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Application of Balancing Methods in Modeling the Penicillin Fermentation

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Summary

This paper shows the application of elementary balancing methods in combination with simple kinetic equations in the formulation of an unstructured model for the fed-batch process for the production of penicillin. The rate of substrate uptake is modeled with a Monod-type relationship. The specific penicillin production rate is assumed to be a function of growth rate. Hydrolysis of penicillin to penicilloic acid is assumed to be first order in penicillin. In simulations with the present model it is shown that the model, although assuming a strict relationship between specific growth rate and penicillin productivity, allows for the commonly observed lag phase in the penicillin concentration curve and the apparent separation between growth and production phase (idiophase–trophophase concept). Furthermore it is shown that the feed rate profile during fermentation is of vital importance in the realization of a high production rate throughout the duration of the fermentation. It is emphasized that the method of modeling presented may also prove rewarding for an analysis of fermentation processes other than the penicillin fermentation.

INTRODUCTION

In recent years great progress has been achieved in the modeling of fermentation processes. For a recent survey of the literature on this subject the reader is referred to Roels and Kossen.[1]

Basically a simple unstructured model for a fermentation process with product formation can be constructed if three rate equations are defined: an equation for the substrate conversion rate; an equation for the biomass production rate; and an equation for the product conversion rate. On introduction of these rate equations in the mass balances for substrate, biomass, and product the time evolution of the concentration of these components can, in principle, be calculated. However, for the design of fermentation equipment and the optimization of the process more information is generally needed,

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i.e.: oxygen consumption rate; carbon dioxide evolution rate; and heat production rate.

In the past there has been a tendency to resort to experimentation to obtain information about the three additional rate processes mentioned above. In recent years it has been shown, however, that valuable and reliable information concerning these processes can be obtained by the application of elementary and enthalpy balances.[1-9] In the next section a limited survey of the literature treating the application of these balancing techniques in fermentation technology will be presented. In the subsequent sections these techniques will be combined with literature data on the relevant kinetic equations and a simple unstructured model for the production of penicillin by fermentation will be shown to be the result.

APPLICATION OF ELEMENTARY AND ENTHALPY BALANCES IN FERMENTATION PROCESS MODELING

In recent years the concept of elementary balances has become an increasingly valuable tool in fermentation process modeling. Numerous applications have been reported with regard to the modeling of the production of single cell protein (SCP).[2-5] The production processes for bakers' yeast and citric acid[6,7] have also been treated in this way. Herbert[8] and Stouthamer[9] report applications in more specific cases. Roels and Kossen[1] presented a rather general treatment of the principle. Cooney and Acevedo[10] used the elementary balance principle to calculate theoretical penicillin yields.

The enthalpy balance has also received considerable attention in fermentation modeling. Cooney et al.[11] reported the existence of direct proportionality between the heat production and oxygen consumption rates. Minkevich and Eroshin[12] and Imanaka and Aiba[13] derived the relationship between heat production and oxygen consumption on a more or less theoretical basis by combining enthalpy and elementary balances.

STRUCTURE OF THE MODEL FOR THE PENICILLIN FERMENTATION

In this section the structure of the model for the production process for penicillin will be defined. Then elementary balances will be used to derive a set of useful relationships between the various rates. Mass balances for the relevant compounds will be formulated

and the necessary kinetic equations will be introduced into these balance equations.

The first step in the construction of the model is to decide which compounds are relevant for the present, unstructured description of the penicillin fermentation. The set of compounds given in Table I is proposed. In Table I the mycelial dry matter is represented as a compound of a fixed chemical composition that can be conveniently represented by its elementary composition formula. This formula is based on an average for microorganisms.[14] Glucose is assumed to be the sole carbon and energy source, ammonia is the nitrogen source, and sulfuric acid and *o*-phosphoric acid are the sulfur and phosphorus sources, respectively. The precursor for the product penicillin-G is phenylacetic acid. The product of the hydrolysis of penicillin, penicilloic acid, is also introduced, thus taking account of the fact that penicillin-G is a relatively unstable compound that slowly hydrolyzes to yield penicilloic acid.[15]

The molar enthalpies presented in Table I are in part directly taken from thermodynamic tables, in part calculated.

The enthalpies of mycelial dry matter, phenylacetic acid (PAA), penicillin G, and penicilloic acid (PA) were calculated from the heats of combustion of these compounds. The heats of combustion were calculated using the observation of Kharash[16] that each mole of oxygen consumed on combustion of a compound results in a release of 106 kcal.

Having defined the relevant compounds, the elementary and en-

TABLE I
Table of Relevant Compounds in Penicillin Fermentation

Compound	Chemical Formula	Mol weight (g)	Molar enthalpy (kcal/mol)	Conversion rate (mol/hr)
Glucose	$C_6H_{12}O_6$	180	−303	r_s
Oxygen	O_2	32	0	r_o
Carbon dioxide	CO_2	44	− 94	r_c
Water	H_2O	18	− 68	r_w
Penicillin[a]	$C_{16}H_{18}O_4N_2S$	334	−115	r_p
Ammonia	NH_3	17	− 19	r_n
Sulfuric acid	H_2SO_4	98	−194	r_{su}
Phosphoric acid	H_3PO_4	98	−319	r_{ph}
Phenylacetic acid	$C_8H_8O_2$	136	− 69	r_{pa}
Penicilloic acid	$C_{16}H_{20}O_5N_2S$	352	−183	r_{po}
Mycelium	$CH_{1.64}O_{0.52}N_{0.16}S_{0.0046}P_{0.0054}$	24.52	− 28.1	r_x

[a] One mol penicillin G = 0.594 billion units penicillin G.

thalpy balances can now be formulated. Because there are, according to Table I, six relevant elements, six elementary balances, for the elements C, H, N, O, S, P and the enthalpy balance can be formulated. This results in the following relationships between the conversion rates defined in Table I:

Carbon balance

$$6r_s + r_c + r_x + 16r_p + 8r_{pa} + 16r_{po} = 0 \tag{1}$$

Hydrogen-balance

$$12r_s + 2r_w + 1.64r_x + 18r_p + 3r_n + 2r_{su} + 3r_{ph} + 8r_{pa} + 20r_{po} = 0 \tag{2}$$

Nitrogen-balance

$$0.16r_x + 2r_p + r_n + 2r_{po} = 0 \tag{3}$$

Oxygen-balance

$$6r_s + 2r_o + 2r_c + r_w + 0.52r_x + 4r_p + 4r_{su} + 4r_{ph} + 2r_{pa} + 5r_{po} = 0 \tag{4}$$

Sulfur-balance

$$0.0046r_x + r_p + r_{su} + r_{po} = 0 \tag{5}$$

Phosphorus-balance

$$0.0054r_x + r_{ph} = 0 \tag{6}$$

Enthalpy balance

$$-303r_s - 94r_c - 68r_w - 28.1\,r_x - 115r_p - 19r_n - 194r_{su} - 319r_{ph} - 69r_{pa} - 183r_{po} + r_H = 0 \tag{7}$$

where r_H is the heat production rate (kcal/hr).

It is stressed that the reaction rates are defined with regard to the conversion in the total broth weight and not per unit broth weight. This will prove to be a convenient convention as, the production process for penicillin being of the fed-batch type, broth weight is variable during the course of the fermentation process.

By some algebraic manipulation of eqs. (1)–(7) the following relations for the conversion rates of oxygen (r_o), carbon dioxide (r_c), heat (r_H), ammonia (r_n), sulfuric acid (r_{su}), and orthophosphoric

acid (r_{ph}) are obtained:

$$-r_o = -6r_s - 9.0r_{pa} - 1.044r_x - 18.5r_p - 18.5r_{he} \quad (8)$$

$$r_c = -6r_s - 8r_{pa} - r_x - 16r_p - 16r_{po} \quad (9)$$

$$r_H = -669r_s - 955r_{pa} - 110.1r_x - 1961r_p - 1961r_{po} \quad (10)$$

$$-r_n = 0.16r_x + 2r_p + 2r_{po} \quad (11)$$

$$-r_{su} = 0.0046r_x + r_p + r_{po} \quad (12)$$

$$-r_{ph} = 0.0054r_x \quad (13)$$

From eqs. (8) and (9) it is immediately clear that, for the assumed composition formula of the organism, the respiratory quotient (RQ) always exceeds unity. From eqs. (8) and (10) it follows that oxygen consumption and heat production are proportional for the present case, the proportionality constant ranging from 106 to 111 kcal/mol oxygen.

The equations presented above were derived assuming an average biomass composition. It is, however, known that nitrogen and phosphorus content may vary with growth rate.[8]

Recalculation of the equations for two extremes of the biomass composition results in only a slight variation in the coefficient of r_x as is shown in Table II. As is also shown in Table II the coefficient of r_x is, however, very sensitive to the nature of the nitrogen source used.

If HNO_3 instead of NH_3 is used as the sole nitrogen source, the coefficient rises strongly. A similar conclusion was reached by Stouthamer[9] on the basis of biochemical considerations for growth without product formation. From eqs. (8)–(13) it is clear that r_o, r_c, r_H, r_{su}, and r_{ph} can be calculated if r_s, r_p, r_x, r_{po}, and r_{pa} are known. It is obvious that at least five kinetic equations are needed to complete the model. The following kinetic equations are chosen:

TABLE II
Effect of Biomass Composition and the Nature of the Nitrogen Source

Condition	Composition formula of biomass	Nitrogen source	Coefficient of r_x in eq. (8)
Low specific growth rate	$CH_{1.64}O_{0.52}N_{0.11}S_{0.0046}P_{0.0038}$	NH_3	1.08
High specific growth rate	$CH_{1.64}O_{0.52}N_{0.16}S_{0.0046}P_{0.0054}$	NH_3	1.04
		HNO_3	1.36

an equation for the glucose uptake rate; an equation for the biomass formation rate; an equation for the rate of penicillin formation; an equation for the precursor consumption rate; and an equation for the rate of penicillin hydrolysis.

In the next section the formulation of these kinetic equations will be treated. It will be shown that, using the present formulation of the model structure, only the concentrations of glucose, penicillin, and biomass are relevant with regard to the formulation of these kinetic equations.

Generally the time evolution of the concentration of a compound can be calculated by the formulation of a mass balance with respect to that compound. The following equations can be formulated for glucose, mycelial dry matter, and penicillin:

Mass balance for glucose:

$$\frac{d}{dt}(G \cdot C_s) = C_s \frac{dG}{dt} + G \frac{dC_s}{dt} = r_s + \Phi_s \tag{14}$$

Mass balance for penicillin:

$$\frac{d}{dt}(G \cdot C_p) = C_p \frac{dG}{dt} + G \frac{dC_p}{dt} = r_p \tag{15}$$

Mass balance for mycelial dry matter:

$$\frac{d}{dt}(G \cdot C_x) = C_x \frac{dG}{dt} + G \frac{dC_x}{dt} = r_x \tag{16}$$

where G is the total broth weight (kg); C_s is the concentration of glucose (mol/kg); C_p is the penicillin concentration (mol/kg); C_x is the biomass concentration (mol/kg); and Φ_s is the flow of sugar into the fermentor (mol/hr).

As the fermentation is assumed to be of the fed-batch type the flux of sugar into the fermentor is defined. Penicillin and biomass are assumed to be not exchanged with the environment and their fluxes into or from the fermentor are taken as zero.

In the mass-balance equations the variation in broth weight does appear; it can be calculated by the formulation of a balance equation for total weight:

Weight-balance

$$\frac{dG}{dt} = \sum (\text{feeds–evaporation}) + 0.032 r_o + 0.044 r_c \tag{17}$$

For the reader's convenience the essential steps in the construction of the model have been summarized in the flow chart presented in Figure 1.

From Table I it can be seen that there are 11 unknown reaction rates, of which six are defined by virtue of elementary balances and five by the formulation of kinetic equations. The enthalpy balance provides the biological heat production (no account is taken of the enthalpy exchange due to agitation or aeration, these contributions may, however, be quite significant). In the next section a literature survey will be presented resulting in the identification of the necessary kinetic equations.

FORMULATION OF THE KINETIC EQUATIONS

In the preceding section the conclusion was reached that at least five kinetic equations are needed to complete the present model of the penicillin fermentation. In this section these kinetic equations will be evaluated using literature data.

Equation for the Substrate Uptake Rate

Fishman and Biryukov[17] use a Michaelis–Menten-type of rela-

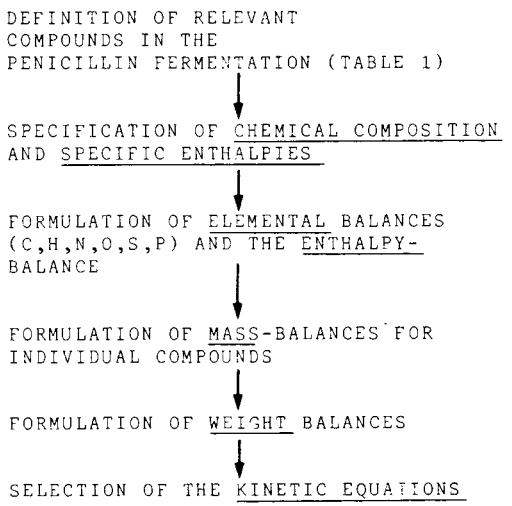

Fig. 1. Flowsheet of the construction of the model.

tionship for the sugar uptake rate:

$$-r_s = q_{s,\max} C_s C_x G/(K_s + C_s) \tag{18}$$

where $q_{s,\max}$ is the maximal value of specific sugar uptake rate g/g biomass dry matter (DM)/hr and K_s is the Michaelis constant (g/kg).

Fishman and Biryukov estimate $q_{s,\max}$ to be 0.12 and K_s to be 1. Righelato[18] mentions the maximum specific growth rate to be 0.12 hr^{-1}, if a maximum growth yield of about 0.5 g DM/g glucose is assumed, this is equivalent to a $q_{s,\max}$ of 0.24. Reasonable estimates of the kinetic constants in eq. (18) thus seem to be $q_{s,\max} = 0.18$, $K_s = 1$.

Transforming to molar units results in the following equation:

$$-r_s = 0.0245\, C_s C_x G/(0.0056 + C_s) \tag{19}$$

In eq. (19) rates and concentrations are expressed in molar units.

Equation for the Biomass Formation Rate

In Figure 2 data on the relationships between the substrate consumption rate and the growth rate, as reported by various investigators,[17–19] have been plotted.

The familiar linear relationship between substrate consumption rate and growth rate is shown to hold to a fair degree of approximation and there is good agreement between the results of different authors. The maximum growth yield, Y_{xs}, is estimated to be 0.5 g biomass/g glucose, the maintenance factor, m_s, is approximately 0.025 g glucose/g mycelial DM hr. In molar units the parameters are given by:

$$\begin{aligned} Y_{xs} &= (0.5 \times 180)/24.52 \\ &= 3.67 \text{ mol mycelial DM/mol glucose} \\ m_s &= 3.4 \times 10^{-3} \text{ mol glucose/mol mycelial DM/hr} \end{aligned} \tag{20}$$

The biomass yield of 3.67 is equivalent to a carbon conversion efficiency of 0.61, the carbon conversion efficiency being defined as the fraction of substrate carbon fixed in biomass dry matter.

For the case of the formation of appreciable amounts of a product not directly associated with the primary energy-producing pathways, Humphrey[20] proposes an extension to the linear equation for growth; a term is added which accounts for sugar used in product formation:

$$-r_s = (1/Y_{xs}) r_x + m_s C_x G + (1/Y_{ps})(r_p + r_{po}) \tag{21}$$

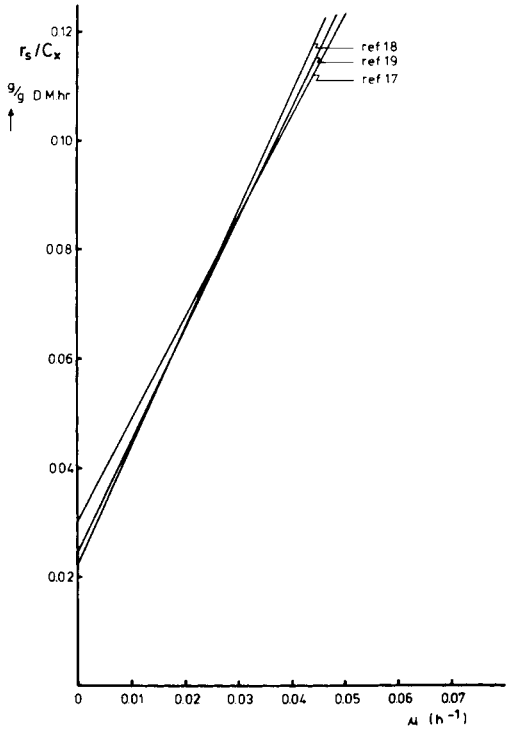

Fig. 2. Literature data on the relation between growth rate and glucose consumption rate.

For Y_{xs} and m_s the parameter values obtained above will be assumed to be valid. This is most probably allowed as these parameters were evaluated from results on low producing strains.

In that case the last term of eq. (21) is of little importance. In eq. (21) Y_{ps} represents the conversion yield for glucose to penicillin. In eq. (21) the total penicillin synthesis is substituted, that is to say the net synthesis rate corrected for penicillin loss due to hydrolysis.

The linear equation [eq. (21)] can be substituted into eqs. (8) and (9) to yield equations for the oxygen uptake rate and the carbon dioxide evolution rate. For this purpose an equation for the precursor consumption rate is also needed. It is obtained by the assumption that the precursor is not used in processes other than penicillin synthesis:

$$-r_{pa} = r_p + r_{po} \tag{22}$$

Substitution of eqs. (21) and (22) into eqs. (8) and (9) results, after

elimination of r_s, in the following equations:

$$-r_o = (6/Y_{xs} - 1.044)r_x + 6m_sC_xG + (6/Y_{ps} - 9.5)(r_p + r_{po}) \quad (23a)$$

$$r_c = (6/Y_{xs} - 1)r_x + 6m_sC_xG + (6/Y_{ps} - 8)(r_p + r_{po}) \quad (23b)$$

From equations (23a) and (23b) the following relationships between yields on substrate and on oxygen are obtained:

$$1/Y_{xo} = 6/Y_{xs} - 1.044 \quad (24)$$

$$1/Y_{po} = 6/Y_{ps} - 9.5 \quad (25)$$

Equations (24) and (25) define the true yields for mycelial dry matter and penicillin on oxygen (Y_{xo} and Y_{po}) expressed as mol/mol oxygen. In Figure 3 the relationships according to eqs. (24) and (25) are shown graphically. The true yields for penicillin and mycelium on substrate are seen to be related to the true yields on oxygen for these components. In the limiting case where there is only a carbon source requirement due to the carbon necessary for the construction of the biomass and the product and no carbon

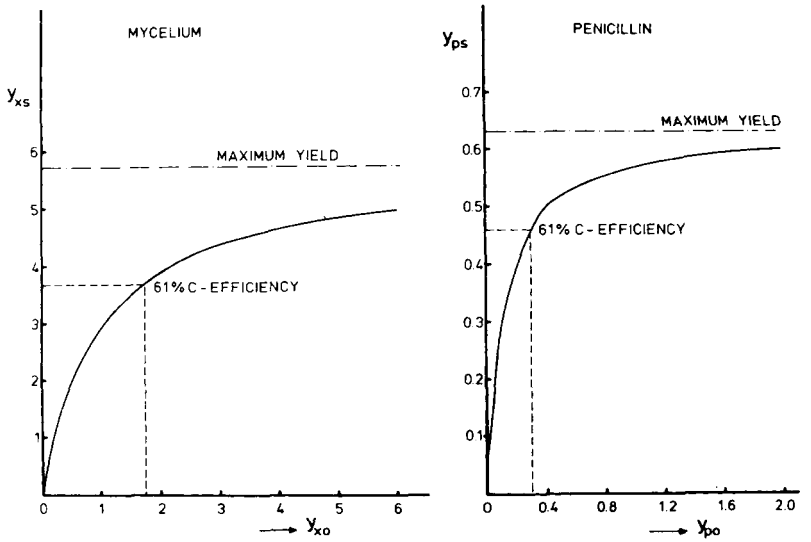

Fig. 3. Relations between yields on glucose and oxygen for mycelium and penicillin.

source is used in energy supplying reactions, Y_{xo} and Y_{po} are infinite. The maximum substrate yields for this case are calculated to be $Y_{xs} = 5.75$ and $Y_{ps} = 0.631$. The value of the penicillin yield according to this maximum yield was derived earlier by Stouthamer[21] on the basis of simple stoichiometric considerations. It is clear that, for carbon efficiencies exceeding 0.6, Y_{xo} and Y_{po} are only of relatively little influence if eqs. (24) and (25) are used to calculate the substrate yields. In these cases the substrate yields are to a large extent determined by the carbon needs for biosynthesis. With this in mind and noting that only a fraction of the sugar is converted to penicillin, it will be clear that a calculation of Y_{ps} on the assumption of a conversion efficiency equal to that for biomass formation will not result in large errors. This assumption allows Y_{ps} to be calculated at 0.46 mol penicillin/mol glucose.

This value can be compared with the value obtained by Cooney and Acevedo.[10] A detailed analysis on the basis of the presumably known metabolic pathways involved in penicillin synthesis resulted in an Y_{ps} value of 0.56.

Kinetic Equation for the Penicillin Production Rate

The literature treating penicillin production kinetics can be classified in two major groups: the first group provides penicillin production rate data in arbitrary units only;[17,22] the second group provides penicillin production rate data in absolute units.[19,23–25] Here attention will be focused on the second group.

In Table III the relevant data have been summarized. Most investigators are seen to define a functional relationship for the specific rate of penicillin synthesis q_p and provide a crude estimate of its maximum value $q_{p,\max}$. A number of investigators assumed the specific penicillin synthesis rate to be maximal if the specific growth rate exceeds a critical value.

This critical value can be estimated to be between 0.006 and 0.014 hr^{-1}. If the specific growth rate is lower than the critical one, Pirt and Righelato[25] observe a decaying penicillin synthesis rate with time. Humphrey and Jefferis[20] assume a stable specific rate of penicillin synthesis to be possible at specific growth rates lower than the critical value, it does, however, decrease linearly with decreasing specific growth rate if the specific growth rate is lower than the critical one. It seems that most data more or less agree with the kinetic behavior proposed by Humphrey. For the critical growth rate a value of 0.01 hr^{-1} seems reasonable. The maximum specific penicillin synthesis rate is about 8 U/mg DM hr. In molar units, and

TABLE III
Survey of Kinetic Data for Penicillin Production

Ref.	Type of kinetic equation	$q_{p,max}$ (U/mg hr)	Gross production rate (U/g fluid hr)	Penicillin titer (U/g) at about 200 hr fed-batch culture	Efficiency of penicillin production (U/mg glucose)	Experimental conditions
24	$q_p = f(C_{ol})$	–	200	25000	130	fed-batch (FB) culture in complex medium
23	$q_p = f$(age)	5	100	11000	73	FB culture
22	$q_p = f$(age)	–	90	9000	–	FB culture
25	q_p = constant $\mu > 0.014$ q_p = unstable $\mu < 0.014$	1.53	–	–	–	continuous culture (CC)
19	–	8.4	–	–	100	CC; $\mu = 0.029$ sucrose limit
	–	10.2	–	–	55	CC; $\mu = 0.029$ P-limit
	–	2.7	–	–	–	CC; $\mu = 0.020$ S-limit
	–	5.3	–	–	–	CC; $\mu = 0.020$ N-limit
20	$q_p/q_{p,max} = 1$	–	–	–	–	$\mu > \mu_{cr}$
	$q_p/q_{p,max} = \mu/\mu_{cr}$	–	–	–	–	$\mu < \mu_{cr}$
18	$q_p/q_{p,max} = 1$	–	–	–	–	$\mu > 0.006$ hr^{-1}

correcting for penicillin G hydrolysis, the rate equation for total penicillin production runs:

$$\begin{aligned} r_p + r_{po} &= 3.3 \times 10^{-4}\, C_x G && \mu \geq 0.01\,\text{hr}^{-1} \\ r_p + r_{po} &= 3.3 \times 10^{-4}\, C_x G(\mu/0.01) && \mu < 0.01\,\text{hr}^{-1} \end{aligned} \tag{26}$$

where μ is the specific growth rate.

Equation for Precursor Conversion Rate

As was already stated the precursor is assumed to be used for penicillin synthesis only. This results in the expression given by eq. (22)

$$-r_{pa} = r_p + r_{po}.$$

Equation for the Rate of Penicillin Hydrolysis

According to Benedict et al.,[15] penicillin hydrolysis to penicilloic acid takes place by a first-order reaction. The first-order rate constant is about 2×10^{-3} hr^{-1} at 25°C and pH = 7. The following kinetic equation is thus obtained:

$$r_{po} = 0.002 C_p \cdot G \tag{27}$$

In the preceding sections the five kinetic equations needed to complete the model were given. In the next section the results of some simulations with the model will be shown.

SIMULATION STUDIES OF THE PENICILLIN FERMENTATION MODEL

In this section some results of simulations with the model for the penicillin fermentation developed will be presented. These simulations have been performed using the continuous systems modeling program (CSMP). The simulations were performed using the operating data presented in Table IV. *ortho*-Phosphoric acid is not supplied as a feed but is present in the batch before inoculation in an amount sufficient for the duration of the fermentation. The required feed rates of ammonia, sulfuric acid, and precursor are calculated on the assumption that they are supplied at rates equal to their conversion rates. The water loss from the broth by evaporation is calculated on the assumption of saturation of the air leaving the fermentor. The computer program is presented in the Appendix.

TABLE IV
Operating Variables

Variable	Vaiue
Broth temperature	25°C
Air flow rate[24]	50 Nm^3/ton/hr
Air pressure	1.5 atm
Broth weight after inoculation	10^5 kg
Initial mycelial concentration	1 g DM/kg
Initial sugar concentration	10 g/kg
Ammonia feed concentration	250 g/kg
Sulfuric acid feed concentration	250 g/kg
Precursor feed concentration	250 g/kg
Duration of the fermentation[24]	200 hr
Sugar concentration in feed	500 g/kg

Three simulations with different glucose feed rate profiles but equal total glucose feed will be discussed. In the first simulation (A) the glucose feed rate was kept constant at a level of 1000 mol/hr (180 kg/hr). This is, according to Giona et al.,[24] a realistic value. In the second simulation (B) the glucose feed rate is assumed to increase linearly according to the equation 500 + 5*TIME (mol/hr).

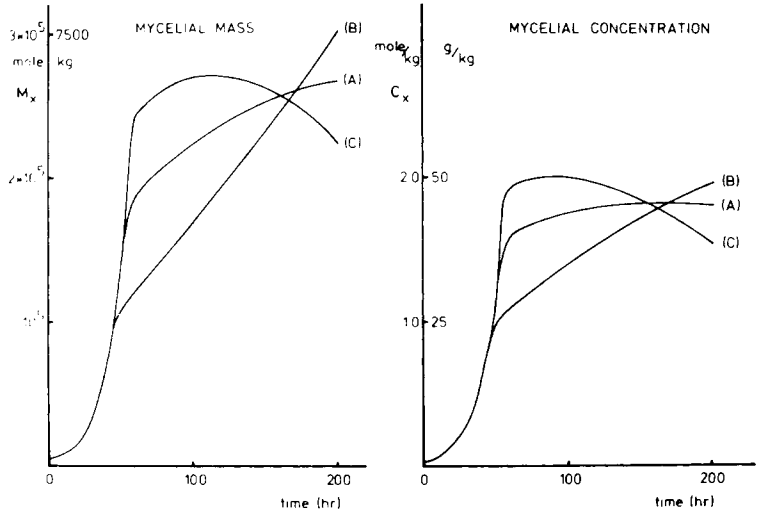

Fig. 4. Mycelial mass and concentration curves for glucose feed rate schemes A, B, C (see text).

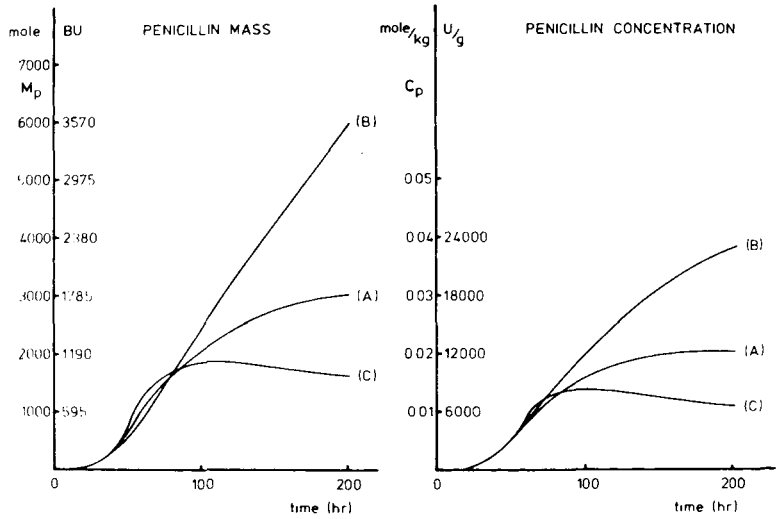

Fig. 5. Penicillin mass and concentration curves for glucose feed rate schemes A, B, C (see text).

The third simulation (C) was performed assuming a decreasing glucose feed rate according to 1500 − 5*TIME (mol/hr).

The results of these simulations are presented in Figures 4–11.

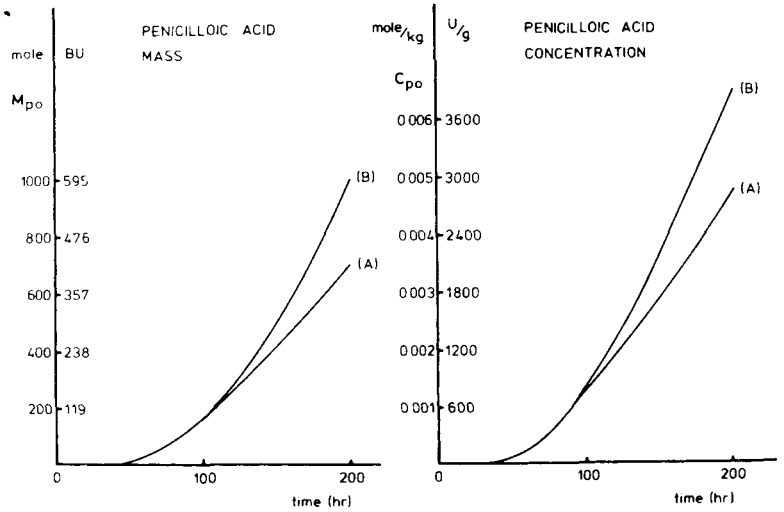

Fig. 6. Penicilloic acid mass and concentration curves for glucose feed rate schemes A, B (see text).

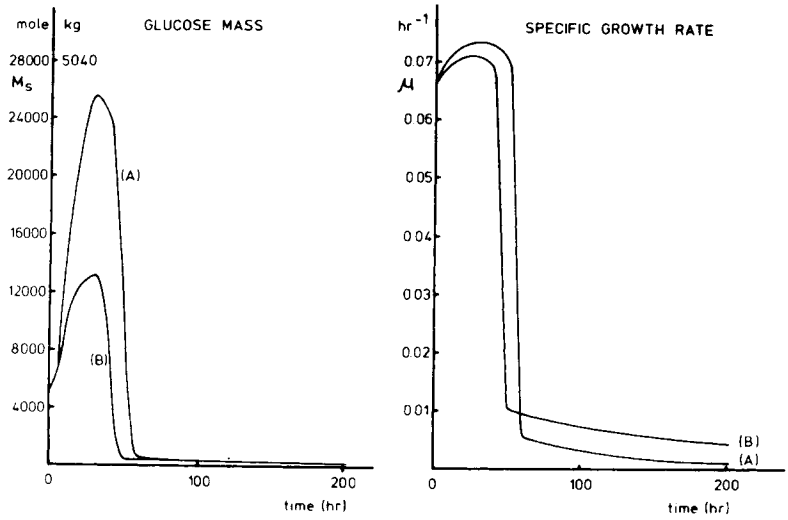

Fig. 7. Glucose mass in the fermentor and specific growth rate curves for glucose feed rate schemes A, B (see text).

General Discussion of the Simulation Results

Before discussing the differences between the results of the various simulation runs some fairly general conclusions will be drawn.

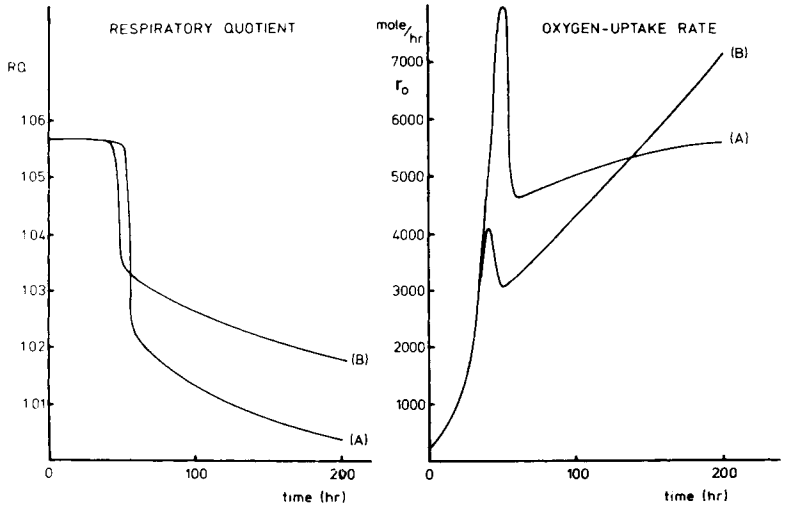

Fig. 8. RQ and oxygen uptake rate for glucose feed rate schemes A, B (see text).

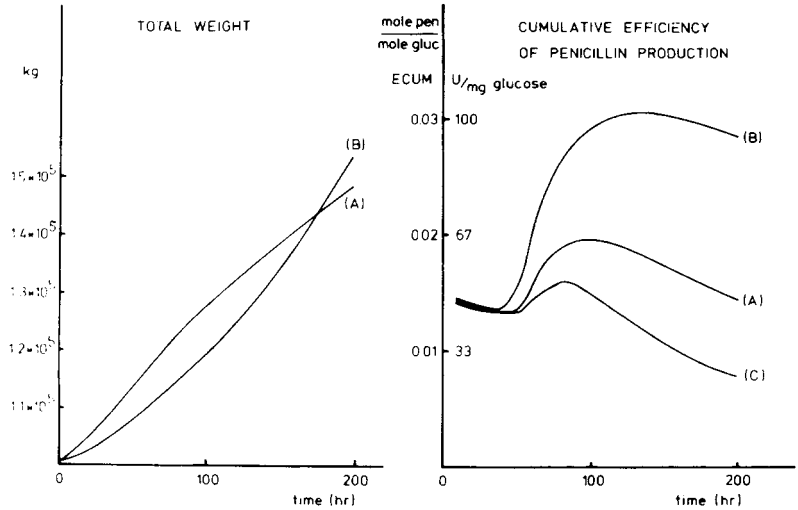

Fig. 9. Total weight for glucose feed rate scheme's A and B and cumulative efficiency for glucose feed rates schemes A, B, C (see text).

From Figures 4 and 5 it is evident that the total amounts of mycelium and penicillin increase much faster than their concentrations due to the increasing total weight caused by the various feeds (Fig. 9). It will be clear that this means that a constant mycelium or penicillin

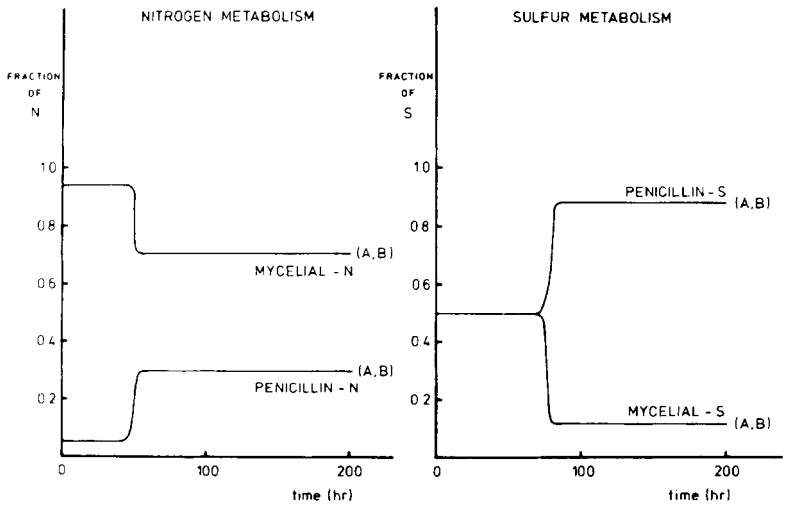

Fig. 10. N and S utilization in the penicillin fermentation.

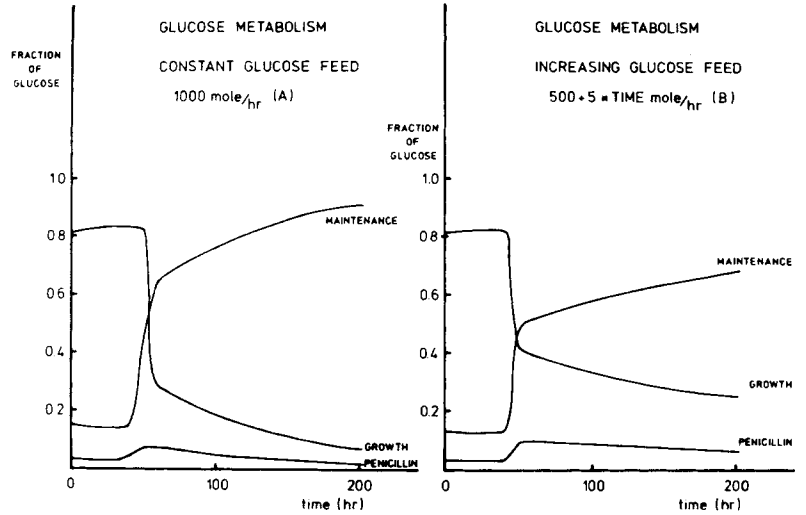

Fig. 11. Glucose utilization in the penicillin fermentation.

concentration or even a decreasing one does not necessarily mean that growth rate or penicillin production rate are zero or negative. It is the opinion of the present authors that this effect has been frequently overlooked in the analysis of the growth and production curves of penicillin fermentation. Up until now the penicillin fermentation was often classified in the group of product formation processes of the non-growth-associated type; the process was assumed to exist of two phases: a phase of rapid growth with no product formation (trophophase) and a phase with no growth in which the product is formed (idiophase).[26]

The simulation results presented in Figures 4 and 5, especially the concentration plots, are seemingly in agreement with this description. It is, however, quite remarkable that our results were obtained on the assumption of a direct coupling between specific growth rate and specific rate of product formation [see eq. (26)]. The specific rate of penicillin production was assumed to be higher at the highest specific growth rate. Therefore it may be concluded that the apparent separation between production and growth phases does not necessarily mean that penicillin production is of the non-growth-associated type. The results commonly observed can be explained perfectly by the present model, which assumes coupling between penicillin production and growth.

Another feature of the simulation results is an apparent lag phase

in the penicillin production curve of about 20 hr. This lag phase in the beginning of the penicillin production was *a priori* introduced in models of the penicillin production process by several authors.[22,23] In our simulations a lag phase was not introduced *a priori* but it can be seen that the simulation results still show an indication of an apparent lag phase, therefore it remains possible that there is in reality no real lag in the commencement of penicillin production.

Another modeling approach that has been followed is the assumption of a relationship between mycelial age and its productivity.[17] In Ref. 17 the product activity was assumed to decrease at high mycelial age. In this way the decreasing slope of the penicillin production curve in the last part of the fermentation can be modeled. Apart from problems in defining the age of a mycelial culture and the fact that it is difficult to imagine how mycelial age, apart from concurrent changes in its composition, can influence productivity, this decreasing slope may very well be explained from one of the following factors: dilution due to increasing broth weight and hydrolysis of penicillin to penicilloic acid.

With these considerations in mind the present authors would like to emphasize that a growth-coupled penicillin production provides an adequate description of most of the observed phenomena, which have often led to the assumption of non-growth-associated or age-dependent penicillin productivity. There appears to be no experimental evidence necessitating the introduction of an age dependency or a time delay in modeling the kinetics of penicillin production.

On inspection of Figures 5 and 6 it becomes clear that a quite significant amount of the penicillin produced hydrolyzes during the course of the fermentation process to yield penicilloic acid. This may amount to 10 to 20% of the total penicillin production.

In Figure 8 the relationship between respiratory quotient (RQ) and time is given, the RQ is about 1.05 at the maximum growth rate and decreases to about 1 with increasing fermentation time.

Figure 10 shows the extent to which N and S are incorporated into penicillin and mycelial dry matter respectively. About 20% of the total N feed and as much as 80% of the total S feed appear as penicillin. This last point stresses the importance of the sulfur metabolism in the production of penicillin as has been pointed out by one of the present authors.[21]

In Figure 11 the fate of the carbon and energy source glucose is analyzed. It can be seen that 20% of the glucose is used in the

production of mycelial dry matter, 10% is used for penicillin synthesis, and as much as 70% is used in maintenance processes. This certainly stresses the importance of the maintenance requirements in the analysis of the penicillin production process.

Effects of Differences in Glucose Feed Rate Schemes

In this section the effects of differences in feed rate scheme at constant total glucose feed will be analyzed. As was already pointed out simulation runs with three kinds of feed rate schemes were performed: (A) a constant rate, 1000 mol glucose/hr; (B) an increasing feed rate with time (500 + 5 TIME mol glucose/hr); (C) a decreasing feed rate with time (1500 − 5 TIME mol glucose/hr). The results of these simulations are shown in Figures 4–11.

The results of the simulations show that these three feed rate schemes do not result in appreciable differences up to 40 hr of fermentation. This is caused by the fact that the specific growth rate in the beginning of the fermentation is limited by the maximum specific growth rate of the organism, the differences in glucose supply rate result in differences in glucose accumulation in the fermentor (Fig. 7). It is also clear that the feed rate schemes according to schemes A and C turn out to be disastrous after 80 hr of fermentation; the penicillin production rate is shown (Fig. 5) to decrease sharply because the large amount of biomass formed cannot be kept at the specific growth rate necessary for optimal penicillin productivity (see Fig. 7) [$\mu = 0.01$, eq. (26)]. From Figure 8 it is also clear that using scheme A leads to a period of very high oxygen demand which may very well exceed the aeration capacity of the fermentation vessel resulting in productivity loss due to underaeration.[24] This also holds for scheme C. In Figure 9 the cumulative efficiency (penicillin produced/glucose consumed) of the synthesis of penicillin is shown. It can be seen that, after a rapid increase until 80 hr there is a steady decrease in efficiency. This is due to the increasing glucose demands put by the maintenance process (also see Fig. 11).

From the foregoing it is clear that the present model allows one to make the conclusion that an increasing glucose feed rate with time will result in higher penicillin productivity with higher efficiency as compared with other possible schemes like a constant feed rate or a decreasing feed rate with time.

Although the present authors did not consider other schemes in detail, it is clear that the present model, in principle, allows the optimization of the glucose feed rate scheme with respect to a

chosen optimality criterion also considering the limitations to the feed rate scheme put by the capacity of the equipment with respect to the supply of oxygen or the removal of heat and carbon dioxide.[20]

Parameter Sensitivity Analysis of the Model

Once a model has been developed it is useful to test the sensitivity of the model outputs to variation of the model parameters.

In general, this presents information on the accuracy with which certain parameters need to be known in order to obtain reliable results. In this paper the simple approach will be used in the analysis of parameter sensitivity and some results obtained in simulations with parameters varied in a certain range will be discussed. In Figures 12–16 the results of parameter variations on the parameters $q_{s,\max}$, m_s, K_s, Y_{xs}, and Y_{ps} are shown. In each of these graphs the variation of the model outputs, penicillin mass, and mycelial mass in response to the variation of one of the parameters are shown. Glucose feed rate scheme B has been used.

In Figure 12 it is shown that the value of $q_{s,\max}$ has a significant effect on the growth curve, especially in the early hours of the fermentation. The final penicillin production, however, is not affected very much. This shows that, at the same total glucose feed,

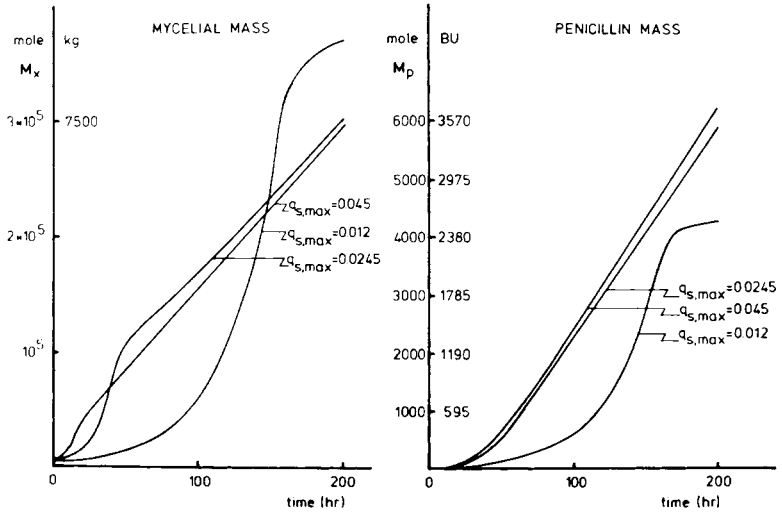

Fig. 12. Effect of variation in $q_{s,\max}$ on mycelium and penicillin production (feed rate scheme B).

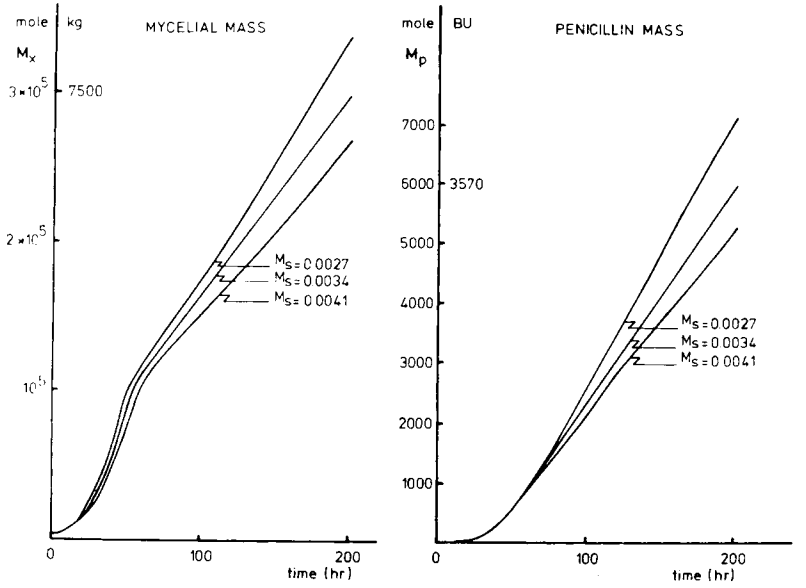

Fig. 13. Effect of variation of m_s on mycelium and penicillin production (feed rate scheme B).

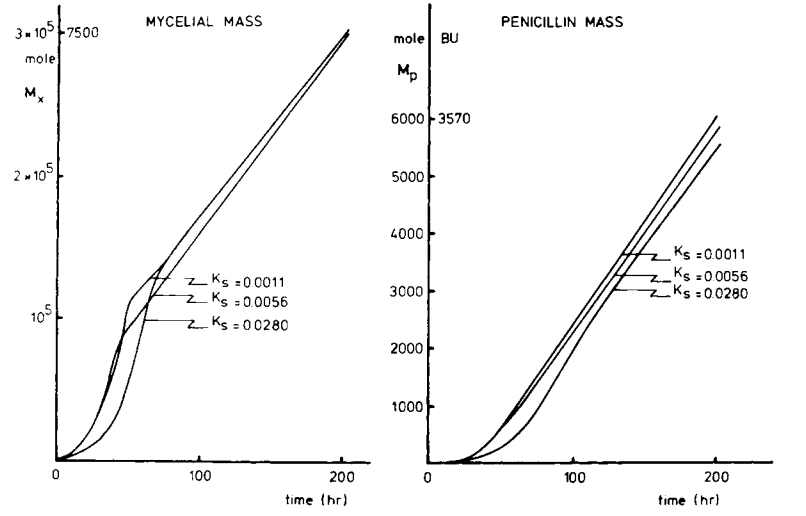

Fig. 14. Effect of variation in K_s on mycelium and penicillin production (feed rate scheme B).

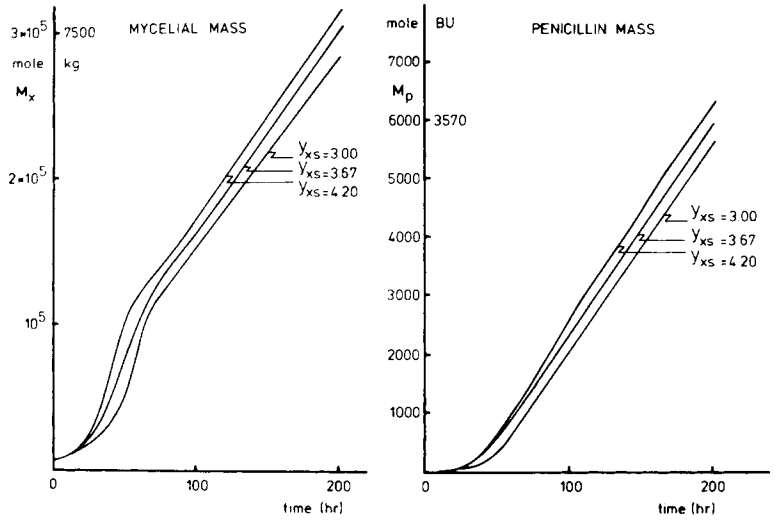

Fig. 15. Effect of variation in Y_{xs} on mycelium and penicillin production (feed rate scheme B).

comparable penicillin yields can be obtained using different growth curves.

In Figure 13 the dramatic effect of the maintenance factor is shown.

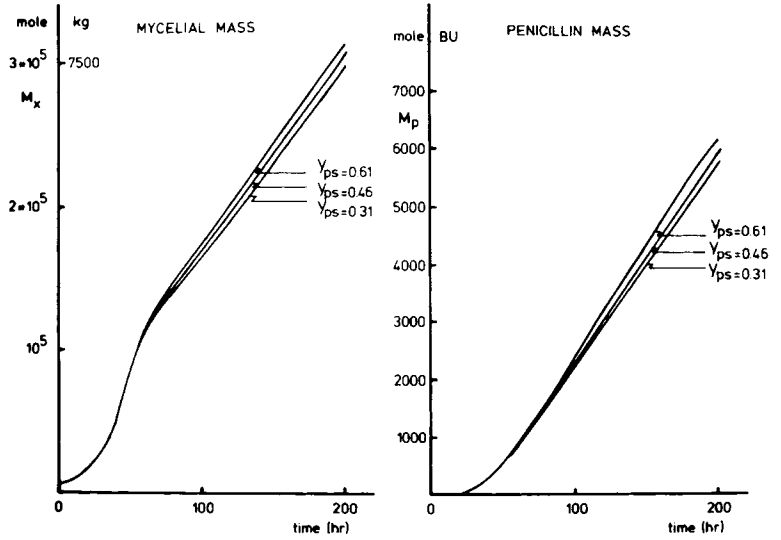

Fig. 16. The effect of variation in Y_{ps} on mycelium and penicillin production (feed rate scheme B).

As could be expected, lowering the maintenance factor results in an increased penicillin yield and a higher mycelial dry matter; an increased maintenance factor has the adverse effect.

Figures 14–16 show that variation of K_s, Y_{xs}, and Y_{ps} results in only minor effects as far as the mycelial dry matter and penicillin profiles are concerned.

CONCLUSIONS

In this paper the concept of elementary balance has been combined with kinetic equations (based on a literature survey) for the consumption of substrate, the production and hydrolysis of penicillin, and a linear equation for the rate of the substrate taken up by the organism. In this way a simple unstructured model for the fed-batch production of penicillin was constructed. The most important results of simulation studies with the model are:

An important feature of the fed-batch penicillin fermentation is the dilution of the broth by the various feeds during fermentation. Neglect of this effect and the effect of first-order penicillin hydrolysis leads to an underestimation of the organisms productivity in the last part of the fermentation and may very well have contributed to the modeling of fermentation in terms of a negative age dependence of productivity.

The assumption of a growth-coupled penicillin productivity, as was assumed in the present model, leads to simulation results in which growth and product formation are apparently separated. Furthermore, an apparent lag is observed in the penicillin concentration curve. This means that the assumption of a lag or the trophophase–idiophase are not necessary to explain the observations on the penicillin production process.

About 10–20% of the penicillin produced is lost through hydrolysis during the course of the fermentation.

In a penicillin fermentation with glucose as sole carbon and energy source RQ exceeds 1.

The N demand for penicillin production accounts for 20% of the total N demand during fermentation; the sulfur demand for penicillin production accounts for 80% of the total S demand.

A very significant feature of microbial growth as far as the examined process for penicillin production is concerned is the maintenance concept; about 70% of the sugar supplied is used for maintenance processes.

In obtaining high penicillin yields the glucose feed scheme is of crucial importance, it was shown that a scheme using an increasing feed rate as a function of time is superior as compared with a constant or a decreasing feed rate.

It is the authors' feeling that the present example has shown that the construction of a simple unstructured model based on some notion of the relevant kinetic equations in combination with the very important concept of elementary balance is of great help in the understanding of the factors relevant in the optimization and control of fermentation processes.

APPENDIX

Listing of the CSMP-Program for Simulation of the Model of the Penicillin Fermentation

```
* CSMP73 INPUT LIST * 27.002.000
0001    PARAM     QS=0.0245,KS=0.0056,YX=3.67,M=3.4E-3, . . .
0002              YP=0.46,QP=3.3E-4,BP=0.002
0003    INCON     MSO=5500,MX0=4000.,GO=1.E5,MPO=0.,MP00=0.
0004    CONST     A=5.,B=500.
0005    INITIAL
0006              MP=MP0
0007              MU=0.1
0008              CS=MSO/GO
0009              MX=MXO
0010    DYNAMIC
0011              RS=-QS*CS*MX/(KS+CS)
0012              RPO=0.002*MP
0013    NOSORT
0014              IF(MU.GT.0.01)  RP=QP*MX-RPO
0015              IF(MU.LE.0.01)  RP=QP*MX*MU/0.01 -RPO
0016    SORT
0017              RX=-RS*YX-M*YX*MX-YX*(RP+RPO)/YP
0018              MU=RX/MX
0019              RO=(6./YX-1.044)*RX+6.*M*MX+ . . .
0020                  (6./YP-9.5)*(RP+RPO)
0021              RC=(6/YX-1.)*RX+6.*M*MX+(6./YP-8.)*(RP+RPO)
0022              RQ=RC/RO
0023              FNX=.16*RX/RN
0024              FNP=1.-FNX
0025              FSX=0.0046*RX/RSU
0026              FSP=1.-FSX
0027              FGX=-RX/(YX*RS)
0028              FGM=-M*MX/RS
0029              FGP=-(RP+RPO)/(YP*RS)
0030              RN=0.16*RX+2.*(RP+RPO)
0031              RSU=0.0046*RX+RP+RPO
0032              E=-RP/RS
0033              FIS=A*TIME+B
```

```
0034                MSDOT=RS+FIS
0035                MS=INTGRL(MSO,MSDOT)
0036                CS=MS/G
0037                MXDOT=RX
0038                MX=INTGRL(MXO,MXDOT)
0039                CX=MX/G
0040                MPDOT=RP
0041                MP=INTGRL(MPO,MPDOT)
0042                CP=MP/G
0043                MPODOT=RPO
0044                MPO=INTGRL(MPO0,MPODOT)
0045                CPO=MPO/G
0046                GDOT=-0.016*G*50.E-3+FIS/2.78-0.044*RC . . .
0047                     +RN/14.71+RSU/2.55+(RP+RPO)/1.44+0.032*RO
0048                G=INTGRL(G0,GDOT)
0049                CUMS=INTGRL(1.0,RS)
0050                ECUM=-MP/CUMS
0051  METHOD        SIMP
0052  TIMER         FINTIM=200.,DELT=0.01,OUTDEL=20.
0053  PRTPLT        CX,CP,CS,CPO,MU,FNX,FNP,FSX,FSP,FGX,FGM,FGP
0054  ENDJOB
```

Nomenclature

C_{po}	penicilloic acid concentration in broth (mol/kg)
C_s	substrate concentration in broth (mol/kg)
C_p	product concentration in broth (mol/kg)
C_x	biomass concentration in broth (mol/kg)
ECUM	cumulative efficiency of penicillin production (mol/mol)
G	total broth weight (kg)
K_s	Michaelis constant for sugar uptake (mol/kg)
m_s	maintenance constant (mol/mol DM hr)
M_p	total penicillin mass in the fermentor (mol)
M_{po}	total penicilloic acid mass in the fermentor (mol)
M_s	total glucose mass in the fermentor (mol)
M_x	total mycelial mass in the fermentor (mol)
q_p	specific penicillin synthesis rate (mol/mol DM hr)
$q_{p,\max}$	maximum specific penicillin synthesis rate (mol/mol DM hr)
$q_{s,\max}$	maximum specific sugar uptake rate (mol/mol DM hr)
r_c	net rate of CO_2 conversion (mol/hr)
r_H	net rate of heat production (kcal/hr)
r_n	net rate of nitrogen source conversion (mol/hr)
r_o	net rate of oxygen conversion (mol/hr)
r_p	net rate of product conversion (mol/hr)
r_{pa}	net rate of phenylacetic acid conversion (mol/hr)
r_{ph}	net rate of conversion of phosphorus source (mol/hr)
r_{po}	net rate of conversion of penicilloic acid (mol/hr)
r_s	net rate of sugar conversion (mol/hr)
r_{su}	net rate of conversion of sulfate source (mol/hr)
r_w	net rate of conversion of water (mol/hr)
r_x	net rate of biomass conversion (mol/DM hr)

Y_{po}	true yield for product on oxygen (mol/mol)
Y_{ps}	true yield for product on substrate (mol/mol)
Y_{xo}	true yield for biomass on oxygen (mol/mol)
Y_{xs}	true yield for biomass on substrate (mol/mol)
μ	specific growth rate (hr^{-1})
μ_{cr}	critical specific growth rate for penicillin production (hr^{-1})
Φ_s	flow rate of substrate to the system (mol/hr)

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