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Calculation errors of time-varying flux control coefficients obtained from elasticity coefficients by means of summation and connectivity theorems in Metabolic Control Analysis

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Abstract

This paper investigates the accuracy of a matrix method proposed by other researchers to calculate time-varying flux control coefficients (dynamic FCCs) from elasticity coefficients by means of summation and connectivity theorems in the framework of metabolic control analysis. A mathematical model for the fed-batch penicillin V fermentation process is used as an example for discussion. Calculated results reveal that this method produces significant calculation errors because the theorems are essentially valid only in steady state, although it may provide rough time-transient behaviors of FCCs. Strictly, therefore, dynamic FCCs should be directly calculated from the differential equations for metabolite concentrations and sensitivities.

Keywords: Dynamic sensitivity; Time-varying flux control coefficient; Elasticity coefficient; Fed-batch fermentation process; penicillin V

1. Introduction

Sensitivity analysis is a powerful means to efficiently characterize biochemical systems [1-10]. If a given system has a steady state, it can be characterized using steady-state sensitivities. Biochemical Systems Theory (BST) provides an efficient algorithm to calculate these values [4-10]. On the other hand, if the system has no steady state or if it is meaningful to characterize the system in a transient state even if the system has a steady state, it is necessary to calculate time-varying sensitivities, *i.e.*, dynamic sensitivities. This calculation must be carried out by simultaneously solving the differential equations for metabolite concentrations and sensitivities [11]. The dynamic sensitivities have various applications such as sensitivity analysis [1-4], simplification of complicated network reactions [12], detection of chaotic behaviors [13], and identification of metabolic bottlenecks [14].

It is known that the calculation method of time-varying flux control coefficients, *i.e.*, dynamic FCCs, in Metabolic Control Analysis (MCA) is an alternative way to calculate the dynamic sensitivities. This method gives the dynamic FCCs and CCCs (concentration control coefficients) from time-varying fluxes (or elasticities) by means of summation and connectivity theorems [15-18]. However, it is questionable to what extent these dynamic FCCs are reliable, because FCCs are originally steady-state properties and the summation and connectivity theorems are valid only at steady state.

In the present work, therefore, an attempt is made to elucidate an applicable limitation of

the calculation method of dynamic FCCs. To achieve this objective, the behaviors of dynamic FCCs are discussed in the fed-batch penicillin V fermentation process [19-21].

2. Theory

2.1. Model system

Figure 1 shows the reaction pathway for the penicillin V production in *Penicillium chrysogenum* [19]. The first step is the condensation of the three precursors, L-α-aminoadipic acid (α-AAA), L-cysteine, and L-valine, to form δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine (LLD-ACV), catalyzed by the enzyme δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine synthetase (ACVS). The second step is the oxidative ring closure of LLD-ACV to form isopenicillin N (IPN) catalyzed by isopenicillin N synthetase (IPNS). The third step is the conversion of isopenicillin N into Penicillin V (PenV) catalyzed by acyl-CoA:isopenicillin N acyltransferase. There are two pathways to form PenV. One is the conversion of isopenicillin N into PenV by one step. The other is the conversion of isopenicillin N via 6-aminopenicillanic acid (6-APA) into PenV. Acyl-CoA:isopenicillin N acyltransferase has two binding sites and is responsible for the reactions catalyzed by the four enzymes acetyl-CoA:isopenicillin N acyltransferase (IAT), isopenicillin N amidohydrolase (IAH), acyl-CoA:6-APA acyltransferase (AAT), and penicillin amidase (PA), which are thus collectively described as acyltransferase (AT). The products LLD-ACV, IPN, Penicillin V, and 6-APA are excreted outside the cell in proportion

to the magnitudes of their concentrations. A more detailed explanation of the metabolic reactions and pathways can be found in the literature [19].

The metabolite concentrations are represented as $X_1 = [\mathrm{ACV}]$, $X_2 = [\mathrm{IPN}]$, $X_3 = [6\mathrm{APA}]$, $X_4 = [\mathrm{PenV}]$, $X_5 = [\mathrm{ACVout}]$ $X_6 = [\mathrm{IPNout}]$, $X_7 = [8\mathrm{HPA}]$, and $X_8 = [\mathrm{OPC}]$, the three enzyme concentrations as $X_9 = X_{\mathrm{ACVS}}$, $X_{10} = X_{\mathrm{IPNS}}$, and $X_{11} = X_{\mathrm{AT}}$, and the liquid volume in the fermenter V, the cell concentrations x, and the glucose concentration s as $X_{12} = V$, $X_{13} = x$, and $X_{14} = s$, respectively. The rate constants, k_1-k_5, and k_8, are represented as Y_1–Y_5, and Y_7, respectively, and the catalytic constant, k_6, is as Y_6, and the secretion constants, k_{ACV}, and k_{IPN}, are as Y_8, and Y_9, respectively. Taking differential mass balances with respect to the cell and fermenter in the penicillin V fermentation process leads to the equations (1)-(17) shown in Table 1.

Since there is no description regarding the differential mass-balance equations for reactor operation (Eqs.(12)-(14)) and the kinetic equations for cell growth (Eq.(17)) in the original model [19], we employ the equations given in another, more recent, model [22,23], where optimization of the same process has been discussed. The initial values for other variables are as follows; $X_{1\text{-}8}$=0.0 mM, X_{12}=4.0 L, X_{13}=4.0 g-cell L^{-1}, and X_{14}=20.0 g L^{-1}. It should be noted that the initial value for X_{14} is larger than the value 3.0 g L^{-1} used in the reference [22]. This is because the use of such a small value caused a quick drop in X_{14} to zero during the operation period of 0-5 h where no feed was supplied, resulting in an underflow error. Despite the

partially different calculation condition, it was possible to successfully reproduce the calculated lines given in the reference [22]. The parameter values used for the calculation are listed in Table 2. The feed rate F was changed according to the pattern shown in Figure 2, which is the same as reported elsewhere [19].

The followings are the major features of the mathematical model given by Eqs. (1)-(17).

1) The concentrations of the three precursors α -AAA, L-cysteine, and L-valine, denoted as [AAA], [CYS], and [VAL], respectively, are assumed constant (see the equation for v_1 in Eq. (15)), meaning that ACV is steadily synthesized during the fermentation process. Thus, it should be noted that the ACV concentration is increased even when the glucose concentration is zero.

2) The activities of the three enzymes ACVS, IPNS, and AT are decreased according to zero order kinetics with respect to the time t [22,24] (see Eqs. (9)-(11)).

3) The intracellular metabolites are excreted into the culture media according to the first-order kinetics (see Eq. (16)).

2.2. Definition of dynamic sensitivity and its computation method

Let us consider the differential equations that express the time rates of change in metabolite concentrations:

$$\frac{dX_i}{dt} = f_i(X_1, X_2, \cdots, X_N; Y_1, Y_2, \cdots, Y_M) = f_i(\mathbf{X}, \mathbf{Y}) \tag{18}$$

with the initial conditions:

$$X_i = X_i(0) \qquad (i = 1, \cdots, N) \tag{19}$$

where X_i $(i = 1, \cdots, N)$ are the dependent concentrations, Y_k $(k = 1, \cdots, M)$ are the independent variables, t is the time, N is the number of dependent variables, and M is the number of independent variables. Partial differentiations of Eqs. (18) and (19) with respect to any independent variable Y_k give

$$\frac{dS_{i,k}}{dt} = \sum_{j=1}^{N} \frac{\partial f_i(\mathbf{X}, \mathbf{Y})}{\partial X_j} S_{j,k} + \frac{\partial f_i(\mathbf{X}, \mathbf{Y})}{\partial Y_k} \quad (i = 1, \cdots, N; k = 1, \cdots, M) \tag{20}$$

$$S_{i,k} = S_{i,k}(0) = 0 \tag{21}$$

where $S_{i,k}$ $(i = 1, \cdots, N; k = 1, \cdots, M)$ represent the gains for a dependent variable to a change in any independent variable, given by

$$S_{i,k} = \frac{\partial X_i}{\partial Y_k} \qquad (i = 1, \cdots, N; k = 1, \cdots, M) \tag{22}$$

The logarithmic (or normalized) gains are preferentially used in the evaluation of the dynamic regulatory structure of the system. These sensitivity values are defined as the percentage responses of dependent variables to an infinitesimal percentage change in any independent variable, given by

$$L_{i,k} = L(X_i, Y_k) = \left(\frac{\partial X_i}{\partial Y_k} \right) \frac{Y_k}{X_i} = S_{i,k} \frac{Y_k}{X_i} \quad (i = 1, \cdots, N; k = 1, \cdots, M). \tag{23}$$

By solving Eqs. (18) and (20) simultaneously and then applying the gains computed at each time point to Eq. (23), one gets the time courses of logarithmic gains, *i.e.*, the dynamic logarithmic gains of metabolite concentrations X_i with respect to enzyme activities Y_k. These values correspond to the dynamic CCCs in MCA. The initial values for Eq. (23) are given as

$$L_{i,k}(0)=0 \quad (i=1,\cdots,N;k=1,\cdots,M). \tag{24}$$

The local flux v_k can be generally given by

$$v_k = f_k(X_1, X_2,\cdots, X_N; Y_1, Y_2,\cdots, Y_M) = f_k(\mathbf{X},\mathbf{Y}) \qquad (k=1,2,\cdots, p). \tag{25}$$

Differentiation of Eq. (25) with respect to any independent variable Y_r ($1\le r\le M$) provides the equation for the gain of a local flux to an infinitesimal change in Y_r:

$$S\left(v_k(t), Y_r\right) = \frac{\partial v_k(t)}{\partial Y_r} = \sum_{l=1}^{N} \frac{\partial f_k(\mathbf{X},\mathbf{Y})}{\partial X_l} S\left(X_l(t), Y_r\right) + \frac{\partial f_k(\mathbf{X},\mathbf{Y})}{\partial Y_r} \tag{26}$$

with the initial value:

$$S\left(v_k(0), Y_r\right) = \left.\frac{\partial f_k(\mathbf{X},\mathbf{Y})}{\partial Y_r}\right|_{t=0}. \tag{27}$$

Thus, insertion of the obtained gain into the following equation provides the logarithmic gain of v_k with respect to Y_r.

$$L\left(v_k(t), Y_r\right) = S\left(v_k(t), Y_r\right)\frac{Y_r}{v_k(t)} \tag{28}$$

These values correspond to the dynamic FCCs in MCA.

The computation of the dynamic logarithmic gains was performed by simultaneously solving the differential equations for metabolite concentrations and sensitivity values, by a

combination of the highly-accurate numerical differentiation method [25] and variable-step, variable-order Taylor-series method [26]. Such computation gives accurate dynamic logarithmic gains by setting only the differential equations for metabolite concentrations.

3. Results and discussion

3.1. Time courses of metabolite concentrations

3.1.1. Non-steady state process

We initially used the same parameter values as given in Conejeros and Vassiliadis [22]. These values are fundamentally the same as those in Pissara et al. [19], except the values associated with the fermenter operation; in the latter paper, there is no description about the parameter values and kinetic expressions associated with the fermenter operation. Equations (1)-(14) were solved using these parameter values. However, since our calculated results differed from those in Figs. 2 and 3 in Pissara et al. [19], the kinetic parameters were adjusted; Table 2 lists the kinetic parameter values determined. Figure 3 shows the time courses of dependent variables calculated using the kinetic parameter values. As the calculated results are in good agreement with those of Pissara et al. [19], we can confirm that the kinetic parameter values and kinetic expressions used in the present work can successfully reproduce the calculated results obtained by Pissara et al. [19].

In Fig.3, the PenV concentration X_4 steadily increases despite the substrate concentration being nearly zero after 60 h. This is not due to instantaneous consumption of the substrate in the medium liquid that is continuously supplied to the fermenter, but mainly due to the LLD-ACV concentration continuously increasing as a result of assuming the concentrations of the three precursors, α-AAA, L-cysteine, and L-valine, which are always constant. However, as this contradiction does not affect the results discussed in the present work, calculations were performed without any modification of the mathematical model.

Figure 3 clearly shows that X_1 continuously increases with the fermentation time and never reaches a pseudo steady state. Although it could be possible to assume a pseudo steady state for X_2 in the range of 10–70 h, it is obviously not possible to do so after 70 h.

3.1.2. Steady state process

Since the condensation flux of α-AAA, L-cysteine, and L-valine to LLD-ACV is constant in the present mathematical model, X_1 and X_2 should be almost constant. In Fig.3, however, these concentrations change with time, which can be considered due to the mathematical model including the terms for enzyme deactivations. Equations (1)-(14) were therefore solved on the assumption that the enzymes were not deactivated. The calculated results shown in Fig.4 indicate that X_1 and X_2 is almost unchanged over a long period of time.

3.2. Examination of a method for calculation of FCCs from elasticity coefficients by means of summation and connectivity theorems in MCA

The elasticity coefficient ε_{X_j,v_i} and FCC $C_{Y_j}^{v_i}$ are defined as follows;

$$\varepsilon_{X_j,v_i} = \frac{\partial v_i}{\partial X_j}\frac{X_j}{v_i} \quad (i=1,\ldots; j=1,\ldots) \tag{29}$$

$$C_{Y_j}^{v_i} = \frac{\partial v_i}{\partial Y_j}\frac{Y_j}{v_i} \quad (i=1,\ldots) \tag{30}$$

where X_i expresses the intracellular metabolite concentration, v_i the flux through the pathway i, and Y_i the enzyme activity in the pathway i. The equation for each elasticity coefficient can be derived by differentiation of the relevant kinetic expression with respect to a given dependent variable. As a result, successive substitution of the relevant metabolite concentrations varying with time, into the resulting expression, provides the time courses of elasticity coefficients.

Applications of the summation and connectivity theorems, which are originally valid only at steady state, to the metabolic pathways in Fig.1 give the following equations.

$$C_{Y_1}^{v_1} + C_{Y_2}^{v_1} + C_{Y_8}^{v_1} = 1 \tag{31}$$

$$\varepsilon_{X_1,v_1}C_{Y_1}^{v_1} + \varepsilon_{X_1,v_2}C_{Y_2}^{v_1} + \varepsilon_{X_1,v_9}C_{Y_8}^{v_1} = 0 \tag{32}$$

$$\varepsilon_{X_5,v_1}C_{Y_1}^{v_1} + \varepsilon_{X_5,v_2}C_{Y_2}^{v_1} + \varepsilon_{X_5,v_9}C_{Y_8}^{v_1} = 0 \tag{33}$$

The summation theorem gives that the sum of the FCCs of local fluxes is equal to unity (Eq. (31)), while the connectivity theorem gives that the sum of the products of flux control

coefficients and elasticity coefficients is zero (Eqs. (32) and (33)). It should be noted that only the elasticity coefficients and FCCs for the incoming flux and outgoing flux at the LLD-ACV (X_1) pool are considered here because all other values are theoretically zero. In addition, ε_{X_5,v_1} and ε_{X_5,v_2} in Eq. (33) are zero. The remaining elasticity coefficients can be calculated using the following equations.

$$\varepsilon_{X_1,v_1} = \frac{X_1 K_{\mathrm{ACV}}^{-1}}{1 + X_1 K_{\mathrm{ACV}}^{-1}} \tag{34}$$

$$\varepsilon_{X_1,v_2} = \frac{K_{\mathrm{m}}(1+[\mathrm{GSH}]K_{\mathrm{i}}^{-1})}{X_1 + K_{\mathrm{m}}(1+[\mathrm{GSH}]K_{\mathrm{i}}^{-1})} \tag{35}$$

$$\varepsilon_{X_1,v_9} = \frac{X_1}{X_1 - X_5} \tag{36}$$

$$\varepsilon_{X_5,v_9} = \frac{-X_5}{X_1 - X_5} \tag{37}$$

Equations (31)-(33) are written in matrix form as

$$\begin{pmatrix} 1 & 1 & 1 \\ \varepsilon_{X_1,v_1} & \varepsilon_{X_1,v_2} & \varepsilon_{X_1,v_9} \\ \varepsilon_{X_5,v_1} & \varepsilon_{X_5,v_2} & \varepsilon_{X_5,v_9} \end{pmatrix} \begin{pmatrix} C_{Y_1}^{v_1} \\ C_{Y_2}^{v_1} \\ C_{Y_8}^{v_1} \end{pmatrix} = \begin{pmatrix} 1 \\ 0 \\ 0 \end{pmatrix} \tag{38}.$$

Taking into consideration that ε_{X_5,v_1} and ε_{X_5,v_2} are zero and then solving Eq.(38) with respect to FCCs gives

$$\begin{pmatrix} C_{Y_1}^{v_1} \\ C_{Y_2}^{v_1} \\ C_{Y_8}^{v_1} \end{pmatrix} = \begin{pmatrix} 1 & 1 & 1 \\ \varepsilon_{X_1,v_1} & \varepsilon_{X_1,v_2} & \varepsilon_{X_1,v_9} \\ 0 & 0 & \varepsilon_{X_5,v_9} \end{pmatrix}^{-1} \begin{pmatrix} 1 \\ 0 \\ 0 \end{pmatrix} \tag{39}.$$

FCCs are therefore obtained by calculating the elasticity coefficients from Eqs. (34)-(37) and then applying their values to Eq. (39).

This matrix method was used to calculate the time courses of elasticity coefficients and FCCs for the fed-batch fermentation process for penicillin V production. The results are shown in Fig.5. From comparison of these results with those by Pissara et al. [19], it is clear that the time courses of elasticity coefficients are almost the same but the FCCs are quite different.

The FCCs are percentage responses of fluxes to an infinitesimal percentage change in a given enzyme activity, and their values can be exactly calculated, according to the procedure described in the Theory section, not only at steady state but also in transient state. For comparison, the dynamic logarithmic gains, which have the same meaning as the FCCs, were calculated by the exact method. The results are shown in Fig. 5. It is clear that the dynamic FCCs are remarkably deviated from the dynamic logarithmic gains.

It is important to check if the summation and connectivity theorems are really valid during the entire fermentation period. For this purpose, we calculated the time courses of the values on the left-hand sides in Eqs. (31)-(33), *i.e.*, the sums of FCCs and those of products of FCC and elasticity coefficient. The results are shown in Fig.6. If the pseudo steady state is satisfactory, the two sums should take values close to unity and zero, respectively. Nevertheless, it is evident that both sums are rather different from their respective values, indicating that the summation and connectivity theorems are not valid for the present fed-batch fermentation process. It is thus clear that the significant differences between the

dynamic logarithmic gains and FCCs in Fig.5 are due to the dynamic FCCs being calculated on an invalid assumption of the pseudo steady state.

In the penicillin V fermentation model considered here, Eqs. (3)-(8) may not be valid because the dilution terms are missing. A comparison of the dynamic FCCs calculated using its modified model with the dynamic logarithmic gains is given in Appendix. It is clear that the calculated lines are quite different.

It is also useful to confirm whether the above method for calculation of dynamic FCCs can be used when a pseudo steady state can be validly assumed. Fortunately, Fig.4 suggests that in the absence of deactivation of enzymes, the LLD-ACV and IPNS concentrations are kept almost constant over a long period of time and it is reasonable to assume a pseudo steady state for the present system. The sums of FCCs and those of products of FCC and elasticity coefficient were therefore calculated again on the assumption that no enzymes are deactivated. The results are shown in Fig.7. Obviously the two sums take values close to unity and zero, respectively, during the entire fermentation period, indicating that the matrix method for calculation of dynamic FCCs can be used in this case.

In their research, Pissara et al. [19] stated that *the characteristic time for the turnover of the LLD-ACV pool is 30 min, whereas the characteristic time for the intracellular accumulation of LLD-ACV is more than 100 h, which clearly shows that, at a given time point, the LLD-ACV concentration can be considered "frozen"*. However, as the enzymes are

deactivated in the present model, it becomes difficult to assume a pseudo steady state. This is clear from Fig.3, where the metabolite concentrations remarkably change with time. In the absence of deactivation of enzymes, the ACV and IPN concentrations do not significantly change at different pseudo steady states despite considerable changes in the feed rate *F*. As shown in Fig.8, therefore, the dynamic FCCs do not remarkably change and agree well with the time course of the corresponding dynamic sensitivity. In this case, it is no longer reasonable to dynamically calculate FCCs, because they are almost constant over a long period of time. Rather, they should be calculated directly through the steady-state analysis by BST or MCA on the assumption of steady state.

4. Conclusion

In the calculation of dynamic FCCs from elasticity coefficients, it is important to certainly confirm the presence of pseudo steady state. In the case of the fed-batch fermentation model investigated here, however, it is not always possible to assume pseudo steady state as the enzymes become deactivated. Even when the pseudo steady state can be validly assumed, furthermore, it would not be advantageous to conduct such a calculation because steady-state analysis makes it easier to calculate FCCs.

The calculated results clearly show that the matrix method produces significant calculation errors because the theorems are essentially valid only at steady state, although it

may provide rough time-transient behaviors of FCCs. Strictly, therefore, the dynamic FCCs should be directly calculated by other direct methods [11], such as the direct differential method used in the present work, finite-difference method, or Green's function method.

Appendix

It should be noted that Eqs. (3)-(8) in the penicillin V fermentation model lack a dilution term that characterizes fed-batch operation. In the present work, however, the model has been used without modification. This is because the main objective of the present work is to elucidate the calculation accuracy of the matrix method based on the summation and connectivity theorems in MCA. Nevertheless, it is useful to show whether or not the result is the same when the comparison is made using the mathematical model with modification.

Equations (3)-(8) with their respective dilution term are given as follows.

$$\frac{dX_3}{dt} = \left(v_3 - v_4 - v_6 + v_8\right)\rho_c X_{13} - \frac{X_3 F}{X_{12}} \tag{A1}$$

$$\frac{dX_4}{dt} = \left(v_4 + v_5 - v_8\right)\rho_c X_{13} - \frac{X_4 F}{X_{12}} \tag{A2}$$

$$\frac{dX_5}{dt} = v_9 \rho_c X_{13} - \frac{X_5 F}{X_{12}} \tag{A3}$$

$$\frac{dX_6}{dt} = v_{10} \rho_c X_{13} - \frac{X_6 F}{X_{12}} \tag{A4}$$

$$\frac{dX_7}{dt} = v_6 - \frac{X_7 F}{X_{12}} \tag{A5}$$

$$\frac{dX_8}{dt} = v_7 \rho_c X_{13} - \frac{X_8 F}{X_{12}} \tag{A6}$$

Several parameters were re-determined so that the calculated values were fitted to experimental data, and they are given in Table 2 (see the values put in parentheses). Figure 9 shows comparisons of the time courses of metabolite concentrations for the current and

modified model. Interestingly, the calculated lines by the modified model are in better agreement with experimental data than those by the current model. As clear from Fig. 10, however, the dynamic FCCs calculated by the matrix method are quite different from the dynamic logarithmic gains, like in the current model.

Nomenclature

$C_{Y_j}^{v_i}$ flux control coefficient defined by Eq. (30)

F = feed flow rate (L h^{-1})

K_{AAA} = saturation constant of ACVS for L-α-aminoadipate (mM)

K_{ACV} = inhibition constant of ACVS for ACV (mM)

K_{CYS} = saturation constant of ACVS for L-cysteine (mM)

K_i = inhibition constant of IPNS for glutathione (mM)

K_m = saturation constant of IPNS for ACV (mM)

$K_{m,6APA\text{-}Poa}$ = saturation constant of AAT for 6-APA with phenoxyacetyl CoA (mM)

$K_{m,IPN}$ = saturation constant of IAH for IPN (mM)

$K_{m,\ IPN\text{-}Poa}$ = saturation constant of IAT for IPN with phenoxyacetyl CoA (mM)

$K_{m,PenV}$ = saturation constant of PA for penicillin V (mM)

$K_{m,Poa}$ = saturation constant of AAT and IAT for phenoxyacetyl CoA (mM)

K_s = saturation constant for the specific growth rate with substrate (g L^{-1})

K_{sm} = saturation constant for the maintenance coefficient expression (g L^{-1})

K_{VAL} = saturation constant of ACVS for L-valine (mM)

k_{ACV} = secretion constant of intracellular ACV (h^{-1})

k_{IPN} = secretion constant of intracellular IPN (h^{-1})

k_1 = rate constant for the reaction 1 catalyzed by ACVS (h^{-1})

k_2 = rate constant for the reaction 2 catalyzed by IPNS (h^{-1})

k_3 = rate constant for the reaction 3 catalyzed by IAH (h^{-1})

k_4 = rate constant for the reaction 4 catalyzed by AAT (h^{-1})

k_5 = rate constant for the reaction 5 catalyzed by IAT (h^{-1})

k_6 = rate constant for the reaction 6, carboxylation of 6-APA into 8-HPA in extracellular medium (h^{-1})

k_7 = rate constant for the reaction 7 catalyzed by PA (h^{-1})

L_{ik} = logarithmic gain or normalized sensitivity for a response of a metabolite concentration with respect to an infinitesimal change in an enzyme activity at time *t*

$L\left(v_k(t), Y_r\right)$ = logarithmic gain or normalized sensitivity for a response of a local flux with respect to an infinitesimal change in an enzyme activity at time *t*

$L(X_i(t), Y_k)$ = logarithmic gain or normalized sensitivity for a response of a metabolite concentration with respect to an infinitesimal change in an enzyme activity at time *t*

M = number of independent variables

m = maintenance coefficient (g g^{-1} h^{-1})

m_M = maximum maintenance coefficient (g g^{-1} h^{-1})

N = number of dependent variables

P_{ACVS} = linear decay constant for ACVS activity (mM^{-1} h^{-1})

P_{AT} = linear decay constant for acyltransferase activity (mM^{-1} h^{-1})

P_{IPNS} = linear decay constant for IPNS activity (mM^{-1} h^{-1})

S_F = substrate concentration in the feed stream (g L^{-1})

s = substrate, extracellular glucose concentration (g L^{-1})

S_{ik} = gain or local sensitivity for a response of a metabolite concentration with respect to an infinitesimal change in an enzyme activity at time *t* (mM) or (mM h)

$S\left(v_k(t), Y_r\right)$ = gain or local sensitivity for a response of a local flux with respect to an infinitesimal change in an enzyme activity at time *t* (mM)

$S(X_i(t), Y_k)$ = gain or local sensitivity for a response of a metabolite concentration with

respect to an infinitesimal change in an enzyme activity at time t (mM h)

t = time (h)

V = volume (L)

v_i = local flux of the ith pathway (mM h^{-1})

X_{ACVS} = ACVS concentration (mM)

X_{AT} = AT concentration (mM)

X_F = concentration of a desired metabolite (mM)

X_{IPNS} = IPNS concentration (mM)

X_1 = ACV concentration (mM)

X_2 = IPN concentration (mM)

X_3 = 6APA concentration (mM)

X_4 = PenV concentration (mM)

X_5 = extracellular concentration of ACV (mM)

X_6 = extracellular concentration of IPN (mM)

X_7 = 8HPA concentration (mM)

X_8 = OPC concentration (mM)

X_9 = ACVS concentration (mM)

X_{10} = IPNS concentration (mM)

X_{11} = AT concentration (mM)

X_{12} = volume (L)

X_{13} = cell concentration (g L^{-1})

X_{14} = extracellular glucose concentration (g L^{-1})

x = cell concentration (g L^{-1})

$Y_{X/S}$ = growth yield (g-cell g^{-1})

$Y^*_{X/S}$ = true growth yield (g-cell g^{-1})

Y_1 = rate constant for the reaction 1 catalyzed by ACVS (h^{-1})

Y_2 = rate constant for the reaction 2 catalyzed by IPNS (h^{-1})

Y_3 = rate constant for the reaction 3 catalyzed by IAH (h^{-1})

Y_4 = rate constant for the reaction 4 catalyzed by AAT (h^{-1})

Y_5 = rate constant for the reaction 5 catalyzed by IAT (h^{-1})

Y_6 = rate constant for the reaction 6, carboxylation of 6-APA into 8-HPA in extracellular medium (h^{-1})

Y_7 = rate constant for the reaction 7 catalyzed by PA (h^{-1})

Y_8 = secretion constant of intracellular ACV (h^{-1})

Y_9 = secretion constant of intracellular IPN (h^{-1})

Greek Symbol

ε_{X_j, v_i} elasticity coefficient defined by Eq. (29)

μ = specific growth rate (h^{-1})

μ_M = maximum specific growth rate (h^{-1})

ρ_c = cell specific volume (mL g^{-1}-cell)

Abbreviations

AAA = α-aminoadipic acid

ACV = L-α-aminoadipyl-L-cysteinyl-d-valine

ACV_{out} = extracellular L-α-aminoadipyl-L-cysteinyl-D-valine

ACVS = L-α-aminoadipyl-L-cysteinyl-D-valine synthetase

AT = acyltransferase

CYS = L-cysteine

GSH = glutathione

HPA = 8-hydroxypenillic acid

IAH = isopenicillin N aminohydrolase

IAT = acetyl CaA to isopenicillin N acyltransferase

IPN = isopenicillin N

IPN_{out} = extracellular isopenicillin N

IPNS = isopenicillin N synthetase

O_2 = dissolved oxygen

OPC = 6-oxopiperidine-2-carboxylic acid

PA = penicillin amidase

PenV = penicillin V

PoaCoa = phenoxyacetyl CoA

VAL = L-valine

6APA = 6-aminopenicillanic acid

8HPA = 8-hydroxypenillic acid

[ ] = metabolite concentration (mM)

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Legend

Fig.1. Metabolic pathway of penicillin V fermentation.

Fig.2. Pattern of a change in feed speed during penicillin V fermentation period.

Fig.3. Time courses of metabolite concentrations during penicillin V fermentation period with enzyme deactivation.

Fig.4. Time courses of metabolite concentrations during penicillin V fermentation period without enzyme deactivation.

Fig.5. Time courses of elasticity coefficients (left) and those of FCCs and logarithmic gains (right) during penicillin V fermentation with enzyme deactivation.

Fig.6. Time courses of the sums of FCCs on the left-hand side of Eq.(31) (left figure) and of products of FCC and elasticity coefficient on the left-hand side of Eq.(32) (right figure) in summation and connectivity theorems during penicillin V fermentation with enzyme deactivation.

Fig.7. Time courses of the sums of FCCs on the left-hand side of Eq.(31) (left figure) and of products of FCC and elasticity coefficient on the left-hand side of Eq.(32) (right figure) in summation and connectivity theorems during penicillin V fermentation without enzyme deactivation.

Fig.8. Time courses of elasticity coefficients (left) and those of flux control coefficients and logarithmic gains (right) during penicillin V fermentation without enzyme deactivation.

Fig.9. Comparisons of time courses of metabolite concentrations for current and modified models during penicillin V production in fed-batch cultivation with deactivations of enzymes

Fig.10. Time courses of elasticity coefficients (left) and those of FCCs and logarithmic gains (right) during penicillin V fermentation. The calculations were conducted using a modified model with enzyme deactivation

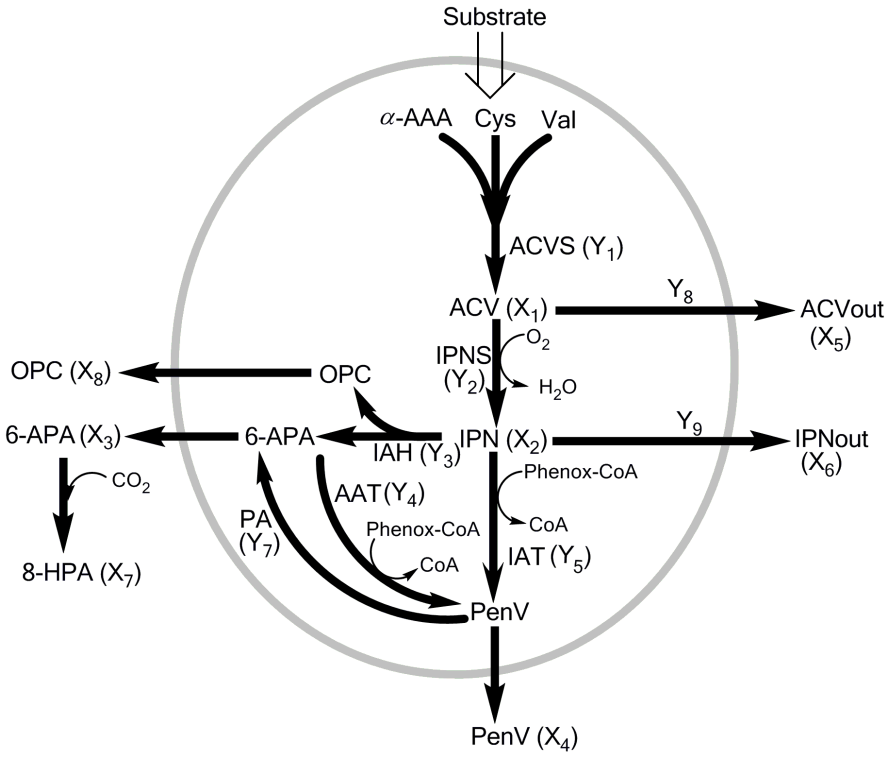


Fig.1. Metabolic pathway of penicillin V fermentation.

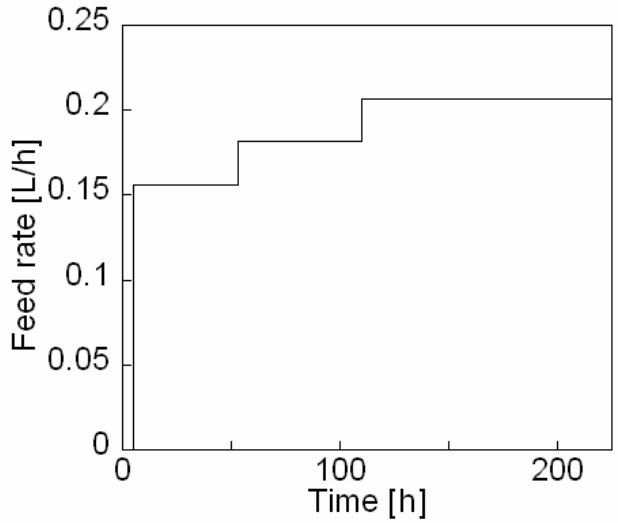


Fig.2. Pattern of a change in feed speed during penicillin V fermentation period.

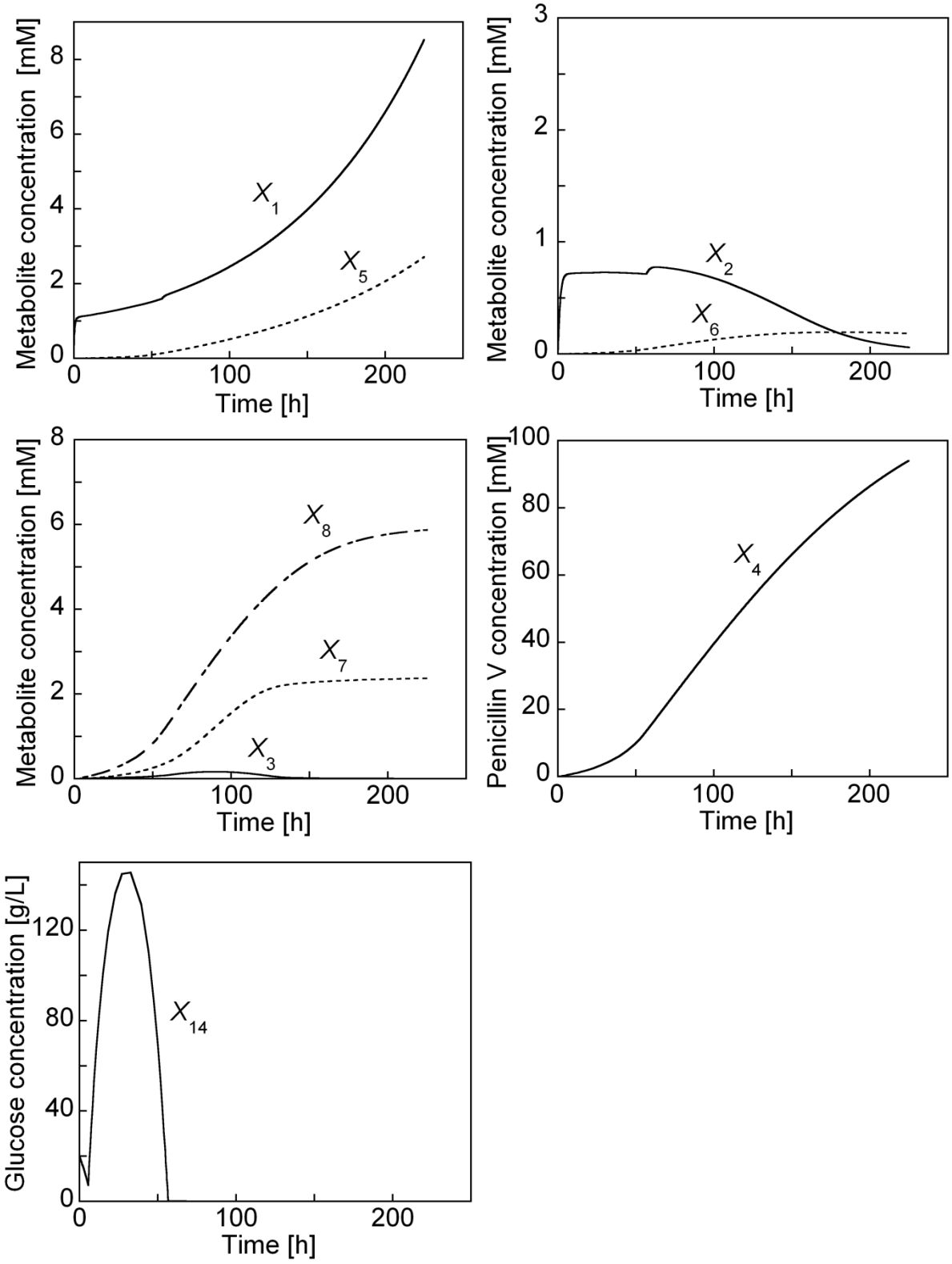


Fig.3. Time courses of metabolite concentrations during penicillin V production in fed-batch cultivation with deactivations of enzymes.

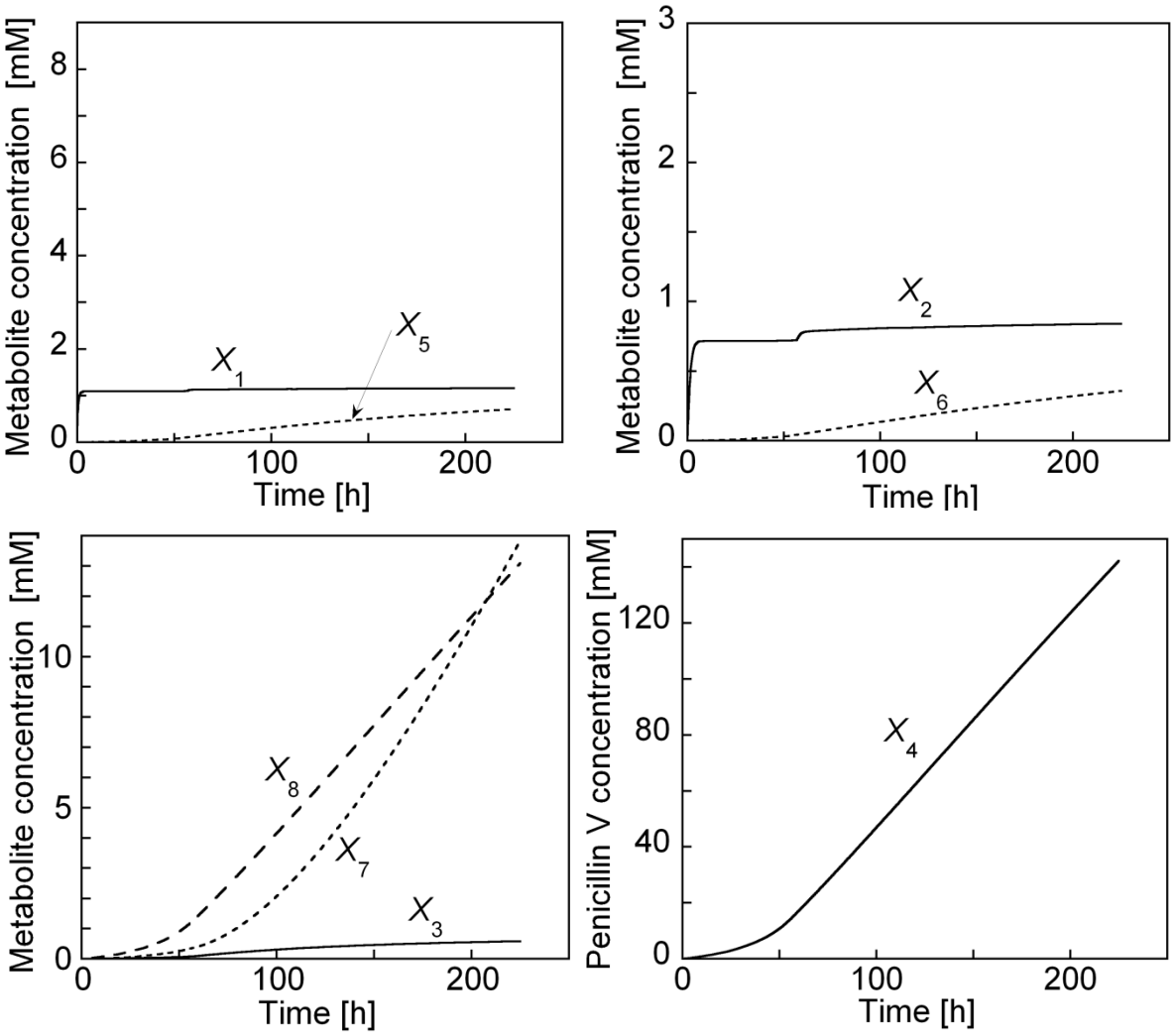


Fig.4. Time courses of metabolite concentrations during penicillin V production in fed-batch cultivation without deactivations of enzymes.

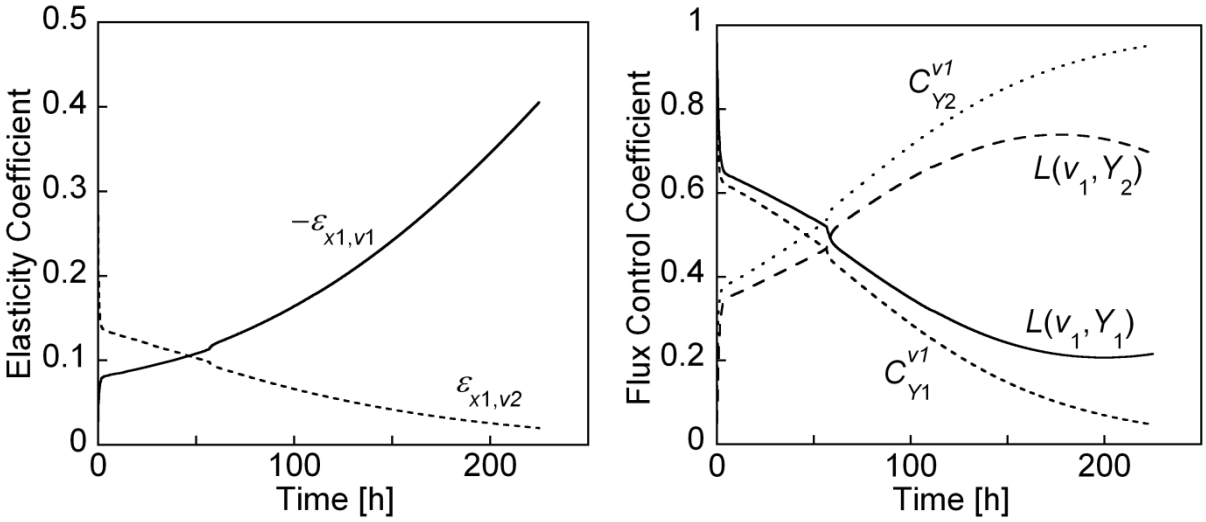
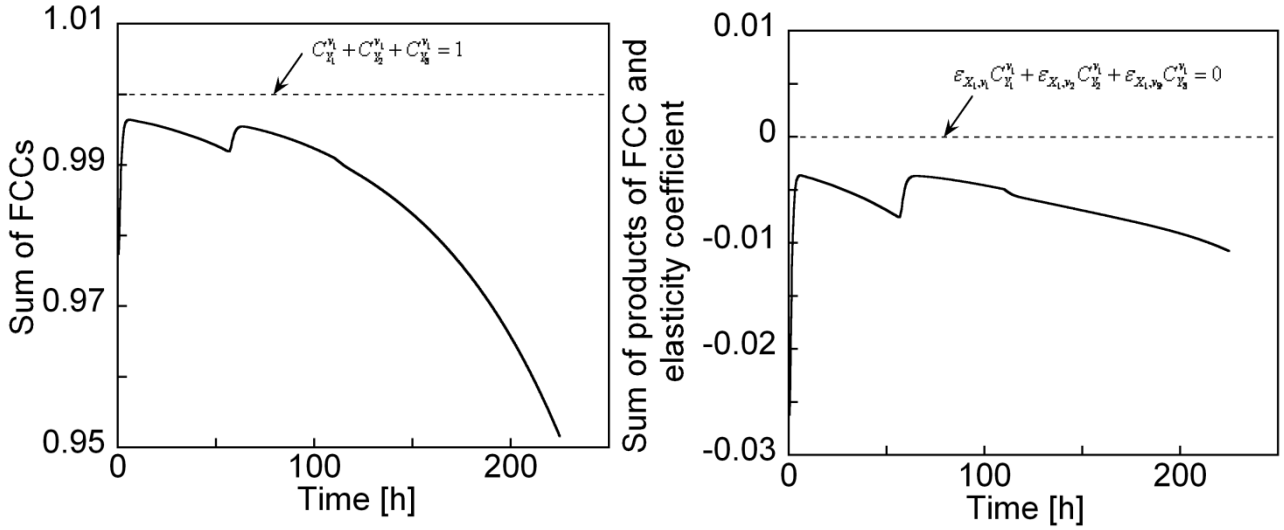


Fig.5. Time courses of elasticity coefficients (left) and those of FCCs and logarithmic gains (right) during penicillin V fermentation with enzyme deactivation.



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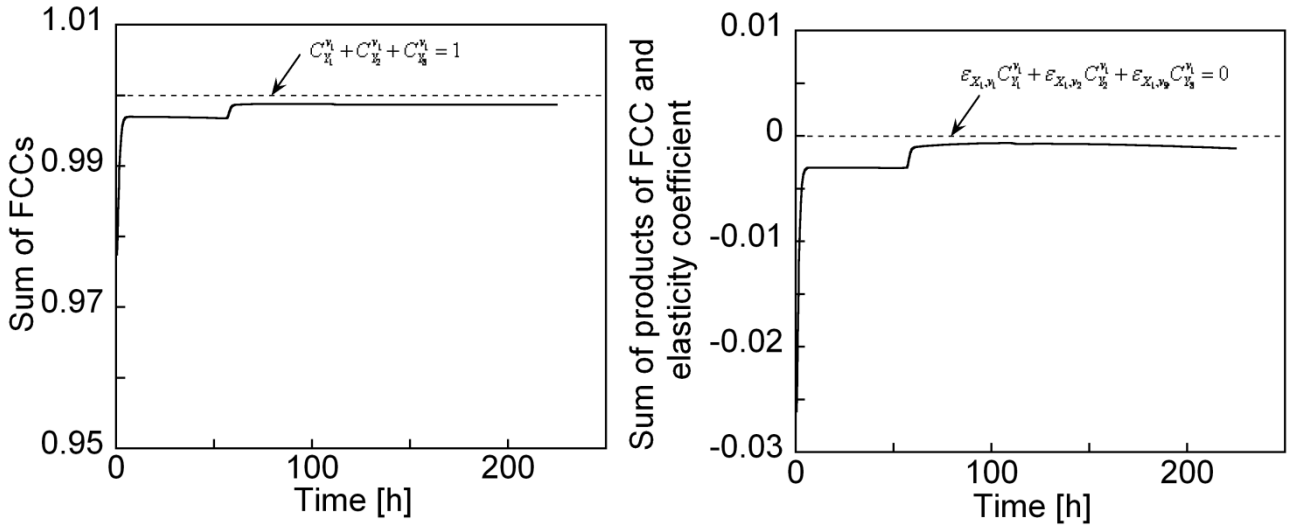

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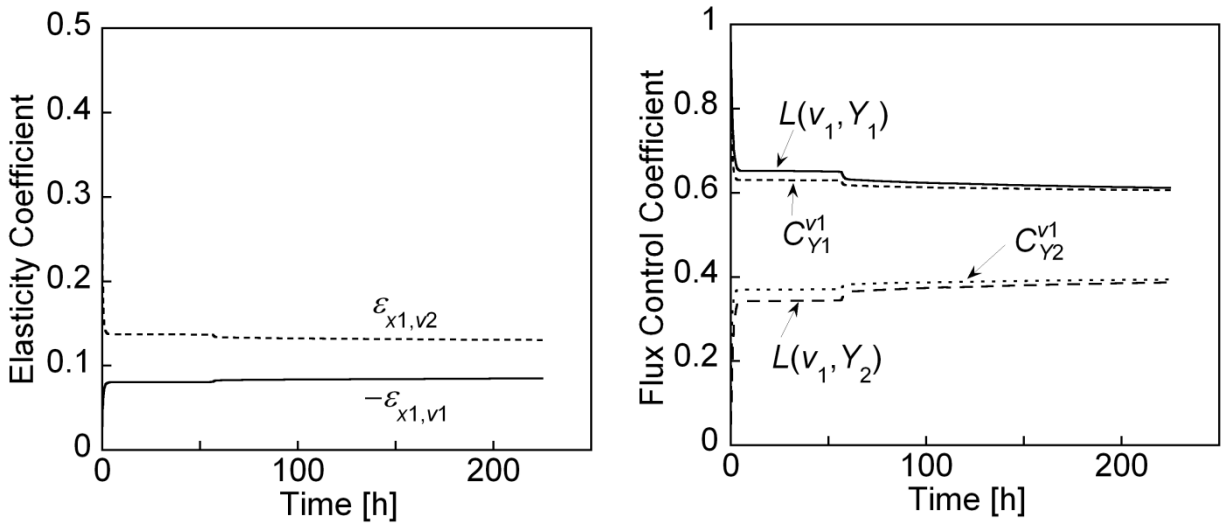

Fig.8. Time courses of elasticity coefficients (left) and those of flux control coefficients and logarithmic gains (right) during penicillin V fermentation without enzyme deactivation.

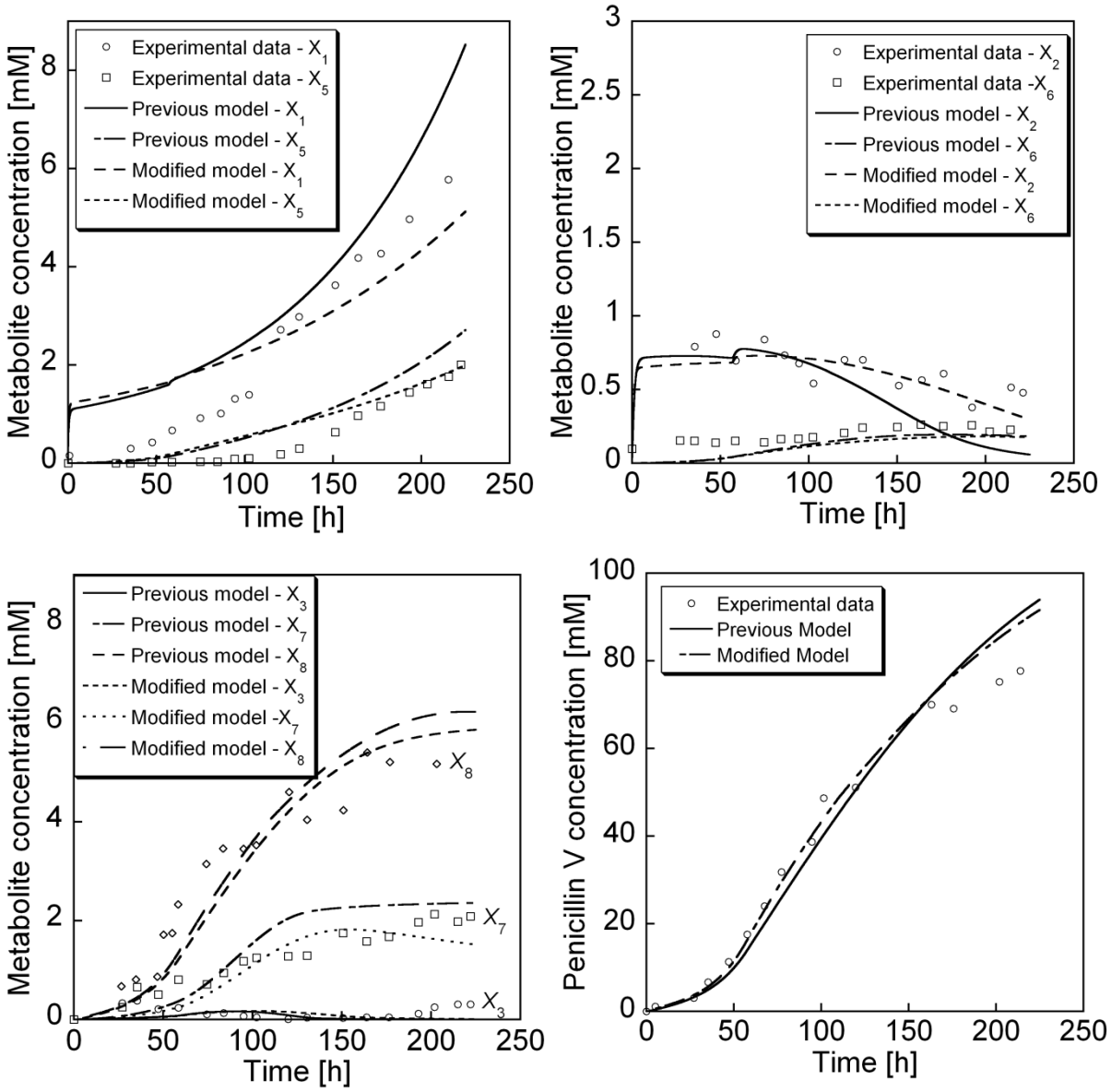

Fig.9. Comparisons of time courses of metabolite concentrations for current and modified models during penicillin V production in fed-batch cultivation with deactivations of enzymes

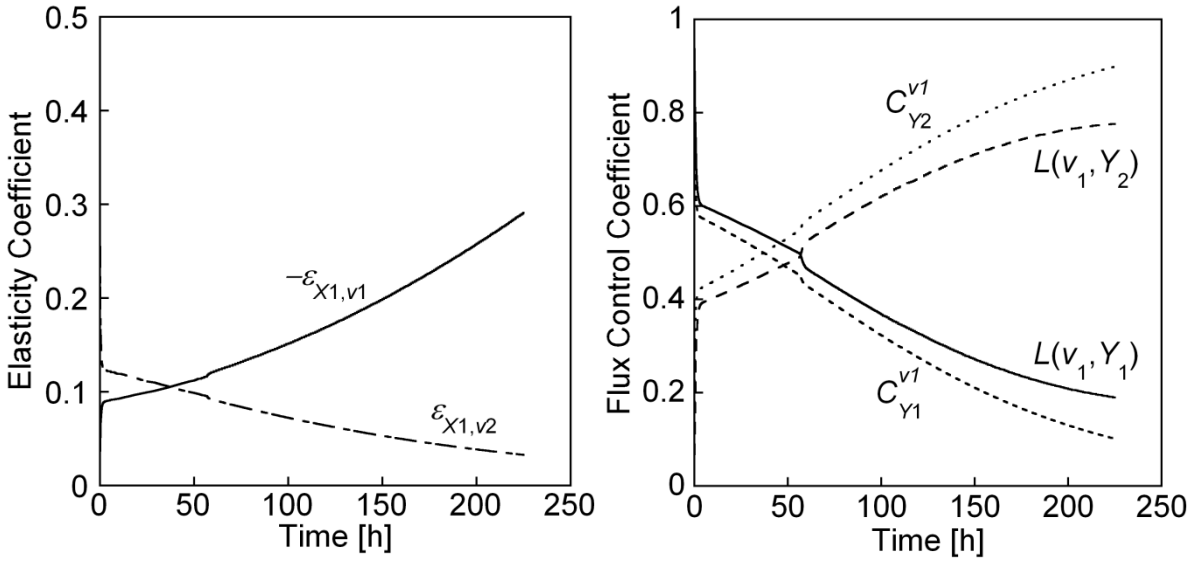

Fig.10. Time courses of elasticity coefficients (left) and those of FCCs and logarithmic gains (right) during penicillin V fermentation. The calculations were conducted using a modified model with enzyme deactivation

Table 1

Model description

Mass-balance equations

$$\frac{dX_1}{dt} = \nu_1 - \nu_2 - \nu_9 - \nu_{12}X_1 \quad (1) \qquad \frac{dX_2}{dt} = \nu_2 - \nu_3 - \nu_5 - \nu_{10} - \nu_{12}X_2 \quad (2)$$

$$\frac{dX_3}{dt} = \left(\nu_3 - \nu_4 - \nu_6 + \nu_8\right)\rho_c X_{13} \quad (3) \qquad \frac{dX_4}{dt} = \left(\nu_4 + \nu_5 - \nu_8\right)\rho_c X_{13} \quad (4)$$

$$\frac{dX_5}{dt} = \nu_9\rho_c X_{13} \quad (5) \qquad \frac{dX_6}{dt} = \nu_{10}\rho_c X_{13} \quad (6)$$

$$\frac{dX_7}{dt} = \nu_6 \quad (7) \qquad \frac{dX_8}{dt} = \nu_7\rho_c X_{13} \quad (8)$$

$$\frac{dX_9}{dt} = -\frac{1}{P_{ACVS}} \quad (9) \qquad \frac{dX_{10}}{dt} = -\frac{1}{P_{IPNS}} \quad (10)$$

$$\frac{dX_{11}}{dt} = -\frac{1}{P_{AT}} \quad (11) \qquad \frac{dX_{12}}{dt} = F \quad (12)$$

$$\frac{dX_{13}}{dt} = \nu_{12}X_{13} - \frac{X_{13}F}{X_{12}} \quad (13) \qquad \frac{dX_{14}}{dt} = \frac{F\,s_F}{X_{12}} - \nu_{12}\nu_{13}X_{13} - \frac{X_{14}F}{X_{12}} \quad (14)$$

Kinetic equations

$$\left.\begin{aligned}
&\nu_1 = \frac{Y_1X_9}{\left(1+\frac{K_{AAA}}{[AAA]}+\frac{K_{CYS}}{[CYS]}+\frac{K_{VAL}}{[VAL]}\right)\left(1+\frac{X_1}{K_{ACV}}\right)}, \quad \nu_2 = \frac{Y_2X_1X_{10}[O_2]}{X_1 + K_m\left(1+\frac{[GSH]}{K_i}\right)}\\
&\nu_3 = \frac{Y_3X_2X_{11}}{X_2 + K_{m,IPN}}, \quad \nu_4 = \frac{Y_4X_3X_{11}[PoaCoa]}{X_3[PoaCoa] + K_{m,6APA\text{-}Poa}[PoaCoa] + K_{m,Poa}X_3}\\
&\nu_5 = \frac{Y_5X_2X_{11}[PoaCoa]}{X_2[PoaCoa] + K_{m,IPN\text{-}Poa}[PoaCoa] + K_{m,Poa}X_2}, \quad \nu_6 = Y_6X_3\\
&\nu_7 = \nu_3, \quad \nu_8 = \frac{Y_7X_4X_{11}}{K_{m,PenV} + X_4}
\end{aligned}\right\} \quad (15)$$

$$\nu_9 = Y_8\left(X_1 - X_5\right), \quad \nu_{10} = Y_9\left(X_2 - X_6\right) \quad (16)$$

$$\nu_{11} = m = \frac{m_M X_{14}}{X_{14} + K_{sm}}, \quad \nu_{12} = \mu = \frac{\mu_M X_{14}}{K_s X_{13} + X_{14}}, \quad \nu_{13} = \frac{1}{Y_{X/S}} = \frac{1}{Y^*_{X/S}} + \frac{\nu_{11}}{\nu_{12}} \quad (17)$$

Table 2

Parameter values in a mathematical model for penicillin V production in fed-batch cultivation

Parameter	Value	Parameter	Value[*)]
$K_{\text{mIPN-Poa}}$	0.023 mM	K_{sm}	0.0001 g L^{-1}
K_{mPoa}	0.006 mM	m_{M}	0.02 g g^{-1}-cell
K_{mIPN}	4.0 mM	K_{s}	0.006 g g^{-1}
$K_{\text{m6APA-Poa}}$	0.0093 mM	μ_{M}	0.06 h^{-1}
K_{i}	8.9 mM	Y^{0}	0.13 g-cell g^{-1}
K_{m}	0.13 mM	ρ_{c}	0.0024l mL g^{-1}-cell
K_{AAA}	0.63 mM	S_{F}	450 g L^{-1}
K_{CYS}	0.12 mM	Y_1	17.6 h^{-1} (17.77)
K_{VAL}	0.3 mM	Y_2	74.5 h^{-1} (72.6)
K_{ACV}	12.5 mM	Y_3	4.03 h^{-1}
K_{PenV}	2.0 mM	Y_4	1.95 h^{-1}
P_{ACVS}	615 $mM^{-1}h$	Y_5	13.55 h^{-1} (13.74)
P_{IPNS}	330 $mM^{-1}h$	Y_6	0.2 h^{-1}
P_{AT}	420 $mM^{-1}h$	Y_7	0.4 h^{-1}

[PoaCoa]	0.006 mM	Y_8	0.05 h^{-1} (0.08)
[GSH]	3.0 mM	Y_9	0.03 h^{-1} (0.04)
[O_2]	0.1157 mM	X_9	1.0 (1.8)
[AAA]	1.2 mM (0.6)	X_{10}	1.0 (1.5)
[CYS]	0.25 mM (0.4)	X_{11}	1.0 (1.5)
[VAL]	2.2 mM (1.2)		

*) The parameter values in parentheses represent the values re-determined for a modified penicillin V fed-batch fermentation model.