic equations (from a literature survey) Heijnen et al. (1979) proposed a simple unstructured mathematical model for the penicillin G fed-batch fermentation. In the original model the equation for the total broth weight takes into account dynamic effects resulting from the input of glucose, nitrogen source, sulphate source, oxygen and precursor, and from evaporation and carbon dioxide production. Van Impe et al. (1990) have concluded that the major contribution to the dynamics and final value of the most interesting variable, namely the product P, comes from the input of glucose. As a consequence, the other terms are neglected, resulting in a more simple model. The differential equation for penicilloic acid will not be considered here because penicilloic acid does not appear explicitely in the other equations. In order to obtain compatibility with the other model, the (simplified) model (originally in mol-kg units) has been rewritten in a g-L units system.

Bajpai & Reuß (1980, 1981) based their model on balancing methods and biochemical knowledge. The original model (1980) contained an equation for the oxygen dynamics which has been omitted in a second paper (1981). This simplified model shall be discussed here.

With these modifications both models have the same general structure, as described by the following system of first order differential equations:

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

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

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

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

with t the time, S, X and P respectively the amount of substrate, biomass and product in the broth, V the fermentor volume, F the input substrate feed rate at a concentration s_F, μ, σ and π respectively the specific growth, substrate consumption and production rate and k_h the penicillin hydrolysis or degradation constant. A summary of all the symbols and the appropriate units is given at the end of this paper. The structural differences between both models are due to structural differences in the specific rates μ, π and σ.

The model of Heijnen, Roels & Stouthamer

In the model of Heijnen et al. σ is given by a Monod-type relationship:

$$\sigma = Q_{s,\max}\frac{C_s}{K_s + C_s} \tag{5}$$

with $C_s \triangleq S/V$ the substrate concentration, $Q_{s,\max}$ the maximum specific substrate uptake rate and K_s the Monod saturation constant for substrate limitation for biomass production. π is assumed to be growth-associated and is modeled by a Blackman- type relationship:

$$\pi(\mu) = \begin{cases} Q_{p,\max}\mu/\mu_{\text{crit}} & \text{for } \mu \leq \mu_{\text{crit}} \\ Q_{p,\max} & \text{for } \mu \geq \mu_{\text{crit}} \end{cases} \tag{6}$$

with $Q_{p,\max}$ the maximum specific penicillin synthesis rate and μ_{crit} the critical specific growth rate. μ is defined by an extended Herbert-Pirt relation:

$$\mu = Y_{x/s}(\sigma - m_s - \pi/Y_{p/s}) \tag{7}$$

with $Y_{x/s}$ the cell mass on substrate yield, $Y_{p/s}$ the product on substrate yield and m_s the maintenance constant.

It is clear that this approach assumes that maintenance and production occur at the expense of biomass. This behaviour is called endogenous metabolism (Herbert 1958). The parameter values are given in Table 1.

Table 1. Parameter set used in the model of Heijnen et al.

$Q_{s,\max}$	[g/g DM h]	0.18
K_s	[g/L]	1.0
$Y_{x/s}$	[g DM/g]	0.5
$Y_{p/s}$	[g/g]	0.854
$Q_{p,\max}$	[g/g DM h]	0.0045
μ_{crit}	[h^{-1}]	0.01
k_h	[h^{-1}]	0.002
m_s	[g/g DM h]	0.025

The model of Bajpai & Reuß

Bajpai & Reuß assumed a maintenance metabolism (Pirt 1965) in which σ is given by:

$$\sigma = \mu/Y_{x/s} + m_s + \pi/Y_{p/s} \tag{8}$$

with $Y_{x/s}$, $Y_{p/s}$ and m_s as above. μ is modeled by Contois-kinetics:

$$\mu = \mu_x \frac{C_s}{K_x C_x + C_s} \tag{9}$$

with $C_x \triangleq X/V$ the cell mass concentration, μ_x the maximum specific substrate to biomass conversion rate and K_x the Contois saturation constant for substrate limitation of biomass production. Penicillin synthesis is thought to be inhibited or repressed by high amounts of glucose. Therefore a Haldane substrate inhibition kinetics of the following form is used:

$$\pi = \mu_p \frac{C_s}{K_p + C_s + C_s^2/K_i} \tag{10}$$

with μ_p the specific production constant, K_p the saturation constant for substrate limitation of product formation and K_i the substrate inhibition constant for product formation. For later purposes it is worthwhile to rewrite Eq. (10) using (9) as

$$\pi = \frac{\mu_p \mu K_x C_x}{(\mu_x - \mu)K_p + \mu K_x C_x [1 + (\mu K_x C_x)/(K_I(\mu_x - \mu))]}$$

$$\text{for} \quad \mu \leq \mu_x \tag{11}$$

Bajpai & Reuß demonstrated a close agreement between model predictions and reported fermentation data by Pirt & Rhigelato (1967) (using parameter Set 1 in Table 2) and by Mou (1979) (using parameter Set 2 in Table 2).

The basic differences beween both models are summarized in Table 3.

Table 2. Parameter sets used by Bajpai & Reuß .

parameter		*Set 1*	*Set 2*
μ_x	[h^{-1}]	0.092	0.11
K_x	[g/g DM]	0.15	0.006
μ_p	[g/g DM h]	0.005	0.004
K_p	[g/L]	0.0002	0.0001
K_i	[g/L]	0.1	0.1
$Y_{x/s}$	[g DM/g]	0.45	0.47
$Y_{p/s}$	[g/g]	0.9	1.2
k_h	[h^{-1}]	0.04	0.01
m_s	[g/g DM h]	0.014	0.029

Biochemical validation of the two models

The present knowledge about the penicillin biosynthesis was recently reviewed by Martin & Liras (1989) and is summarized in Fig. 1. This biosynthetic framework will be used to investigate the structural differences and the reliability of the parameters of both models.

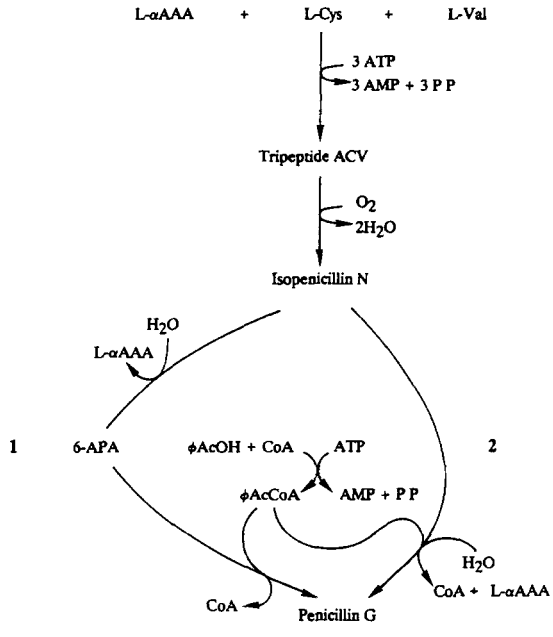

Fig. 1. Pathway of the penicillin G biosynthesis.

Specific production rate kinetics

Heijnen et al. assumed a relation between π and μ, described by Eq. (6), based on continuous culture experiments by Pirt & Rhigelato (1967). These results are in agreement with those obtained by Ryu & Hospodka (1980). However, this Blackman- type kinetics is very different from the substrate-inhibition kinetics (10) used by Bajpai & Reuß which predicts a decrease of π for large values of C_s (and thus of μ).

A major point of difference lies in the value of the specific production rate π for large values of the specific growth rate μ. Already in the early publications (e.g. Jarvis & Johnson 1947) it appeared that high glucose concentrations in the production phase are well correlated with a low penicillin yield (the 'glucose effect'). It has been confirmed recently (Alvarez et al 1987; Banko & Demain 1987; Brunner & Röhr 1975; López-Nieto et al. 1985; Ramos et al. 1985; Revilla et al. 1984) that high glucose concentrations inhibit the synthesis

Table 3. Major differences between the model of Heijnen et al. and Bajpai & Reuß .

	Heijnen et al.	Bajpai & Reuß
Number of parameters	8	9
Specific production rate kinetics	$\pi = f(\mu)$, No substrate inhibition	$\pi = f(C_s)$, Substrate inhibition
Metabolism	Endogenous	Maintenance
Specific substrate to biomass conversion rate	Monod	Contois

of the enzymes of the penicillin pathway, but not the actual penicillin biosynthesis. In other words, glucose represses (and not inhibits) the penicillin biosynthesis.

These findings do not contradict the results of Pirt & Rhigelato (1967) (on which Heijnen et al. based their production kinetics) and of Ryu & Hospodka (1980) which were obtained for continuous culture fermentations. Because for high values of the specific growth rate μ it is most likely (as shall be discussed below) that maintenance metabolism occurs, it can be shown that in steady state continuous culture conditions, and with μ described by a Monod kinetics

$$C_s = K_M \frac{\mu/\mu_x}{1 - \mu/\mu_x} \qquad (12)$$

Pirt & Rhigelato determined π for μ between 0.023 and 0.086 h^{-1}. They also reported a value $\mu_x \approx 0.095\ h^{-1}$, so that for their experiments μ/μ_x is in the range of 0.24 to 0.9. Substituting K_M in Eq. (12) by the value $K_M = 1$ g/L as used by Heijnen et al., one finds with the above equation $0.3 < C_s < 9$ g/L. This agrees well with the work of Revilla et al. (1984), who reported that penicillin biosynthesis repression only occurs at glucose concentrations from $C_s = 10$ g/L on. The conclusion is that the glucose concentrations in the experiments of Pirt & Rhigelato probably were too low for glucose repression to be detected. The experimental data published by Ryu & Hospodka are not detailed sufficiently to permit a similar analysis.

Bajpai & Reuß decided to disregard the differences between time constants for the two regulation mechanisms (glucose repression or inhibition) because of the relatively very long fermentation times, and therefore proposed a Haldane expression for π.

It is interesting that simulations with the Heijnen et al. model for the initial conditions given by these authors indicate that, when the remaining substrate is fed at a constant rate, a considerable and unrealistic amount of penicillin is produced when the glucose concentration is still very high (Van Impe et al. 1990). Simulations with the Bajpai & Reuß model correctly predict almost no penicillin production in similar conditions.

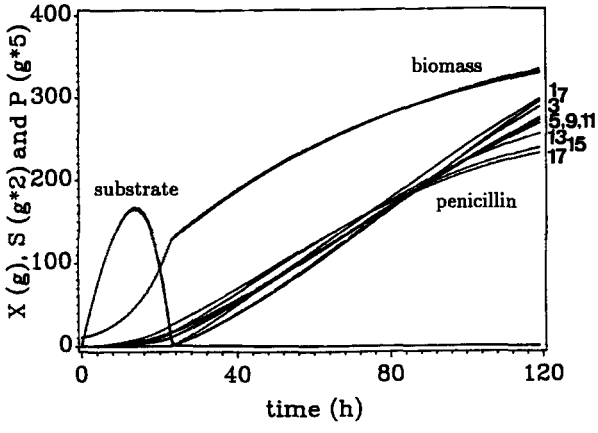

Fig. 2. Substrate, biomass and penicillin profiles during constant feed rate fed-batch fermentation for some K_p, K_i and μ_p combinations of Table 5.

Table 4. Initial values and process parameters for the simulations of Fig. 2.

$S(t=0)$	[g]	0.0
$X(t=0)$	[g]	10.5
$P(t=0)$	[g]	0.0
$V(t=0)$	[L]	7.0
s_F	[g/L]	500.0
F	[L/h]	0.025
t_f (final time)	[h]	120.0
S_{tot} (total amount of substrate added)	[g]	1500.

The parameters of the Bajpai & Reuß model were estimated using a mixture of published data, trial and error and a Nelder-Mead optimization routine. Using parameter Set 2 in Table 2 (which shall be denoted

as the nominal one, used in all simulations in this paper), the maximum specific production rate occurs at $C_s = (K_p K_i)^{1/2} = 3.162\ 10^{-3}$ g/L. At $C_s = 0.9$ g/L, π falls to 10% of its maximum value, and thus far below the lower bound for glucose of approximately $C_s = 10$ g/L at which glucose repression occurs (Revilla et al. 1984). In Fig. 2, the time profiles of biomass, substrate and penicillin resulting from a constant feed rate strategy are plotted for different values of K_p and K_i. For obvious reasons, only values of K_p and K_i greater than the nominal ones are considered. The initial and other operational conditions are summarized in Table 4. The value of μ_p is adjusted so as to minimize the Euclidian distance to the reference (i.e. using the nominal parameter values) concentration profiles, measured at 120 discrete times. Some numerical results are summarized in Table 5, columns 1 to 5 (the final amount of product is denoted with P_{ref}). All computations were done on a *VAX-VMS* system, using *NAG*-routines *E04ABF* for minimization and *D02EBF* for stiff systems integration. The above results indicate that even large variations of K_p and K_i have virtually no influence on the time evolution of biomass and substrate and have only a slight influence on the penicillin time profile. The adjusted μ_p values are of the same order of magnitude as the reported ones ($\mu_p = 0.004$ g/g DM h (Set 2 in Table 2) to $\mu_p = 0.005$ g/g DM h (Set 1)). From Eq. (10), the maximum specific production rate is found to be $\pi_{\max} = \mu_p/(1 + 2(K_p/K_i)^{1/2})$, which is almost equal to μ_p if $K_i \gg K_p$, as is the case for the values of Table 2. This agrees well with the value of $Q_{p,\max} = 0.0045$ g/g DM h used by Heijnen et al., and with the value of 0.006 g/g DM h used by Nestaas & Wang (1983).

Maintenance or endogenous metabolism

Bajpai & Reuß assumed that the energy for the maintenance and production requirements of the mould are directly obtained from the combustion of substrate. As a consequence of this maintenance metabolism concept, the system equations (1)–(4) indicate that for $F = 0$ (e.g. during a batch growth phase) C_s decreases monotonically, and may even become negative, which is of course physically impossible. It would rather be expected that under severe environmental limiting conditions, the energy requirements of the cell are fulfilled by combustion of the cellular storage compounds such as trehalose and polyols (Ballio et al. 1964; Thevelin 1984) or cell debris in the fermentation medium. This metabolic behaviour is called endogenous metabolism (Herbert 1958).

On the other hand, for high substrate concentrations it can be expected that organisms directly use the substrate for their energy and production requirements, instead of first synthesizing biomass and then combusting or transforming this into penicillin. Therefore, the model of Heijnen et al. (which relies on the endogenous metabolism hypothesis) can be expected to behave well for low C_s values; for high C_s-regions, the maintenance metabolism of the Bajpai & Reuß model is at least conceptually more attractive. It should be stressed that optimization studies with a modified Bajpai & Reuß model which allows for a smooth transition beween endogenous metabolism in the low C_s region and maintenance metabolism in the high C_s region showed that the realizable gains are very dependent on the nature of the metabolism (Van Impe et al. 1992).

The maintenance coefficient used by Heijnen et al. ($m_s = 0.025$ g/g DM h) corresponds well to the value $m_s = 0.029$ g/g DM h (Set 2 of Bajpai & Reuß), to the value $m_s = 0.024$ g/g DM h reported by Ryu & Hospodka (1980), and to the value used by Nestaas & Wang ($m_s = 0.022$ g/g DM h) (1983). However, these values differ from the value in Set 1 of Bajpai & Reuß ($m_s = 0.014$ g/g DM h). It is not clear where this difference originated from. Simulations indicated that the dynamic behaviour of the model is rather sensitive with respect to the value of m_s.

In the model van Heijnen et al., at severe substrate limitation conditions, and thus most probably corresponding to endogenous metabolic behaviour, the biomass consumption due to maintenance and production requirements may exceed the conversion of substrate into biomass and μ eventually may become negative. This situation may occur at the end of the growth phase during a fed-batch fermentation. For these conditions π is not defined. A straightforward extension of the $\pi(\mu)$ kinetics (10) could be $\pi(\mu \leq 0) = 0$, but there are some biochemical indications that the penicillin biosynthesis actually does not stop in that case.

From Fig. 1 it is clear that, in order to produce penicillin, there is need for the amino acid precursors L-Lys and L-Val, L-α-AAA, ATP, ϕAc, O_2, some cofactors and of course the relevant enzymes. It can readily be assumed that the amino acid precursors are always present in the amino acid pool of the living cell. It has also been confirmed that L-α-AAA is released unaltered after the incorporation of ϕAc, and thus could be recycled for penicillin synthesis (Friedrichs & Demain 1978), although there are also indications that not L-α-AAA but 6-oxo-piperidine-2-carboxylic acid (a cyclisation product of L-α-AAA) is released in the final step (Brundidge et al. 1980). However, L-α-AAA can be synthesized

Table 5. Estimation of μ_p for different values of K_p and K_i for the Bajpai & Reuß model with constant feeding strategy, and corresponding final penicillin amount – optimal initial substrate level and optimal penicillin amount.

Simulation	K_p [g/L]	K_i [g/L]	μ_p [g/g DM h]	$P(t_f)_{\text{ref}}$ [g]	$P(t_f)_{\text{opt}}$ [g]	G [%]	$S_{0,\text{opt}}$ [g]
1	0.0001	0.1	0.00400	59.651	63.461	6.4	280
2		1.0	0.00351	57.610	58.045	0.8	192
3		10.0	0.00338	53.073	56.922	0.5	178
4		100.0	0.00331	55.930	56.777	1.5	219
5		1000.0	0.00330	55.842	56.954	2.0	235
6		10000.0	0.00329	55.688	56.835	2.0	237
7	0.0010	0.1	0.00426	58.156	58.272	0.2	56
8		1.0	0.00374	56.069	56.069	0.0	0
9		10.0	0.00360	55.141	55.141	0.0	0
10		100.0	0.00352	54.439	54.439	0.0	0
11		1000.0	0.00350	54.224	54.224	0.0	0
12		10000.0	0.00350	54.233	54.233	0.0	0
13	0.0100	0.1	0.00652	51.604	51.604	0.0	0
14		1.0	0.00584	49.851	49.851	0.0	0
15		10.0	0.00549	48.310	48.310	0.0	0
16		100.0	0.00529	47.352	47.352	0.0	0
17		1000.0	0.00525	47.137	47.137	0.0	0
18		10000.0	0.00525	47.150	47.150	0.0	0

de novo from α-ketoglutaric acid and Acetyl Coenzyme A. These compounds, just as ATP and the necessary cofactors, can readily be assumed to be always present in a living cell. ϕAc and O_2 are supposed to be supplied at a sufficient rate. From this it can be concluded that even when $\mu \leq 0$, all compounds necessary for the penicillin biosynthesis are present. To the authors' knowledge there is no biochemical evidence for regulation mechanisms that repress or inhibit the relevant enzymes at low μ conditions.

This is also clear from the experiments of Pirt & Righelato (1967) which show that penicillin production in continuous cultures decays linearly at a rate of only 1 or 2% h^{-1} (depending on the previous value of μ) when μ was decreased to zero, so that for relatively short glucose depletion periods (up to a few hours) π remains positive. The decay of penicillin production rate for larger glucose limitation periods may be a result of the decay of the enzymes involved in penicillin biosynthesis in that case. The data of Ryu & Hospodka seem to indicate a positive π for $\mu = 0$ (which the authors assumed to be caused by experimental error or an erroneous linearity assumption) but are too scattered at low μ values to give a decisive answer. Pirt (1990) recently showed that penicillin hydrolysis could be responsible for the apparent low π levels in low μ conditions in continuous cultures, suggesting that the real value of π is higher than the observed one.

Specific substrate to biomass conversion rate

The fraction of the total specific growth rate representing substrate to biomass conversion, is called the specific substrate to biomass conversion rate μ_{substr}.

Bajpai & Reuß incorporated Contois kinetics argumenting that at high biomass concentrations the fermentation broth becomes very thick and limits either oxygen or nutrient diffusion into the fungal cells (Hosler & Johnson 1953). For this reason C_x appears in the nominator of equation (9). Heijnen et al used Monod kinetics which can clearly be motivated from enzyme kinetics studies (Roels 1983). Nihtilä & Virkkunen (1977) proved that both Monod and Contois kinetics can describe fungal growth well. Simulations with optimal feeding policies (Bonte et al. 1989; Vanimpe et al. 1990, 1991) have indicated also that the exact analytical expression of the substrate to biomass conversion rate has a negligible influence on the optimal penicillin production.

In the two parameter sets used by Bajpai & Reuß (Table 2), there is only a small difference in the maximum specific substrate to biomass conversion rate μ_x: 0.092 h^{-1} to 0.11 h^{-1}. These values also agree with the elsewhere reported maximum rates (0.095 h^{-1} to 0.15 h^{-1})

Table 6. Estimation of μ_p for different values of K_x for the Bajpai & Reuß model with constant feeding strategy, and corresponding final penicillin amount – optimal initial substrate level and optimal penicillin amount.

Simulation	K_x [g/g DM]	μ_p [g/g DM h]	$P(t_f)_{\text{ref}}$ [g]	$P(t_f)_{\text{opt}}$ [g]	G [%]	$S_{0,\text{opt}}$ [g]
1	0.150	0.01322	75.641	167.858	122.9	284
2	0.100	0.01038	72.226	150.148	107.9	305
3	0.050	0.00720	67.703	109.734	62.1	335
4	0.010	0.00432	60.907	68.457	12.4	314
5	0.007	0.00408	59.988	64.735	7.9	291
6	0.006	0.00400	59.651	63.461	6.4	280
7	0.005	0.00392	59.281	62.159	4.9	261
8	0.001	0.00370	57.057	57.057	0.0	0

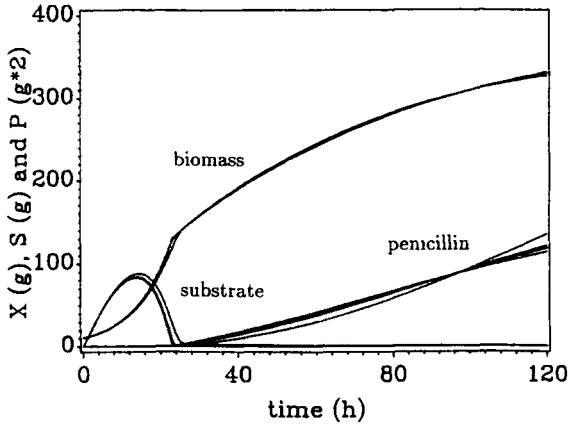

Fig. 3. Substrate, biomass and penicillin profiles during constant feed rate fed-batch fermentation as predicted by the model of Bajpai & Reuß for some K_x and μ_p combinations of Table 6 ($S_0 = 0.$ g).

(Pirt & Rhigelato 1967; Ryu & Hospodka 1980), and with the maximum specific substrate to biomass conversion rate $Q_{s,\max} \times Y_{x/s} = 0.09\ \text{h}^{-1}$ used by Heijnen et al. The differences are probably due to the different strains used in the experiments for parameter estimation.

A more pronounced difference exists between the reported K_x values. For the continuous culture experiments of Pirt & Rhigelato, on which the value $K_x = 0.15$ g/g DM (Set 1 of Bajpai & Reuß) was based, steady-state values of $C_x = 4$ up to 14 g/L have been reported. From (9) it is clear that under these experimental conditions Contois kinetics can be related to an equivalent Monod kinetics, by setting $K_M = C_x K_x$. This results in a K_M value between 0.6 and 2.1 g/L, which agrees well with the value $K_M = 1$ g/L as used by Heijnen et al. However, there is a striking difference between the K_x value of Set 1 ($K_x = 0.15$ g/g DM) and the one of Set 2 ($K_x = 0.006$ g/g DM) reported by Bajpai & Reuß . In Table 6 the results of a similar parameter estimation study as described above are given. K_x values greater than 0.05 g/g DM result in a rather poor fit on the reference fermentation data generated with Set 2, and in unacceptably high μ_p values (> 0.01 g/g DM h). For K_x values from 0.001 to 0.05 g/g DM the μ_p values are in the range of 0.003 to 0.007 g/g DM h and the fit is good, as illustrated in Fig. 3. The cause of the contradiction between the two parameter sets remains unclear. However, in Section 4 it shall be shown that these large differences in K_x may result in a completely different behaviour of the model towards optimization.

The remaining model parameters

$Y_{x/s}$ is almost the same in both models, and corresponds to the yield coefficient measured by Ryu & Hospodka (1980) (0.45 g DM/g). Nestaas & Wang (1983) determined $Y_{x/s}$ to be 0.6 g DM/g, which is somewhat higher than typically reported. This was caused by the presence of some complex nutrients in the growth medium.

$Y_{p/s}$ varies from 0.85 g/g to 1.2 g/g. The last value was based upon the maximum theoretical value of 1.1 g/g calculated by Cooney & Acevedo (1977) (also used by Heijnen et al. (1979) and Nestaas & Wang (1983)), using the biochemical knowledge present at that time. Hersbach et al. (1984) reviewed these calculations, and based on recent biochemical evidence about cysteine-biosynthesis, they concluded that the theoretical maximum $Y_{p/s}$ value may be somewhere between 0.41 g/g and 0.925 g/g, depending on some additional assumptions. These authors however also wrongly assumed that NADP and FAD are required for the oxydative cyclisation of ACV. In similar parameter estimation studies as

described above, several values of $Y_{p/s} < 1.2$ g/g have been considered. $Y_{p/s}$ values below 0.6 g/g resulted in a very poor fit even when a larger weight was assigned to the penicillin fitting errors. This was also the case for an increase of the hydrolysis constant k_h from 0.01 h^{-1} (Set 2 of Bajpai & Reuß) to 0.04 h^{-1} (Set 1), but an acceptable fit has been obtained using $k_h = 0.002$ h^{-1} (Heijnen et al.) ($\mu_p = 3.18\ 10^{-3}$ g/g DM h). Nestaas & Wang (1983) used the value $k_h = 0.003$ h^{-1}.

Effect of the model parameters on feeding strategy optimization

In this section, a feeding strategy of the following form shall be optimized, in order to investigate the possible effects of the model parameters. The total amount of substrate available for fermentation is fixed (1500 g). The initial amount of substrate S_0 is free, the initial volume V_0 follows then from $V_0 = 7 + S_0/s_F$ L (glucose is added as a solution with concentration s_F). The remaining amount of substrate is fed at a constant rate, with the final time t_f being fixed at $t_f = 120$ h so that a one-dimensional optimization problem has been obtained. All simulations were carried out using *NAG*-routines *E04ABF* (optimization routine) and *D02EHF* (stiff system solver). For the other initial conditions the values mentioned in Table 4 have been used.

The biochemical evidence presented in the previous section suggests that, as long as C_s remains positive, the Bajpai & Reuß model structure reflects the actual fermentation process better than the model of Heijnen et al. For this reason all further computations have been performed with the Bajpai & Reuß model. In order to prevent C_s from becoming negative μ and σ are modified as follows:

$$\mu_{\text{substr}} = \mu_x \frac{C_s}{K_x C_x + C_s} \tag{13}$$

$$\mu = \mu_{\text{substr}} - Y_{x/s}(1 - H(C_s))(m_s + \pi/Y_{p/s}) \tag{14}$$

$$\sigma = \mu_{\text{substr}}/Y_{x/s} + H(C_s)(m_s + \pi/Y_{p/s}) \tag{15}$$

with $H(C_s)$ a unit step function:

$$H(C_s) = 1, \quad C_s > 0 \tag{16}$$

$$= 0, \quad C_s \leq 0 \tag{17}$$

As long as $C_s > 0$ Eqs. (14) and (15) reduce to Eqs. (8) and (9), representing a pure maintenance metabolism. If C_s becomes equal to zero Eqs. (14) and (15) can be written as

$$\mu = \mu_{\text{substr}} - Y_{x/s}(m_s + \pi/Y_{p/s}) \tag{18}$$

$$\sigma = \mu_{\text{substr}}/Y_{x/s} \tag{19}$$

which represents an endogenous metabolism as used by Heijnen et al. The important influence of the metabolism assumptions on the realizable gain has recently been shown elsewhere (Van Impe et al. 1992). Below the influence of the model parameters is investigated. Because no actual fermentation data were available for parameter estimation purposes, parameter set 2 was used for the simulations, although the values for K_p, K_I, K_x and k_h may be not correct as described above.

In order to make a comparison between the models with different parameters possible, a gain G is defined as:

$$G = \frac{P_{\text{opt}} - P_{\text{ref}}}{P_{\text{ref}}} 100\ (\%) \tag{20}$$

with P_{ref} the final amount of penicillin for $S_0 = 0$ g and P_{opt} the final amount of penicillin for S_0 optimized.

The optimization results for the different combinations of K_p, K_i and corresponding μ_p values, are given in columns 6–8 of Table 5. It is clear that the gain generally decreases for increasing values of K_i with K_p fixed.

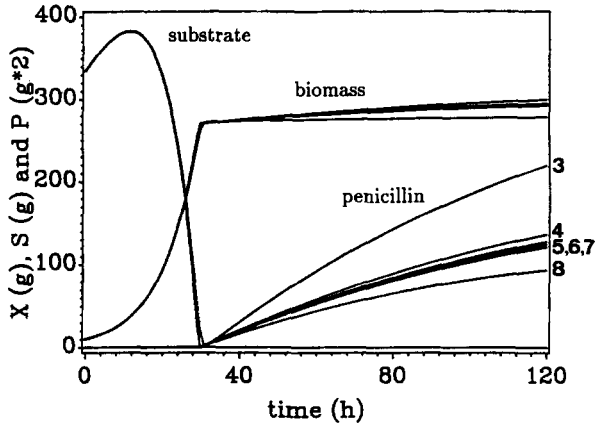

Fig. 4. Substrate, biomass and penicillin profiles during constant feed rate fed-batch fermentation as predicted by the model of Bajpai & Reuß for the K_x and μ_p combinations of Table 6 ($S_0 = 335$ g).

In Table 6, the optimization results for different K_x and corresponding μ_p combinations are given. K_x values below 0.05 g/g DM give an excellent fit (Fig. 3) with acceptable μ_p values (see Fig. 3). It is clear that the K_x value has a very important influence on optimization which can also easily be seen by inspection of Eq. (11). In Fig. 4, the time evolution of the (adjusted) Bajpai & Reuß model using different K_x and μ_p combinations

of Table 6 is given for an initial amount of substrate of 335 g.

A decrease in m_s from 0.029 g/g DM h (Set 2 of Bajpai & Reuß) to 0.014 g/g DM h (Set 1) results in an increase of the realizible gain from 6.4% to 35.9%. However, as mentioned above, the fit of the model with this low m_s value to the reference data is rather poor. When the k_h value is increased from $k_h = 0.01\ h^{-1}$ to $0.04\ h^{-1}$, the fit is also poor and the corresponding μ_p values are unacceptable. In this case the gain has been lost completely. On the other hand, for $k_h = 0.002\ h^{-1}$ as used by Heijnen et al., the fit is acceptable with a corresponding μ_p value equal to 0.0032 g/g DM h. The realizible gain increases to 9.9%.

The influence of the other parameter differences upon optimization is less important.

These simulations indicate that both modeling and a corresponding parameter estimation of biotechnological processes has to be done with great care. Using model based process optimization techniques' fermentation conditions can be found which may reveal an eventual estimation error of a particular parameter or model feature.

It is important that the conclusions drawn from these simple optimization studies agree very well with the conclusions drawn from more complicated optimization studies (Van Impe et al. 1991, 1992).

Conclusions

A careful investigation of both the model of Bajpai & Reuß and the model of Heijnen et al. revealed some biochemical and physical inconsistencies.

Heijnen et al. assumed an endogenous metabolism, which is appropriate in low substrate concentration conditions. At high substrate concentrations, a maintenance metabolism as in the Bajpai & Reuß model seems conceptually more attractive. However, this assumption can lead to (physically impossible) negative substrate concentrations, in cases where the glucose feeding rate becomes too small.

For the specific production kinetics Heijnen et al. have extrapolated continuous culture data to fed-batch conditions. This may obscure substrate inhibition or more probably repression effects. Further, their model predicts penicillin production becoming zero if the net growth rate decreases to zero. This is in contradiction with some experiments of Pirt & Righelato (1977) for limited time ranges. For negative growth rates, a straightforward extension of this production rate kinetics (π=0 for $\mu \leq 0$) may not be valid. The Bajpai & Reuß substrate inhibition production kinetics is believed to reflect biochemical evidence better, although an accurate set of corresponding parameters is not available up to now. Simulation studies reveal that in this model, for simple constant feeding strategies with a zero initial amount of substrate, the time profiles of biomass, substrate and penicillin are quite insensitive towards large changes in K_p, K_i and K_x, which may be a source of large errors in the estimation of these parameters. The reported values of m_s, k_h differ quite a lot. Simulation reveals that the dynamic behaviour of the model is quite sensitive to these variations. The other model parameters agree fairly well with each other, and with the reported literature data.

A simple optimization study has been carried out. For this purpose the model of Bajpai & Reuß has been modified so that when C_s becomes equal to zero, the mould switches from maintenance to endogenous metabolism, and thus prevents C_s from becoming negative.

The realizable gains proved to be dependent on variations of K_p, K_i and especially K_x, although the time profiles of biomass, substrate and penicillin for constant feed rate fermentations with zero inital substrate amount (on which the parameter estimation was based) have been shown to be insensitive to even large variations of these parameters. Variations in the other parameters have a less important influence on optimization results.

It is suggested that model based process optimization techniques can be used to determine experimental fermentation conditions which may reveal some model features and may be helpful in reducing estimation errors of the corresponding parameters.

Nomenclature

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)
F	input substrate feed rate (L/h)
G	gain due to initial amount of substrate optimization (%)
H	unit step function
k_h	penicillin hydrolysis or degradation constant (h^{-1})
K_i	substrate inhibition constant for product formation (g/L)
K_M	Monod saturation constant for an equivalent Monod-kinetics (g/L)

K_p	saturation constant for substrate limitation of product formation (g/L)
K_s	Monod saturation constant for substrate limitation of biomass production (g/L)
K_x	Contois saturation constant for substrate limitation of biomass production (g/g DM)
m_s	maintenance constant (g/g DM h)
μ	specific growth rate (h^{-1})
μ_{crit}	critical specific growth rate (h^{-1})
μ_p	specific production constant (g/g DM h)
μ_{substr}	specific substrate to biomass conversion rate (h^{-1})
μ_x	maximum specific substrate to biomass conversion rate (h^{-1})
P_{ref}	final amount of penicillin (g) for $S_0 = 0$ g
P_{opt}	final amount of penicillin (g) for S_0 optimized
π	specific production rate (g/g DM h)
$Q_{p,max}$	maximum specific penicillin synthesis rate (g/g DM h)
$Q_{s,max}$	maximum specific substrate uptake rate (g/g DM h)
s_F	substrate concentration in feed stream (g/L)
σ	specific substrate consumption rate (g/g DM h)
t	time (h)
V	fermentor volume (L)
$Y_{x/s}$	cell mass on substrate yield (g DM/g)
$Y_{p/s}$	product on substrate yield (g/g)

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