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0273-2289/89/2203—0351$02.00

Kinetic Regularities of Methane Production by a Methanogenic Association

Mathematical Modeling

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Received November 4, 1988; Accepted April 5, 1989

ABSTRACT

Kinetic models for methanol and glucose conversion under the effect of a methanogenic association have been suggested. Mathematical models of the processes have been evolved from the kinetic models. The adequacy of the proposed model to the experiment has been verified through a system of differential equations describing the kinetics of the processes. The reverse problem—that of determining the kinetic parameters of methane generation processes from experimental data—has likewise been solved. Sensitivity of the suggested models with respect to all the kinetic parameters has been analyzed. This has allowed to simplify the original model in the case of glucose conversion.

Index Entries: Kinetics; methanogenic association; kinetic scheme; kinetic model; mathematical model; parameters.

INTRODUCTION

In the previous work (*1*), we investigated the kinetics of methanol and glucose conversion in the presence of a model methanogenic association. Thus, we succeeded in accumulating considerable experimental material that awaited its orderly theoretical generalization. With this aim in view, we carried out a mathematical modeling of the investigated methane formation processes. We proceeded from the kinetic schemes suggested

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in previous work (*1*). Besides several assumptions, logically related to the experiment, were made.

1. All the chemical reactions proceed with the participation of the association's enzymes and obey the Michaelis-Menten (2) kinetics or the Monod equation (*3,4*).
2. The concentration of the enzymes and microorganisms are not constant values but obey the microbial kinetics equation (4).

$$dM_i / dt = \mu_i M_i$$

where M_i is the present concentration of the enzyme (microorganism) responsible for the i-reaction, μ_i the specific growth rate for the microorganism M_i, with $\mu_i = \mu^i m S_i / K_S^i \div S_i$, where μ^i_m is the maximal specific growth rate of the i microorganism; S_i, substrate for the i microorganism; and K_S^i, the half-saturation constant.

3. Nearly all the basic chemical reactions are, as a rule, pH-dependent and regulated by corresponding laws of chemical enzymology

RESULTS AND DISCUSSION

Methanol Conversion Modeling

The kinetic model of methanol conversion by a methanogenic association is based on the kinetic scheme described in a previous work (*1*) and has the form

$$4CH_3OH + M_1 \xrightarrow[Y_1 \cdot K_{a1} \cdot K_{b1}]{q_1 \cdot K_1} CH_3COO^- + 2CH_4 + 2H_2O + H^+ + 2M_1 \quad (1)$$

$$CH_3COO^- + H_+ + M_2 \xrightarrow[Y_2 \cdot K_{a2} \cdot K_{b2}]{q_2 \cdot K_2} CH_4 + CO_2 + 2M_2 \quad (2)$$

$$[H^+] = f([CH_3COO^-]) \quad (3)$$

where Y_1 and Y_2 are the cell growth yield, q_1 and q_2 are the maximal specific consumption rates (metabolic coefficients) of methanol and acetate according to reactions (1) and (2), respectively, with $\mu_1 = Y_1 q_1$ and $\mu_2 = Y_2 q_2$ (μ_1 and μ_2, the maximal specific growth rates). K_1 and K_2, the half-saturation constants, K_{a1}, K_{b1}, K_{a2}, and K_{b2}, the dissociation constants for groups of enzymes of M_1 and M_2, respectively. $[H^+]$, is the current concentration of hydrogen ions in solution. Equation (3) reflects the fact that pH in the system is not constant because of reactions (1) and (2). The function *f* has a sufficiently complex form, but it can be easily evolved upon some reasonings and transformations with due account of the rules and laws of physical chemistry.

$$f = A / 2 + \sqrt{A^2 / 4 + B} \text{ (pH 5.0–8.0), where}$$

$$A = K_{Ac}([F] + [Ac] - [K]) + K_F(2[F] - [K]) / ([K] - [F]) \quad (4)$$

$$B = K_{Ac}K_F(2[F] - [K] + [Ac]) / ([K] - [F]) \text{ whereby}$$

K_{Ac} = the dissociation constant for acetic acid, $K_{Ac} = 1.74 \times 10^{-5}$; K_F = the dissociation constant for $H_2PO_4^-$, $K_F = 6.2 \times 10^{-8}$; F = the overall concentration of phosphate in solution; and K = the concentration of K^+ ions that form phosphate salts, $Ac - [CH_3COO^-]$. Complete evolution of the function f is shown in previous works (*5*).

Mathematical description (mathematical model) of the kinetics of the process (1–3) takes on the form

$$d[CH_3OH] / dt = -4V_1 \quad (5)$$

$$d[Ac] / dt = V_1(1-Y_1) - V_2 \quad (6)$$

$$d[CO_2] / dt = V_2(1 - Y_2) \quad (7)$$

$$d[CH_4] / dt = 2V_1(1 - Y_1) + V_2(1 - Y_2) \quad (8)$$

$$dM_1 / dt = Y_1V_1 \quad (9)$$

$$dM_2 / dt = Y_2V_2 \quad (10)$$

$$[H^+] = f([Ac]), \text{ where} \quad (11)$$

$$V_1 = q_1M_1[CH_3OH] / ((K_1 + [CH_3OH])(1 + [H^+] / K_{a1} + K_{b1} / [H^+]) \quad (12)$$

$$V_2 = q_2M_2[Ac] / ((K_2 + [Ac])(1 + [H^+] / K_{a2} + K_{b2} / [H^+])) \quad (13)$$

Thus, the system of Eq. (5–13) obtained from qualitative conclusions about the mechanism of methane production by methanogenic association should give a qualitative description of the dynamics of the process. These equations were numerically solved with respect to the variables. It was shown that resultant reactions describe the characteristic features of the curves for substrate consumption and products formation: the experimental time dependences of the CH_3OH, Ac, and CH_4 concentrations and the retarded (with a lag-period growth of the CO_2 concentration. The system (5–13) was solved by using several sets of numerical values; the range of the changes of these parameters was assessed from independent experiments, literary data, or assumed *a priori*.

Finally, to obtain comprehensive information on the process under investigation (and to make use of this information for prognosis and control), the reverse problem had to be solved: proceeding from the kinetic measurement data, we had to determine the numerical values of unknown parameters describing the kinetic curves within the accuracy of their measurement. The difficulty is in a large number (eight for methanol conversion and 19 for glucose) of sought parameters and in the absence of kinetic data about the growth of microorganisms. We not only had to find a set of parameters describing the experimental curves sufficiently well, but also try to assess the sensitivity of the model to each of the parameters.

Table 1
Numerical Values of Parameters for Methanol Conversion by Methanogenic Association

Parameters	Numerical values	Parameters	Numerical values
q_1, h^{-1}	0.516	pK_{a1}	5.0
q_2, h^{-1}	0.298	pK_{b1}	7.35
K_1, m*M*	77.1	pK_{a2}	5.64
K_2, m*M*	24.2	pK_{b2}	7.14
Y_1, g/g	0.097	y_2, g/g	0.179

To obtain such parameters, we minimized the accuracy criterion (calculation vs experiment) for the entire pool of experimental data. The function

$$F = \sum_{i=1}^{n} \sum_{j=1}^{k} (X_{ij}^{c} - X_{ij}^{e})^2 \tag{14}$$

was used as such criterion, n= the number of experimental point, k=the number of measured concentrations, and X_{ij}^{c}, X_{ij}^{e}=the calculated and experimental value of substance X_j concentration, respectively. Detailed strategy of the search was described in previous works (*6*). Two methods of minimization were used in our computer program: gradient and random search.

Numerical values were calculated for parameters that provide a satisfactory description for measurements at different pH and starting concentrations of methanol. It is necessary to draw attention to the nonuniqueness of a solution of the problems of kinetic parameters reconstitution (*7,8*). Searching for such a number of parameters (this is also related to glucose, we dealt with their inherent correlativeness and with the possibility of some their combinations existing under the similar quality of description. To avoid this, we accurately chose starting approaches of sought parameters and analyzed the calculated ones in accordance with a physical sense. The numerical values of calculated parameters are cited in Table 1. Figure 1 illustrates the quality of the calculated description of the experiment. We would like to note here that acetate concentration passes through a maximum. The calculation pinpoints the temporal position of this maximum and its magnitude. Thus, the description of the experiment via calculation was quite satisfactory. Besides, the abovedescribed procedure allows to calculate growth curves for microorganisms M_1 and M_2 that we could not obtain experimentally.

Let us now proceed to our next question: the sensitivity of the model to some of the parameters. Accordingly, we calculated indeterminacy intervals for all the eight parameters of Table 1, i.e., we searched for such ranges in the change of these parameters in the region of their values, set in Table 1, that did not distort the quality of description (*6–9*). Say, if the

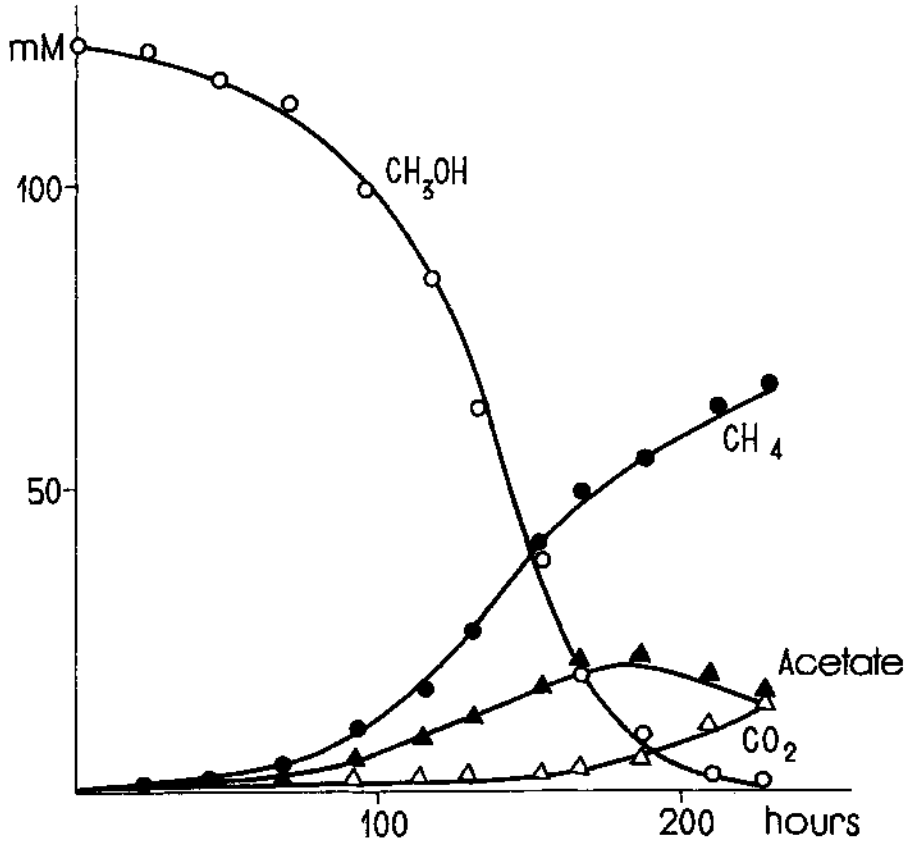

Fig. 1. Comparison of the calculated data with the experimental ones for methanol conversion by methanogenic association, pH 7.0, 124 m*M* CH_3OH. Solid lines, the calculated curves; dots, the experimental data.

required boundary of such an interval proves to be equal to zero, this would mean that, proceeding from the given pool of measurements, a corresponding parameter could not be identified. The model is redundant according to this parameter. From the original complex model, we could obtain a smaller one that could describe the measurements with sufficient accuracy.

A comparison of K_1 and K_2 parameters (Table 1) shows that K_1 makes a larger contribution to the description than does K_2. A change of these parameters from values indicated in Table 1 to zero results in a 30 and 15% difference in the criterion, respectively. It is clear that the description in this case does not lie within the experimental error (10%) and that one should not neglect parameters K_1 and K_2 by simplifying the model.

Similar procedures were performed for assessing the effect of the other parameters listed in Table 1 on the quality of description. We found that all these parameters largely affect the quality of description. More or less identifiable from among the available mass of experimental data, they should not be neglected.

Consequently, the proposed kinetic model (4–12) cannot be simplified with no detriment to the quality of description. Being the simplest one, it provides a satisfactory description of methanol conversion by the given methanogenic association.

A Modeling of Glucose Conversion

The kinetic model—based on the kinetic scheme given in previous work (*1*)—takes this form

$$C_6H_{12}O_6 + H_2O + M_3 \xrightarrow[K_{a3}\cdot K_{b3}]{q_3\cdot K_3\cdot Y_3} C_2H_5OH + 2CO_2 + 2H_2 + CH_3COO^- + H^+ + 2M_3 \quad (15)$$

$$C_2H_5OH + H_2O \xrightarrow[M_3]{k\cdot K_4} CH_3COO^- + H^+ + 2H_2 \quad (16)$$

$$CH_3COO^- + H^+ + M_2 \xrightarrow[Y_2\cdot K_{a2}\cdot K_{b2}]{q_2\cdot K_2} CH_4 + CO_2 + 2M_2 \quad (17)$$

$$CO_2 + 4H_2 + M_4 \xrightarrow[K_{a4}\cdot K_{b4}\cdot Y_4]{q_4\cdot K_5\cdot K_6\cdot K_7} CH_4 + 2H_2O + 2H_2O + 2M_4 \quad (18)$$

$$[H^+] = f([CH_3COO^-]) \quad (19)$$

where Y_2, Y_3, and Y_4 = the cell growth yield; q_2, q_3, and q_4 = the metabolic coefficients, $K_{2\text{-}7}$, the half-saturation constants; $K_{a2\text{-}4}$ and $K_{b2\text{-}4}$ = the dissociation constants for respective groups of enzymes of microorganisms M_2, M_3, and M_4 that regulate the pH activity of corresponding microorganisms; and k = the kinetic constant of reaction (15). Equation (19), as in the case of methanol, shows a correlation between pH and acetate concentration.

A mathematical description of the kinetics of the process obeying the scheme (15–19) assumes the form

$$d[G] / dt = -V_3 \quad (20)$$

$$d[Et] / dt = V_3(1 - Y_3) - V_4 \quad (21)$$

$$d[Ac] / dt = V_3(1 - Y_3) + V_4 - V_2(1 - Y_2) \quad (22)$$

$$d[CO_2] / dt = 2V_3(1 - Y_3) + V_2(1 - Y_2) - V_5(1 - Y_4) \quad (23)$$

$$d[H_2] / dt = 2V_3(1 - Y_3) + 2V_2 - 4V_5(1 - Y_4) \quad (24)$$

$$d[CH_4] / dt = V_2(1 - Y_2) + V_5(1 - Y_3) \quad (25)$$

$$dM_2 / dt = Y_2V_2 \quad (26)$$

$$dM_3 / dt = Y_3V_3 \quad (27)$$

$$dM_4 / dt = Y_4V_5 \quad (28)$$

Table 2
Numerical Values of Parameters for Glucose Conversion by Methanogenic Association

Parameters	Numerical values	Parameters	Numerical values
q_2, h^{-1}	0.332	pK_{a2}	5.8
q_3, h^{-1}	0.713	pK_{b1}	7.4
q_4, h^{-1}	2.05	pK_{a3}	5.0
k, h^{-1}	0.178	pK_{b3}	8.3
K_2, m*M*	16.3	pK_{a4}	5.0
K_3, m*M*	49.4	pK_{b4}	8.2
K_4, m*M*	34.6	y_2, g/g	0.149
K_5, m*M*	19.9	y_3, g/g	0.064
K_6, m*M*	0.5	y_4, g/g	0.074
K_7, m*M*	12.4		

$$[H^+] = f([Ac]). \tag{29}$$

where $[G] = [C_6H_{12}O_6]$, $[Et] = [C_2H_5OH]$

$$V_2 = q_2 [M_2] [Ac] / ((K_2 + [Ac]) (1 + [H^+] / K_{a2} + K_{b2} / [H^+])) \tag{30}$$

$$V_3 = q_3 [M_3] [G] / ((K_3 + [G]) (1 + [H^+] / K_{a3} + K_{b3} / [H^+])) \tag{31}$$

$$V_4 = k [Et] [M_3] / (K_4 + [Et]) \tag{32}$$

$$V_5 = q_4 [M_4] [H_2] [CO_2] / ([K_5 + K_6 [H_2] + K_7[CO_2] + [H_2] [CO_2]) (1 + [H^+] / K_{a4} + K_{b4} / [H^+]) \tag{33}$$

Numerical integration shows that the description (20–33) is adequate with respect to the process of glucose conversion to methane by the given methanogenic association. The reverse problem is solved as in the case of methanol conversion. We calculated numerical values for parameters that furnish a satisfactory description of the measurements at different pH and starting concentrations of glucose (Table 2). Figure 2 illustrates the quality of description via calculation. We must note here that hydrogen, ethanol, and acetate concentrations pass through a maximum. The calculation pinpoints the position of these maxima and their magnitude. The induction period of methane accumulation—taking place under our experimental conditions—is also described by calculation. Thus, the obtained parameters give a satisfactory description of experimental measurements in the system (20–33) and do not contradict the available experimental data. Besides, the abovedescribed procedure enabled us, as in the case of methanol, to calculate growth curves for microorganisms M_2, M_3, and M_4.

Analysis of the sensitivity of the given model to some of the parameters showed that parameter K_6 makes a far less contribution to the description (14) compared with the other constants of the model (20–33). A change of

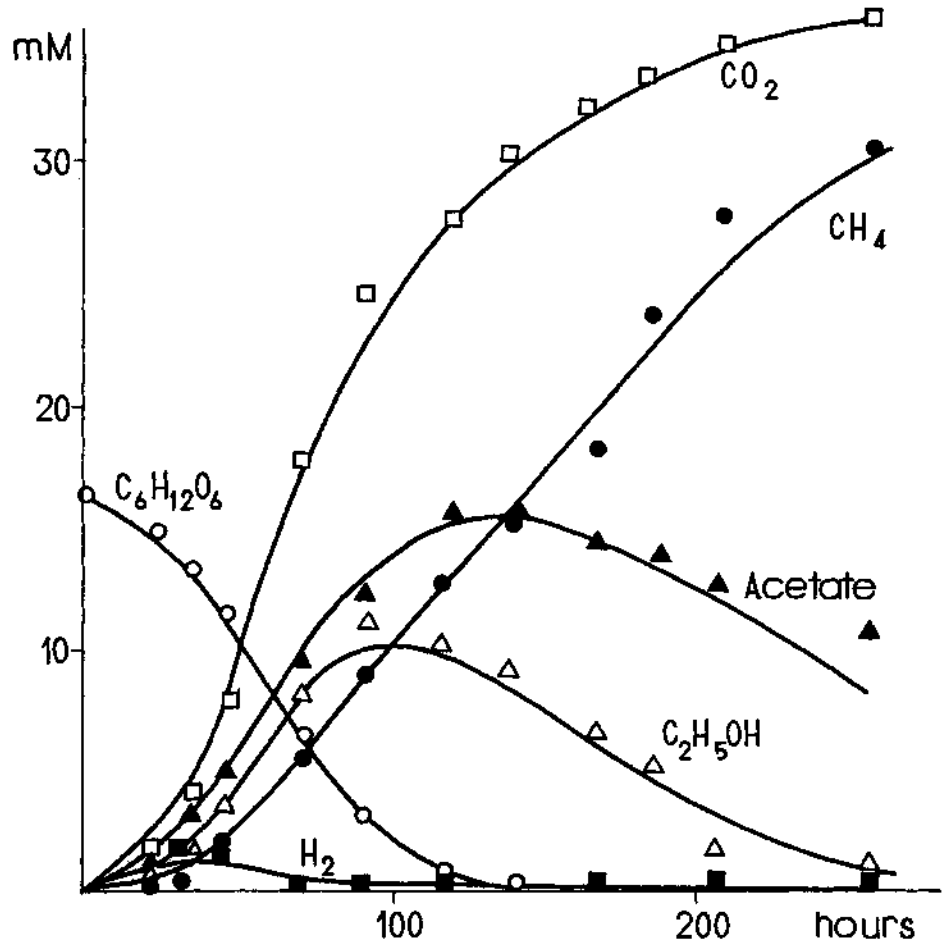

Fig. 2. Comparison of the calculated data with the experimental ones for glucose conversion by methanogenic association, pH 7.0, 16.5 m*M* $C_6H_{12}O_{16}$. Solid lines, the calculated curves; dots, the experimental data.

parameter K_6 from the values indicated in Table 2 to zero results in a 2% difference in the criterion. The description is within the experimental error (< 10%). Consequently, parameter K_6 is not identifiable according to the available data, and it could be neglected in further constructions of the model. Thus, our analysis has shown that the model (20–33) may be simplified somewhat without any detriment to the quality of description. Equation (33) will be modified as

$$V_4 = q_4 [M_4] [H_2] [CO_2] / ((K_5 + K_7[CO_2] + [H_2] [CO_2]) \cdot(1 + [H^+] / K_{a4} + K_{b4} / H^+])) \quad (33)$$

Let us try to compare the values of the kinetic parameters obtained by us with the literary data (Table 3). From this table, it follows that the calculated kinetic parameters are in sufficiently good agreement with those cited in the literature. Numerical values for the maximal specific growth

Table 3
Kinetic Parameters for the Growth of Methanogenes on Different Substrates[a]

Methanogenes	Substrate	m, h^{-1}	K_s, m*M*	Y, g/g	Reference
M. barkeri 227	Acetate	0.038	29.2	–	(*10*)
M. barkeri 227	Acetate	–	–	0.09–0.11	(*11*)
M. barkeri 227	Acetate	0.021	5.0	0.12–0.21	(*12*)
M. barkeri MS	Acetate	0.063	7.7	–	(*10*)
M. barkeri MS	Acetate	–	–	0.1–0.13	(*13*)
M. barkeri TM1	Acetate	0.058	4.5	0.1–0.12	(*14*)
M_2 from *M. kuzneceovii* (CH_3OH)	Acetate	0.053	24.2	0.179	
M_2 from *M. kuzneceovii* ($C_oH_{12}O_6$)	Acetate	0.049	16.3	0.149	
M. thermoautotrophicum	H_2CO_2	0.49–0.69	0.1(H_2) 1.5(CO_2)	0.1–0.19	(*15*)
M. thermoautotrophicum	H_2/CO_2	0.14–0.28	–	0.04–0.1	(*12*)
M_4 from *M. kuzneceovii* ($C_6H_{12}O_6$)	H_2/CO_2	0.152	0.5(H_2) 7.2(CO_2)	0.064	
M. barkeri 227	Methanol	0.07	–	0.28	(*16*)
M. barkeri TM1	Methanol	0.07–0.1	–	0.27–0.3	(*16*)
M_1 from *M. kuzneceovii* (CH_3OH)	Methanol	0.05	77.1	0.097	

[a]Some values of K_s and Y have been calculated in measurement units of Table 3.

rates of microorganisms M_1, M_2, M_3, and M_4 from the methanogenic association *Methanobacillus kuzneceovi* were obtained in accordance with the correlation: $\mu = q\,Y$.

CONCLUSION

Thus, kinetic schemes have been evolved for the process of methanol and glucose conversion by a methanogenic association. All the steps of this process have been investigated and corresponding mathematical models, giving a satisfactory descriptions of the available experimental results, were built. The calculated parameters are in good consistency with those described in the literature.

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