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An Improved Mathematical Model of Methanogenesis of Glucose

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ABSTRACT

An improved mathematical model of methanogenesis of glucose is evolved on the basis of novel kinetic experiments. In reference to our previous model, the improved one additionally includes growth and metabolism of the fourth group of microorganisms, so-called obligate proton reducers, and some regulations dealing with these bacteria. The parameter values of the model have been calculated, sufficiently describing the experimental regularities. The improved model is especially advantageous for describing the experiments with high initial substrate concentrations. The additional model experiments revealed some principal factors of methanogenesis.

Index Entries: Kinetics; kinetic scheme; methanogenic consortium of microorganisms; mathematical model; parameters.

INTRODUCTION

Methanogenesis is a complex multistage process of organic compound decomposition to methane and carbon dioxide by action of numerous anaerobic microflora. Understanding of its kinetic regularities and microbial interactions is important for practical application of anaerobic digestion, especially for fuel production and waste utilization. In this connection, mathematical modeling of this process is essential (*1–10*). In recent work (*10*), we developed a mathematical model of methanogenesis of glucose, one of the key natural organic compounds, involving interaction

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of three groups of microorganisms (acid producers, acetate-, and hydrogen-utilizing methane producers) and an inducible enzyme responsible for conversion of butyrate. The model well describes experimental data under moderate (up to 2 g/L) glucose concentrations over long periods of time (600 h and more). At the same time, computer simulation of cases with higher glucose or its decomposition intermediate concentrations revealed some limitations of this model that, in general, were related to the description of the butyrate conversion step. The aim of the present article is to work out a more comprehensive mathematical model for describing the methanogenesis of glucose.

MATERIALS AND METHODS

Bacterial suspension from a lab UASB reactor operating on synthetic medium, not adapted to the substrates, was used as a source of inoculum. Kinetic investigations (batch cultivation) were carried out at 35°C in hermetic 525-mL flasks, using nutrient medium previously described (*11*) under argon atmosphere without stirring. The volume of liquid phase was 200 mL (the volume of introduced inoculum was 10 mL), and the substrates for anaerobic fermentation were added in concentrations of 1–5 g/L.

Concentrations of methane, hydrogen, and carbon dioxide in gaseous phase and ethanol and volatile fatty acids (VFA) in liquid phase were determined by gas-chromatographic methods as described previously (*11*). Solubility of hydrogen and methane in the medium was neglected. The overall content of CO_2 in the reactor was calculated on the basis of its solubility in the medium depending on temperature, pH, and pressure. The pressure in the reactor, increasing constantly in the course of reaction, was measured with a manometer. Concentration of glucose was determined spectrophotometrically as also described previously (*11*). The average values of metabolite concentrations in the figures below were obtained from four replicates.

RESULTS AND DISCUSSION

Our previous mathematical model of methanogenesis of glucose included: the growth and metabolism of three kinds of microorganisms, i.e., acid producers (*1*), acetate- (*2*), and hydrogen-utilizing (*3*) methane producers; processes of cell lysis; an enzyme located in acid producer and responsible for conversion of butyrate; and some regulations depending on the concentration of intermediates of glucose decomposition (*10*). Postulation (*3*) of this model is most vulnerable, because conversion of butyrate is carried out by a separate bacterial group—obligate proton reducers.

Of course, we understood this from the first steps of the development of the model, but such assumptions enabled us to start with a simpler model and further complicate it. The shortcoming of a model dealing with a postulation (3) is not revealed under computer simulations with moderate substrate concentrations. However, as mentioned in the introduction, this model cannot accurately describe the cases with high (more than 2 g/L) glucose or its decomposition intermediate concentrations, and the limitations of the model are related to the description of the butyrate conversion step. To clarify the regularities of this step, additional experiments were carried out, some of which are discussed below.

Kinetic Investigations

A new set of kinetic studies has been carried out in two stages. In the first stage, we investigated methanogenesis of glucose and its intermediates at various initial concentrations. These experiments were not made earlier (*10*). Here it is necessary to mention that in comparison with the previous kinetic study (*10*), we used more active inoculum (larger initial cell concentration); therefore, the conversion processes proceeded two to three times faster. Investigation of glucose methanogenesis under these conditions showed that the main intermediates (acetate, butyrate, ethanol, hydrogen) and regularities of the process remained practically unchanged, although transient propionate concentration was increased (data are not shown). The conversion of glucose and its intermediates to methane and carbon dioxide was practically complete with initial glucose concentrations of 1–5 g/L. Further increase of glucose concentration led to inhibition of the process by excess of VFA.

The results of the variation of initial butyrate concentration are presented in Fig. 1. It is shown that the initial rate of methane formation during the first 100 h is slightly inhibited by butyrate. With exhaustion of the butyrate level, the inhibition is eliminated. At the same time, at initial butyrate concentrations of more than 4 g/L, the inhibition effect becomes stronger, and the conversion process proceeds over a much longer time. Varying the initial ethanol concentration, we obtained the same results, although the inhibition effects began to appear at concentrations under 4 g/L of ethanol (data are not shown). The inhibition effect of acetate was not apparent at concentrations up to 5 g/L (data also are not shown).

In the second stage of our kinetic investigation, we studied methanogenesis of various substrate mixtures to reveal their mutual influence. Two of these kinetic patterns are presented in Figs. 2 and 3. The conversion of the glucose–butyrate mixture was practically complete after 300 h (Fig. 2). The initial increase in butyrate level is related to its formation under acidogenic glucose decomposition. The two initial substrates are digested successively, and the overall conversion process is composed of two stages—glucose methanogenesis (up to 100 h) followed by butyrate methanogenesis. This is well reflected in the carbon dioxide and acetate

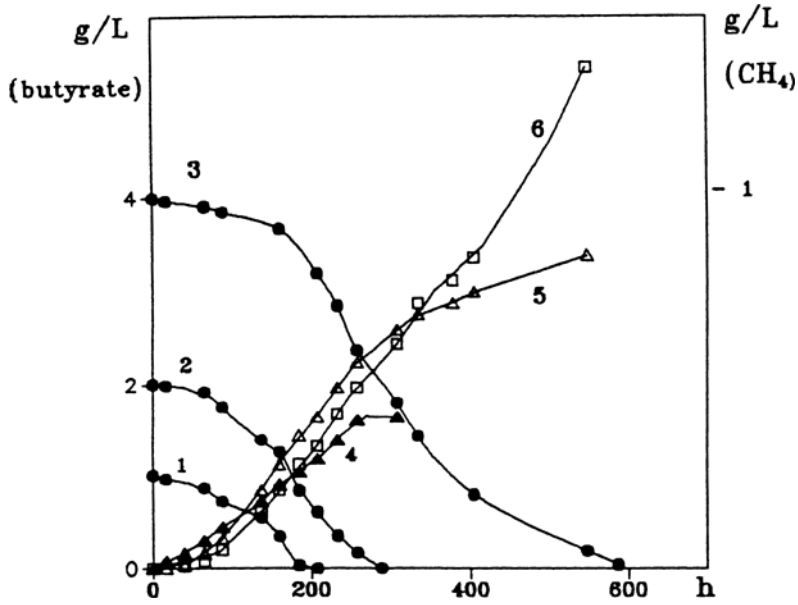

Fig. 1. Kinetics of anaerobic conversion of butyrate at various initial concentrations: 1 g/L (1–butyrate, 4–methane); 2 g/L (2–butyrate, 5–methane); 4 g/L (3–butyrate, 6–methane).

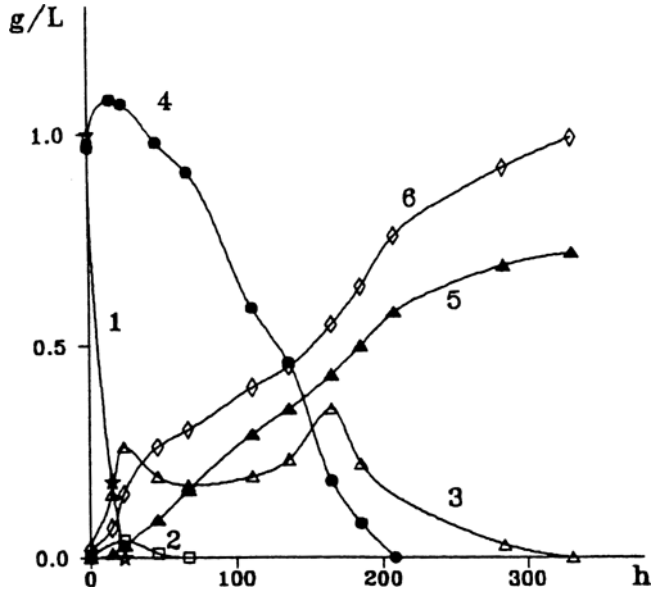

Fig. 2. Kinetics of anaerobic conversion of a mixture of glucose (1 g/L) and butyrate (1 g/L): 1–glucose; 2–ethanol; 3–acetate; 4–butyrate; 5–methane; 6–carbon dioxide.

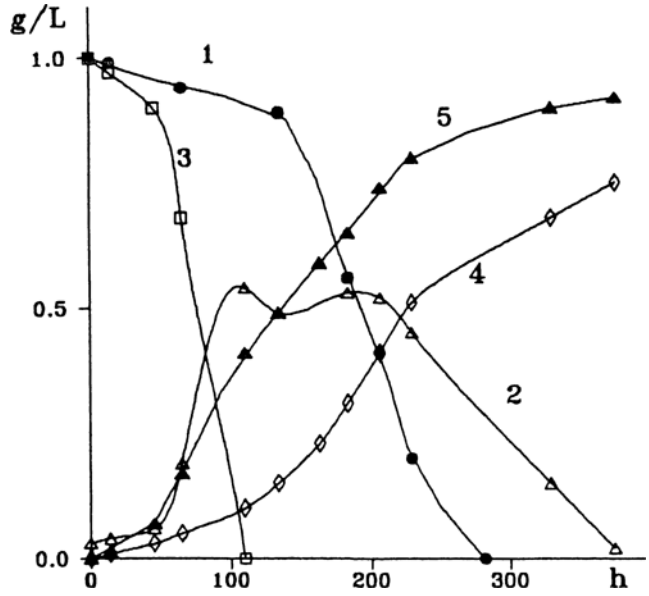

Fig. 3. Kinetics of anaerobic conversion of a mixture of ethanol (1 g/L) and butyrate (1 g/L): 1–butyrate; 2–acetate; 3–ethanol; 4–carbon dioxide; 5–methane.

curves. It should be especially noticed that the latter curve has two maximums—the first maximum is derived from glucose decomposition, and the second one from butyrate conversion.

Interesting results were obtained from the study of ethanol–butyrate mixtures (Fig. 3). It is shown that butyrate was practically not converted until the ethanol level was exhausted. Apart from ethanol inhibition, the main reason for the delayed butyrate conversion is related to hydrogen, which plays an essential role in methanogenic systems. The acetogenic step of conversion of both substrates is described by the following equations:

$$C_2H_5OH + H_2O \rightarrow CH_3COOH + 2H_2 \quad (1)$$

$$C_3H_7COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2 \quad (2)$$

Thermodynamic calculations show that reactions (1 and 2) may proceed at partial pressures of hydrogen < 0.15 and 0.002 atm, respectively, when concentrations of other substances are within the physiological range. The hydrogen level in methanogenic systems is determined by the balance between the rates of hydrogen evolution and consumption (the latter is carried out almost exclusively by hydrogen-utilizing methanogens). Returning to the ethanol–butyrate case, one can say that increased hydrogen evolution according to reaction (1) may create a hydrogen level sufficient to block reaction (2). This is really so, because experiments showed that the partial pressure of hydrogen exceeded 0.002 atm, whereas the ethanol level was high. It should be noticed that similar results were obtained

under the study of methanogenesis of ethanol-propionate mixtures in the work (*12*).

These and similar kinetic data were used for developing an improved mathematical model of the process. The other kinetic information was taken from our previous study (*10*).

Description of Mathematical Model

The model was developed using the approach suggested in the previous studies (*10,13*). Taking the previous model (*10*) as a basis, we modified its postulate (3) by introducing growth and metabolism of a fourth bacterial group—obligate proton reducers. Thus, the improved model includes interaction of four groups of microorganisms: X_1, X_2, X_3, and X_4. Group X_1 contains all acid producers and acetogenes for ethanol conversion; X_2, all acetate-utilizing methanogenes; X_3, all hydrogen-utilizing methanogenes; and X_4, obligate proton reducers for butyrate conversion. For simplicity, the groups of microorganisms X_1, X_2, X_3, and X_4 will be called cultures X_1, X_2, X_3, and X_4. As in the previous model, the present model does not take into account the three-phasic character of the reaction medium and the influence on the biological systems of such physicochemical factors as pH, solubility of gases, and so on.

Using the chemical scheme of the previous study (*10*), the kinetic scheme of the model of glucose methanogenesis in weight concentrations (g/L) appears as the following (where $S_1 = C_6H_{12}O_6$, $S_2 = C_2H_5OH$, $S_3 = CH_3COOH$, $S_4 = C_3H_7COOH$, $S_5 = CH_4$, $S_6 = CO_2$, $S_7 = H_2$):

$$S_1 \xrightarrow{w_1, X_1} 0.074S_2 + 0.487S_3 + 0.166S_4 + 0.278S_6 + 0.011S_7 \quad (3)$$

$$S_1 \xrightarrow{W_1, X_1} X_1 \quad (4)$$

$$S_2 \xrightarrow{w_2, X_1} 1.304S_3 + 0.087S_7 \quad (5)$$

$$S_2 \xrightarrow{W_2, X_1} X_1 \quad (6)$$

$$S_4 \xrightarrow{w_4, X_4} 1.364S_3 + 0.045S_7 \quad (7)$$

$$S_4 \xrightarrow{W_4, X_4} X_4 \quad (8)$$

$$S_3 \xrightarrow{w_3, X_2} 0.267S_5 + 0.733S_6 \quad (9)$$

$$S_3 \xrightarrow{W_3, X_2} X_2 \quad (10)$$

$$S_6 + 0.182S_7 \xrightarrow{w_5, X_3} 0.367S_5 \quad (11)$$

$$S_6 + 0.091S_7 \xrightarrow{W_5, X_3} X_3 \quad (12)$$

where W_i—the growth rates of corresponding cultures X_1–X_4; w_i—the rates of fermentation activity of corresponding cultures X_1–X_4.

It is necessary to notice that according to the proposed scheme (3–12), the growth rate and the rate of fermentation activity of each culture should be described by different functions in the model.

According to the scheme and our assumption, the growth of culture X_1 on substrates S_1 and S_2 proceeds independently and simultaneously, and is described by the following equation:

$$dX_1 / dt = m_1W_1 + 1.304m_2W_2 - a_1X_1 \quad (13)$$

where the growth rates on each substrate are described by the functions:

$$W_1 = S_1X_1/(L_1 + S_1 + L_2X_1 + nS_7) \quad (14)$$

$$W_2 = S_2X_1M_6/[(L_3 + S_2 + L_4X_1 + M_5S_7)(M_6 + S_2)] \quad (15)$$

Description of the growth rate of the culture is based on the classical Monod equation, complicated by self-inhibition of cells when their concentration is high and by inhibition of their growth by hydrogen and ethanol (only for Eq. [15]). The negative term in the growth equations here and further on) accounts for cell lysis as in the previous model (*10*).

Fermentation activity of culture X_1 consists of catalyzing the reactions (3, 5). Hence, the rates of the processes are described by the following equations:

$$w_1 = V(X_1)S_1/[K_1 + S_1 + M_1(S_2 + S_3 + S_4 + S_7)] \quad (16)$$

$$w_2 = V(X_1)S_2/[K_8 + S_2 + M_2S_3 + M_9S_7) \quad (17)$$

here and further on, $V(Y) = Y/(K_{10} + Y)$. The rate of glucose conversion is proportional to the fermentation activity of the culture X_1, which, in turn, is described by the classical Michaelis equation with inhibition by products of reaction (3). Factor $V(X_1)$ accounts for the fact that only a fraction of cells X_1 possesses fermentation activity and its value changes constantly during the cell growth. The rate of ethanol transformation is similarly determined taking into account the inhibition by acetate and hydrogen.

Thus, the growth of culture X_1 on glucose and fermentation of the latter determines the acidogenic step. The kinetic equation for glucose in this case appears as:

$$dS_1/dt = - l_1w_1 - m_1W_1 \quad (18)$$

The corresponding kinetic equation for ethanol is the following:

$$dS_2/dt = 0.074w_1 - l_2w_2 - m_2W_2 \quad (19)$$

Butyrate formed in the acidogenic step is further utilized by growing culture X_4. This process is described by the following equation:

$$dX_4/dt = 1.364m_4W_4 - a_4X_4 \quad (20)$$

where the growth rate of culture X_4 appears in the form:

$$W_4 = S_4X_4M_{10}/[L_8 + S_4 + L_9X_4 + K_2S_7)\,(M_{10} + S_4 + M_{13}S_2)] \quad (21)$$

The growth of culture X_4 is inhibited by high concentrations of butyrate, hydrogen, ethanol, and X_4.

The rate of butyrate transformation into acetate and hydrogen depends on biosynthesis of enzymatic systems (enzyme E in the model) located in culture X_4, and also inhibition by acetate and hydrogen:

$$w_4 = V(E)S_4/(K_9 + S_4 + M_4S_3 + M_7S_2) \quad (22)$$

The rate of the induced biosynthesis of enyzme E is represented by the following equation:

$$dE/dt = E_0V(X_4)S_4^2N_2/[(b + S_4^2)\,(N_2 + S_7 + N_1S_4)] - a_5E \quad (23)$$

where E_0 is the rate of biosynthesis of enzyme E; b is a parameter regulating the induction by butyrate; N_1 and N_2 are parameters responsible for repression of biosynthesis by butyrate and hydrogen; and a_5 is a parameter for inactivation of enzyme E.

Finally, the kinetic equation for butyrate appears as:

$$dS_4/dt = 0.166w_1 - l_4w_4 - m_4W_4 \quad (24)$$

Acetate, carbon dioxide and hydrogen accumulated at acid- and acetate-producing steps are further consumed by growing methanogenic cultures X_2 and X_3; the processes are described by the following kinetic equations:

$$dX_2/dt = m_3W_3 - a_2X_2 \quad (25)$$

$$dX_3/dt = 0.682m_5W_5 - a_3X_3 \quad (26)$$

where the growth rates of cultures X_2 and X_3 appear in the form:

$$W_3 = S_3X_2M_{11}/[(L_5 + S_3 + L_6X_2 + L_7S_4 + M_8S_7)\,(M_{11} + S_3 + M_{12}S_2)] \quad (27)$$

$$W_5 = S_6S_7X_3/(L_{10} + L_{11}S_6 + L_{12}X_3 + L_{13}S_7 + L_{14}S_6S_7) \quad (28)$$

The growth of culture X_2 is inhibited by high concentrations of butyrate, hydrogen, ethanol, acetate, and X_2. The growth of culture X_3 depends on concentrations of two substrates and involves only mutual inhibition of cells.

The rates of the fermentation reactions carried out by cultures X_2 and X_3 are described by the following equations:

$$w_3 = V(X_2)S_3/(K_3 + S_3 + M_3S_7) \quad (29)$$

$$w_5 = V(X_3)S_6S_7/(K_5 + K_6S_6 + K_7S_7 + S_6S_7) \quad (30)$$

The rate of conversion of acetate into methane is inhibited by hydrogen, and we assume that utilization of hydrogen and carbon dioxide proceeds without any complications.

The equations for methanogenic substrates appear in the form:

$$dS_3dt = 0.487w_1 + 1.304l_2w_2 - l_3w_3 - m_3W_3 + 1.364l_4w_4 + a_6R \quad (31)$$

$$dS_6dt = 0.278w_1 + 0.733l_3w_3 - l_5w_5 - m_5W_5 + a_7R \quad (32)$$

$$dS_7dt = 0.011w_1 + 0.045(l_4w_4 + m_4W_4 + 0.087(l_2w_2 + m_2W_2) - 0.182l_5w_5 - 0.91m_5W_5 + a_8R \quad (33)$$

The term $R = (a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4)$ determines the increase in concentrations of acetate, carbon dioxide, and hydrogen at the expense of cell lysis. As in the previous model (*10*), we assume that destruction of biomass finally results in formation of only the latter three substances according to the following equation:

$$\text{Biomass}(CH_2O) + H_2O \rightarrow a_6CH_3COOH + a_7CO_2 + a_8H_2 \quad (34)$$

Finally, the kinetic equation for methane production appears in the form:

$$dS_5dt = 0.267l_3w_3 + 0.364l_5w_5 \quad (35)$$

In the section below, the system of Eqs. (13–33 and 35) will be used for describing the methanogenesis of glucose.

Application of the Model

Verification of the described model (identification to the experiment) and selection of the parameters values were realized by the computer simulations of development of the methanogenic biosystem from different initial states on the concentrations S_1, S_2, S_3, and S_4 (i.e., by means of multiple numerical solutions of Eqs. [13–33 and 35]) without using any regression procedure). As first approximations of the parameters values, we took previous model data and literature data. Later on, starting from the first approximations, we selected the parameters values that allowed us to describe qualitatively the characteristic features of the experiments, including its temporal requirements. In selecting the specific growth rates and metabolic coefficients, we also tried to obtain values of growth yields not exceeding 10% that correspond to the literary data on accumulation of biomass during methanogenesis (*14*).

The values of parameters satisfying to the model of glucose methanogenesis developed by us are listed below; dimensions of the parameters are combinations of concentrations (in g/L) and time (in h):

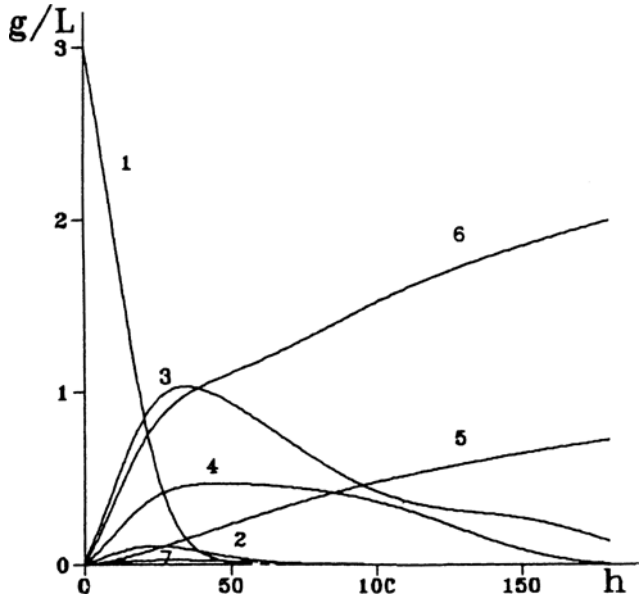

Fig. 4. Computer simulation of anaerobic conversion of glucose (3 g/L): 1-glucose; 2-ethanol; 3-acetate; 4-butyrate; 5-methane; 6-carbon dioxide. 7-hydrogen.

$l_1 = 0.5$; $l_2 = 0.19$; $l_3 = 0.3$; $l_4 = 0.22$; $l_5 = 0.2$; $K_1 = 0.1$; $K_2 = 10$; $K_3 = 0.35$; $K_4 = 0.8$; $K_5 = 0.015$; $K_6 = 0.08$; $K_7 = 0.03$; $K_8 = K_9 = K_{10} = 0.1$; $N_1 = 0.13$; $N_2 = 0.006$; $b = 0.02$; $n = 0.1$; $M_1 = 3$; $M_2 = 3$; $M_3 = 38$; $M_4 = 3.5$; $M_5 = 3$; $M_6 = 0.07$; $M_7 = 50$; $M_8 = 1$; $M_9 = 2$; $M_{10} = 0.15$; $M_{11} = 0.03$; $M_{12} = 1$; $m_1 = 0.012$; $m_2 = 0.03$; $m_3 = 0.04$; $m_4 = 0.019$; $m_5 = 0.01$; $a_1 = a_2 = a_3 = 0.004$; $a_4 = 0.002$; $a_5 = 00.001$; $L_1 = L_3 = L_8 = 0.024$; $L_2 = 0.4$; $L_4 = 0.5$; $L_5 = 0.05$; $L_6 = L_{14} = 0.1$; $L_7 = 2$; $L_9 = 0.2$; $L_{10} = 0.002$; $L_{11} = 0.007$; $L_{12} = 0.03$; $L_{13} = 0.02$ (36)

As in the previous model (*10*), coefficients for Eq. (34) were chosen by assuming that 60% of the lysed biomass transforms into acetate, i.e., $a_6 = 0.6$. Other coefficients are obtained unambiguously from Eq. (34) taking into account the conditions of electron balance: $a_7 = 0.59$; $a_8 = 0.04$.

Let us fix the initial state of bioreactor when it contains only the inoculum and substances introduced with it (all in g/L):

$$S_1^\circ = S_2^\circ = S_4^\circ = S_5^\circ = S_7^\circ = 0;\ S_3^\circ = S_6^\circ = 0.01;\ X_1 = X_3^\circ = 0.01;$$
$$X_2^\circ = 0.014;\ X_4^\circ = 0.0075;\ E^\circ = 0.001 \quad (37)$$

Let us consider some characteristic kinetic situations for methanogenesis starting from different initial states. Taking the concentration of glucose for initial state (37), $S_1^\circ = 3$ g/L, it can be seen from Fig. 4 that anaerobic fermentation proceeds through a number of steps. Formation

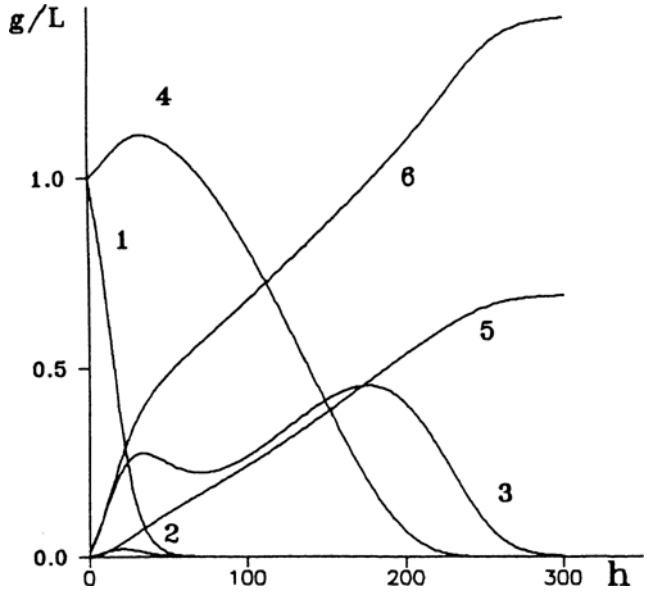

Fig. 5. Computer simulation of anaerobic conversion of a mixture of glucose (1 g/L) and butyrate (1 g/L): 1–glucose; 2–ethanol; 3–acetate; 4–butyrate; 5–methane; 6–carbon dioxide.

of methane is completed within 180 h, the acidogenic step proceeds during the first 50 h, and acetogenesis proceeds through 140 h, which corresponds well to the experimental data. When the process was completed, 8% of the substrates for the growth of the culture X_1 (S_1 + S_2), 3% of the substrate for the culture X_3 (S_3), 7% of the substrates for the culture X_3 (S_6 + S_7), and 8% of the substrate for the culture X_4 were consumed, which agrees with the literature data (*14*).

Figure 5 shows numerical results for development of methanogenesis starting from the initial state (37) when $S_1^0 = S_4^0 = 1$ g/L. The computer simulation shows an initial increase in butyrate level, two maximums in the acetate curve, and a slightly undulating carbon dioxide curve that corresponds in general to the experimental data (Fig. 2). It is necessary to notice that the model results for carbon dioxide production exceed the experimental results. It may be related to following. In spite of taking into account solubility of CO_2 in the medium, we practically always observed deficiency of overall CO_2 content in the reactor in reference to its calculated value from mass and electron balance. It is likely that the CO_2 transfer from liquid phase, where it is formed, to gas phase, where we determine its concentration, proceeds with impediment under unstirred conditions of our experiments. The model does not take into account this impediment.

A similar picture is observed in a computer simulation starting from the initial state (37) when $S_2^0 = S_4^0 = 1$ g/L (Fig. 6). The two initial substrates

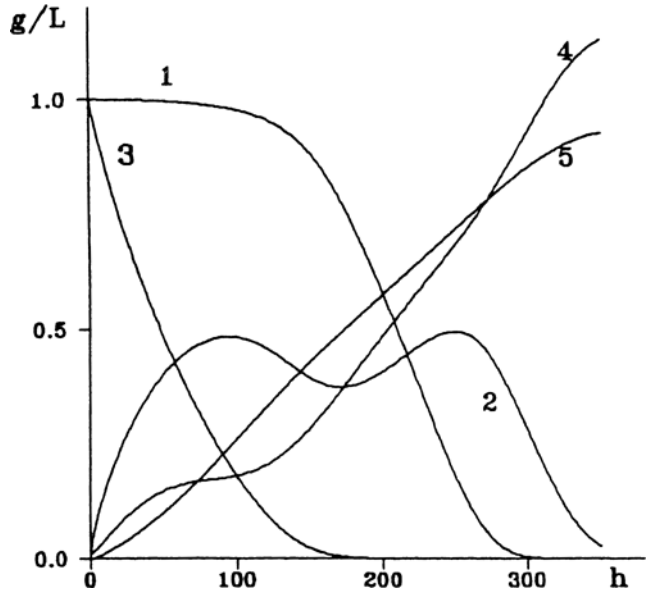

Fig. 6. Computer simulation of anaerobic conversion of a mixture of ethanol (1 g/L) and butyrate (1 g/L): 1–butyrate; 2–acetate; 3–ethanol; 4–carbon dioxide; 5–methane.

are digested successively, and the overall conversion process is composed of two stages—ethanol decomposition (up to 120 h) followed by butyrate conversion. It is noticed that butyrate is not converted until the ethanol concentration is essentially depleted. This is also the reason for two maximums in the acetate curve—the first maximum is derived from ethanol conversion and the second one from acetogenesis of butyrate. The results of the simulation shown in Fig. 6 also correspond to the experimental data in Fig. 3, except for data for CO_2 because of the reason mentioned in the previous paragraph.

Besides describing qualitatively the characteristic features of the kinetic experiments, it is essential to put down a model in its temporal framework. For this purpose, we have carried out many computer simulations varying the initial concentration of all substances that take part in various steps of methanogenesis. Figure 7, as an example, presents numerical data dealing with butyrate variation. Comparison of these data with those of Fig. 1 shows that the model really corresponds the experiment temporal requirements. Figure 7 also presents analogous numerical data obtained with using the previous model (*10*). It is shown that, in this case, agreement between simulation and experiment is significantly worse at high butyrate concentrations. Similar results were obtained for other substrates.

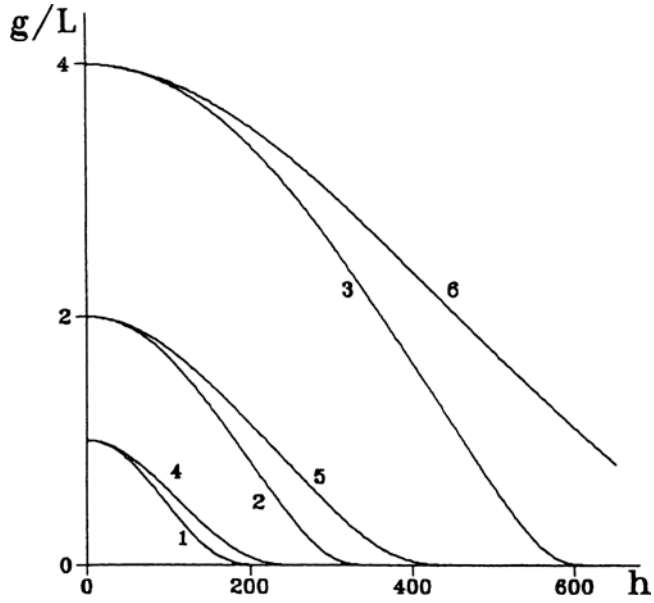

Fig. 7. Numerical simulations of butyrate decomposition kinetics at various initial concentrations: 1 g/L (1–present model, 4–previous model [*10*]); 2 g/L (2–present model, 5–previous model [*10*]); 4 g/L (3–present model, 6–previous model [*10*]).

Let us also illustrate one example of how the principal regulating factors of methanogenesis can be revealed and described based on the model. The above-mentioned high sensitivity of the kinetics of butyrate conversion in response to the presence of hydrogen may be shown in numerical simulations with the help of parameter N_2. Decrease of this parameters increases the repression of biosynthesis of enzyme E (*see* Eq. 23) and, thus, decelerates the butyrate conversion rate. This results in a decrease in methane formation rate (Fig. 8). Parameter N_2 and some others determine the time for the acidogenic step at different initial conditions. Thus, comparing the times with experimental values, we find the proper value that is stated in the list (*37*). Similarly, a choice of other parameters of the suggested model is made, and relations between them are established.

CONCLUSION

In conclusion, it should be mentioned that the model describes adequately the experimental data and can be used for a more thorough study of regularities of methanogenesis. Further improvement of the model

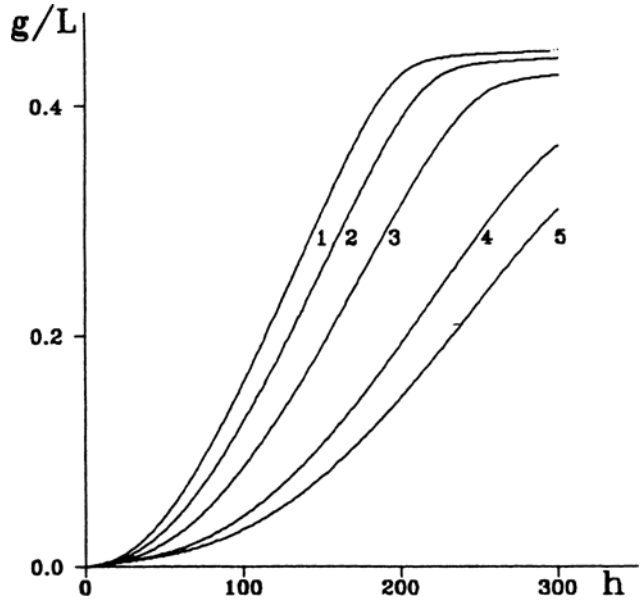

Fig. 8. Numerical simulations of the kinetics of methane formation from butyrate decomposition (1 g/L) with varying value of parameter N_2: 1–0.01; 2–0.006; 3–0.003; 4–0.001; 5–0.006.

may be based on consideration of the three-phasic character of the reaction system, pH-dependencies, and induction of enzymes of the cellulase complex in the case of methanogenesis of cellulose. Other applications of the model and its development will be described in further publications.

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