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PII:S0960-8524(96)00052-1

MODELING OF VOLATILE FATTY ACIDS DEGRADATION KINETICS AND EVALUATION OF MICROORGANISM ACTIVITY

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Abstract
Particular cases of Monod kinetics (zero order and exponential growth) were used for the description of VFA degradation by acetate and glucose pregrown inocula measured by Aguilar et al. (1995), and kinetic constants were evaluated. The equations with inhibition were used for description of the degradation kinetics of VFA mixtures measured by Mawson et al. (1991) and by Ozturk (1991). A good agreement between the model and the batch data with synthetic media and molasses pregrown inoculum was obtained and microorganism activity was evaluated. Using the generalized ⟨METHANE⟩ model of anaerobic digestion described earlier, the Monod and Haldane kinetics were tested for the experimental data of Noike et al. (1985), where mesophilic conversion of acetate was studied in a continuous-flow reactor under a wide range of acetate-loading values. The system failed at high influent acetate concentration because of pH inhibition for Monod kinetics and of unionized-acetate inhibition for Haldane kinetics. However, the model could not describe the change of effluent acetate concentration. To describe that, the corrected Monod and Haldane function with changeable half-saturation coefficients was introduced. Copyright © 1996 Elsevier Science Ltd.

Key words: VFA, Batch kinetics, continuous-flow reactor, inhibition, inoculum, microorganism activity, Monod equation, Haldane equation, allowable acetate loading, population shift.

INTRODUCTION

The slowest step in anaerobic digestion with dissolved waste is generally believed to be methanogenesis (Pavlostathis & Giraldo-Gomez, 1991). Acetate is the precursor of more than 75% of the methane produced in anaerobic digesters

(Mountfort & Asher, 1978; McCarty & Smith, 1986; Moletta et al., 1986). Propionate and butyrate are other important volatile fatty acids which may be further converted into acetate and hydrogen.

When digestion is proceeding satisfactorily, the concentration of volatile fatty acids will be less than 250 mg/l (Metcalf & Eddy, 1991). High acid concentrations are associated with digester failure (Hobson et al., 1974; Hill et al., 1987); so degradation of VFA is essential to control of the process.

Monod kinetics was assumed by Lawrence and McCarty (1969) for bacterial growth on acetate:

$$r_b = \mu_m \frac{S}{K_s+S} \qquad (1)$$

where r_b is the bacterial growth rate, S is the total acetate concentration, B is the biomass concentration, μ_m is the maximum growth rate of biomass, K_S is the half-saturation coefficient. Equation (1) has been used for descriptions of acetate degradation in a number of papers (Smith & Mah, 1978; Zehnder et al., 1982; Wandrey & Aivasidis, 1983; Chang et al., 1983) considering the total acetate concentration (ionized and unionized). Haldane kinetics was assumed by Andrews and Graef (Andrews, 1969; Graef & Andrews, 1973) with the unionized acetate concentration S_n as a real substrate:

$$r_b = \mu_m \frac{S_n}{K_{sn}+S_n+S_n^2/K_{in}} \qquad (2)$$

where K_{in} is the inhibition constant, K_{sn} is the half-saturation coefficient for unionized substrate. Evidently, eqn (2) transforms into eqn (1) at $K_{in} \to \infty$. Assuming a constant pH one can obtain a constant ratio between unionized and total (unionized and ionized) acetate concentrations. Several authors have proved the Haldane kinetics (Lee & Donaldson, 1985; Bolle et al., 1986; Moletta et al., 1986; Yang & Okos, 1987; Attal et al., 1988; Fukuzaki et al., 1990a; 1990b; Morvai et al., 1992).

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Equations (1) and (2) are also used for degradation of VFA other than acetate (Lawrence & McCarty, 1969; Andrews, 1969; Chang et al., 1983; Heyes & Hill, 1983; Gujer & Zehnder, 1983; Whitmore et al., 1985; Denac, 1986; Attal et al., 1988; Fukuzaki et al., 1990a; 1990b; Kos & Wiesmann, 1995).

In structured models of anaerobic digestion the pH inhibition has been either incorporated with substrate inhibition using Haldane kinetics (Graef & Andrews, 1973; Moletta et al., 1986) or by assuming some empirical-type correction of Monod kinetics (Mosey, 1983; Costello et al., 1991a; Siegrist et al., 1993).

In this study the kinetics of volatile fatty acids degradation were modeled to help in developing a new generation of the ⟨METHANE⟩ model which was described earlier (Vasiliev et al., 1993; Vavilin et al., 1993; 1994a).

METHODS

Mathematical modeling was used as a method of analysis of volatile fatty acids degradation kinetics. The development of any simulation model involves numerous simplifications concerning the processes that take place in the system. The complexity of interactions in anaerobic systems is very high. Nevertheless, an adequate model may help to give a general insight to the phenomena investigated. A scheme of microbial ecology is the basis of this simulation model of anaerobic digestion. It includes a selected set of variables, the defined equations involved for the description of substrate limitation and inhibition and the corresponding values of model coefficients.

Three years have passed since the generalized model ⟨METHANE⟩ of anaerobic digestion of complex organic matter was constructed and now a new generation of the model is being developed. In the first version of the ⟨METHANE⟩ model a sum of non-ionized and ionized forms of acetate, and the total short-chain volatile fatty acids other than acetate, were selected as the two types of VFA variables. In the new version of the ⟨METHANE⟩ model three types of VFA including acetate are involved and the mutual inhibition processes of VFA are taken into account.

In a number of papers (see review by Pavlostathis & Giraldo-Gomez, 1991) it is concluded that, with the exception of the hydrolysis step, all subprocesses of anaerobic treatment have been successfully modeled by Monod kinetics. However, a rather high dispersion of the measured values of Monod kinetic coefficients of VFA degradation has been found for various mixed cultures. In some papers mentioned in the Introduction, Haldane kinetics [eqn (2)] with non-ionized forms as the real substrates, has been verified for descriptions of VFA degradation. The values of Haldane kinetic coefficients were much

more constant (Fukuzaki et al., 1990a; 1990b; Kos & Wiesmann, 1995). In the new version of the ⟨METHANE⟩ model both equations were involved. This was easy to do because the detailed pH computing subprogram had been developed earlier (Vavilin et al., 1995).

RESULTS AND DISCUSSION

Monod kinetics of VFA degradation for evaluation of microorganism activity
Particular cases of the Monod model
The Monod model was used traditionally for a description of dissolved-substrate removal and biomass growth:

$$\frac{dS}{dt} = -\frac{\mu_m}{Y}\frac{SB}{K_s+S}, \quad \frac{dB}{dt} = \mu_m\frac{SB}{K_s+S} - K_dB \quad (3)$$

where S is the limiting substrate concentration, B is the biomass concentration, μ_m is the maximum specific growth rate of biomass, K_d is the biomass decay coefficient, Y is the yield coefficient, K_S is the half-saturation coefficient. The biomass equation can be written in the form

$$\frac{dB}{dt} = Y\frac{ds}{dt} - K_dB \quad (4)$$

Neglecting biomass decay a biomass concentration can be written as

$$B = B_0 + Y(S_0 - S) \quad (5)$$

where B_0, S_0 are the initial concentrations of biomass and substrate, respectively. In the particular cases of high B_0 with low biomass change, $\Delta B \ll B_0$ and rather high $S \gg K_S$, the substrate kinetics reduces to a zero-order reaction:

$$\frac{dS}{dt} \approx -\frac{\mu_m}{Y}B_0 \quad (6)$$

From eqn (6) the substrate concentration is

$$S = S_0 - \frac{\mu_m}{Y}B_0t \quad (7)$$

From eqn (7) is obtained

$$\frac{\mu_m}{Y}B_0 = \frac{S_0 - S}{t} \quad (8)$$

In the particular cases of a low initial biomass concentration (B_0) and rather high $S \gg K_S$ and $\mu_m \gg k_d$, the substrate kinetics are written as

Table 1. Summary of values of Monod kinetic coefficients for anaerobic VFA degradation by various mixed cultures[a]

Substrate	Reference	Process	t (°C)	μ_m (per day)	K_S (mgCOD/l)	Y (mgVSS/mgCOD)	K_d (per day)
Acetate	Lawrence & McCarty, 1969	C	25	0.25	930	0.05	0.011
	Lawrence & McCarty, 1969	C	30	0.275	356	0.054	0.037
	Lawrence & McCarty, 1969	C	35	0.357	165	0.041	0.015
	Kugelman & Chin, 1971	C	35	0.34	185	0.04	0.036
	van den Berg, 1977	B	35	0.08–0.09	—	0.02	—
	Massey & Pohland, 1978	C	37	0.43	393	—	—
	Chang *et al.*, 1983	C	35	0.26	57	0.108	0.283
	Siegrist *et al.*, 1993	C/S	35	0.95	30	0.025	0.1
	Vavilin *et al.*, 1994b	C/S	33	0.84	180	—	—
Propionate	Lawrence & McCarty, 1969	C	25	0.358	1145	0.051	0.040
	Lawrence & McCarty, 1969	C	35	0.313	60	0.042	0.010
	Chang *et al.*, 1983	C	35	0.28	15	0.043	0.092
	Gujer & Zehnder, 1983	C	33	0.155	246	0.025	—
	Whitmore *et al.*, 1985	C	37	—	672	—	—
	Siegrist *et al.*, 1993	C/S	35	0.80	15	0.05	0.10
n-Butyrate	Lawrence & McCarty, 1969	C	35	0.354	13	0.047	0.027
	Chang *et al.*, 1983	C	35	0.175	42	0.022	0.005

[a]C = chemostat data, B = batch data, C/S = simulation model used with experimental chemostat data.

$$r = \frac{dS}{dt} \approx -\frac{\mu_m}{Y} B_0 e^{(\mu_m - k_d)t} t \approx -\frac{\mu_m}{Y} B_0 e^{\mu_m t} \quad (9)$$

From eqn (9) the substrate concentration is

$$S = S_0 - \frac{B_0}{Y} e^{\mu_m t} - 1 \quad (10)$$

The kinetic coefficients are determined from eqns (9) and (10):

$$\mu_m = \frac{1}{\Delta t} \ln \frac{r_2}{r_1} \quad (11)$$

$$\frac{B_0}{Y} = \frac{S_0 - S(t)}{e^{\mu_m t} - 1} \quad (12)$$

where r_1, r_2 are the substrate removal rates at $t = t_1$ and $t = t_2$, respectively, $\Delta t = t_2 - t_1$, t is the current time. So, the substrate kinetic curve may be used for a graphic determination of the model constants.

The Monod kinetic coefficients for anaerobic VFA degradation, taken from the literature, are summarized in Table 1. According to Pavlostathis and Giraldo-Gomez (1991) the range of values of VFA kinetic constants for various substrates utilized in mesophilic anaerobic treatment processes with pure and mixed cultures are as follows:

$$K_S = 11–421 \text{ mg COD/l}, \quad \mu_m = 0.08–0.7/\text{day},$$

$$Y = 0.01–0.045 \text{ g VSS/g COD (acetate)}$$

$$K_S = 12–500 \text{ mg COD/l}, \quad \mu_m = 0.13–1.2/\text{day},$$

$$Y = 0.025–0.047 \text{ g VSS/g COD}$$

(other VFA)

o acetate
□ propionate
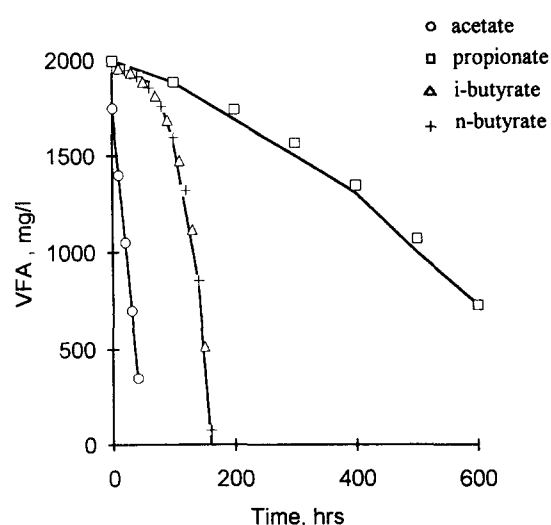▲ i-butyrate
+ n-butyrate

Fig. 1. Progress curves of acetic, propionic, i- and n-butyric acids degradation by an acetate-pregrown inoculum: lines—model predictions [acetate, equation (7), propionate and i,n-butyrate, equation (10)], symbols—experimental data (Aguilar *et al.*, 1995).

Table 2. The maximum specific biomass growth rates of microorganisms μ_m, the ratio between initial biomass concentrations and yield coefficient B_0/Y and their product for acetate pregrown inoculum

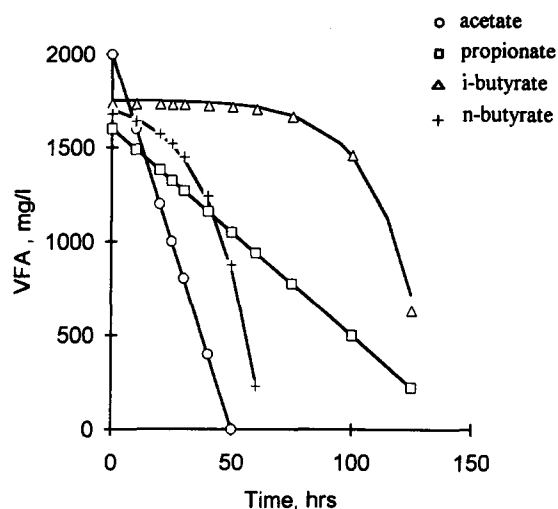| Microorganisms	Parameter		
	μ_m (per h)	B_0/Y (mg/l)	$\mu_m B_0/Y$ [mg/(l.h)]
Methanogens	—	—	35
Propionic syntrophs	0.0022	462	1.0
i-Butyric syntrophs	0.026	30	0.78
n-Butyric syntrophs	0.026	30	0.78

Fig. 2. Progress curves of acetic, propionic, i- and n-butyric acids degradation by glucose pregrown inoculum: lines—model predictions [acetate and propionate, equation (7), i,n-butyrate, equation (10)]; symbols—experimental data (Aguilar *et al.*, 1995).

Acetate pregrown inoculum

In the first series of batch experiments at 37°C Aguilar *et al.* (1995) have used an acetate-pregrown inoculum taken from a chemostat in steady-state at HRT = 4 days, pH = 7.5 and at the influent acetate concentration of 6 g/l. Filamentous microorganisms were dominant in the reactor.

In Fig. 1 the degradation curves of acetic, propionic, n-butyric and i-butyric acids are shown. If acetate kinetics follows the zero-order eqn (7), propionate, n-butyrate and i-butyrate follow the plate curve kinetics (10). There is no difference in n- and i-butyrate degradation curves. Some parameters of the model [eqn (10)] are summarized in Table 2. Aguilar *et al.* (1995) noted that substrate inhibition was not detected in the range of parameters studied.

Glucose-pregrown inoculum

In the second series of batch experiments at 37°C Aguilar *et al.* (1995) used a glucose-pregrown inoculum taken from a chemostat in steady-state at HRT = 18 days, pH = 7.2 and at the influent glucose concentration of 10 g/l. A complex mixture of microorganisms (cocci, rods, filamentous forms) was found in the reactor. In Fig. 2 the degradation

Table 3. The maximum specific biomass growth rates of microorganisms, μ_m, the ratio between initial biomass concentrations and yield coefficient, B_0/Y and their product for glucose pregrown inoculum

Microorganisms	Parameter		
	μ_m (per h)	B_0/Y (mg/l)	$\mu_m B_0/Y$ [mg/(l.h)]
Methanogens	—	—	40
Propionic syntrophs	—	—	11
i-Butyric syntrophs	0.05	2	0.1
n-Butyric syntrophs	0.05	76	3.8

curves of acetic, propionic, n-butyric and i-butyric acids are shown. If acetate and propionate kinetics follow the zero-order eqn (7), n-butyrate and i-butyrate follow the plate curve kinetics (10). Some parameters of the model are summarized in Table 3. From Fig. 2 it can be concluded that the value of the half-saturation coefficient for acetoclastic bacteria is low ($K_S < 50$ mg/l).

Comparing Fig. 1 and Fig. 2 one can see the difference in dynamics of VFA degradation, especially in propionate concentration. The main cause of that is the difference of initial concentrations of propionate degraders. According to Zoetemeyer *et al.* (1981), under the conditions mentioned above acetate and propionate are the main intermediates during glucose conversion.

In the acetate system modeled propionate forms through biomass decay, but the concentrations of it in the reactor and the degrader are both low. Comparing the maximum specific growth rate of propionic syntrophs given in Tables 1 and 2 one can see a rather low μ_m value obtained from Aguilar's data. However, van Kuijk and Stams (1995) reported the values of maximum specific growth rate of pure cultures of propionate oxidizing bacteria to be in the range of 0.024–0.23/day at 37°C.

n-Butyrate may be an intermediate product of carbohydrate, protein or lipid degradation but i-butyrate is produced from a particular substrate degradation (Allison, 1978). Angelidaki and Ahring (1995) showed that the specific degradation rate of n-butyrate is in the range of 0.52–1.39/day and of i-butyrate is in the range of 0.46–1.15/day at 37°C. Isomerization processes of n-butyrate to i-butyrate and back occur in that case. The values of maximum specific biomass growth rate of n-butyric syntrophs shown in Tables 2 and 3 are in the range of values summarized in Table 1.

A potential methanogenic activity for anaerobic sludge may be evaluated through the methane production rate (SMA test), or coenzyme F_{420} content in the sludge, using acetate as a substrate in batch experiments (Dolfig & Mulder, 1985; James *et al.*, 1990; Colleran *et al.*, 1992). An acetoclastic pathway of methanogenic activity may be evaluated using eqns (11) and (12).

Volatile fatty acids degradation kinetics with inhibition by other VFA

Model with inhibition effect

The cause of the plate curve kinetics of degradation of volatile fatty acids may be not only a low initial biomass concentration but an inhibition effect by other VFA. Propionate and butyrate are degraded by syntrophic consortia of acetogenic bacteria and methanogenic bacteria (Schink, 1992). A product inhibition of acetogenic bacteria can happen (Fukuzaki *et al.*, 1990b). For successful degradation of the short-chain fatty acids, efficient removal of the hydrogen produced is necessary.

Introducing non-competitive inhibition by another substrate the following system of equations may be written instead of eqn (3):

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -\frac{\mu_m}{Y}\frac{SB}{K_s+S}\frac{1}{1+I/K_i}$$

$$\frac{\mathrm{d}B}{\mathrm{d}t} = \mu_m\frac{SB}{K_s+S}\frac{1}{1+I/K_i} - K_d B \qquad (13)$$

where I is the inhibitor concentration. Neglecting biomass decay and assuming the simple zero-order kinetics of inhibitor removal with a rate constant k, the following solution for substrate concentration can be written instead of eqn (10) from (eqn 13) at $S K_s$ and $t \le I_0/k$:

$$S = S_0 - \frac{B_0}{Y}\left\{\frac{1+I_0/K_i}{1+I_0/K_i-k/K_i t}\right\}^{\mu_m K_i/k} \qquad (14)$$

where I_0 is the initial inhibitor concentration. At $t \le I_0/k$, when the inhibitor concentration becomes equal to zero, the substrate concentration follows eqn (9). Evidently, a lag-phase time for substrate degradation depends on the value of I_0. The following system (15) was written instead of eqn (13) for degradation kinetics of a mixture of VFA:

$$\frac{\mathrm{d}S_a}{\mathrm{d}t} = -\frac{\mu_{ma}}{Y_a}\cdot B_a\cdot\frac{S_a}{K_{SA}+S_a}\cdot\frac{1}{1+S_b/K_{ib1}}$$

$$+\gamma_b\cdot\frac{\mu_{mb}}{Y_b}\cdot B_b\cdot\frac{S_b}{K_{SB}+S_b}$$

$$+\gamma_p\cdot\frac{\mu_{mp}}{Y_p}\cdot B_p\cdot\frac{S_p}{K_{SP}+S_p}$$

$$\cdot\frac{1}{1+S_a/K_{ia}}\cdot\frac{1}{1+S_b/K_{ib2}}$$

$$\frac{\mathrm{d}Q}{\mathrm{d}t} = \alpha_a\cdot\frac{\mu_{ma}}{Y_a}\cdot B_a\cdot\frac{S_a}{K_{SA}+S_a}$$

$$\cdot\frac{1}{1+S_b/K_{ib1}}+\alpha_b\cdot\frac{\mu_{mb}}{Y_b}$$

$$\cdot B_b\cdot\frac{S_b}{K_{SB}+S_b}+\alpha_p\cdot\frac{\mu_{mp}}{Y_p}$$

$$\cdot B_p\cdot\frac{S_p}{K_{SP}+S_p}\cdot\frac{1}{1+S_a/K_{ia}}$$

$$\frac{1}{1+S_b/K_{ib2}}$$

$$\frac{\mathrm{d}S_p}{\mathrm{d}t} = -\frac{\mu_{mp}}{Y_p}\cdot B_p\cdot\frac{S_p}{K_{SP}+S_p}$$

$$\cdot\frac{1}{1+S_a/K_{ia}}\cdot\frac{1}{1+S_b/K_{ib2}}$$

$$\frac{\mathrm{d}S_b}{\mathrm{d}t} = -\frac{\mu_{mb}}{Y_b}\cdot B_b\cdot\frac{S_b}{K_{SB}+S_b}$$

$$\frac{\mathrm{d}B_a}{\mathrm{d}t} = \mu_a\cdot B_a\cdot\frac{S_a}{K_{SA}+S_a}\cdot\frac{1}{1+S_b/K_{ib1}}-K_{da}\cdot B_a$$

$$\frac{\mathrm{d}B_p}{\mathrm{d}t} = \mu_p\cdot B_p\cdot\frac{S_p}{K_{SP}+S_p}\cdot\frac{1}{1+S_a/K_{ia}}\cdot\frac{1}{1+S_b/K_{ib2}}$$

$$-K_{dp}\cdot B_p$$

$$\frac{\mathrm{d}B_b}{\mathrm{d}t} = \mu_b\cdot B_b\cdot\frac{S_b}{K_{SB}+S_b}-K_{db}\cdot B_b \qquad (15)$$

where S_a, S_b, S_p are the concentrations of acetate, butyrate and propionate, respectively; B_a, B_b, B_p are the concentrations of acetoclastic methanogens, butyrate-utilizing syntrophs and propionate-utilizing syntrophs, respectively; Q is the methane volume released per unit volume of reactor; μ_{ma}, μ_{mb}, μ_{mp} are the maximum specific growth rates of the corresponding bacteria; k_{da}, k_{db}, k_{dp} are the biomass-decay coefficients of the corresponding bacteria; Y_a, Y_b, Y_p are the yield coefficients of the corresponding bacteria; K_{SA}, K_{SB}, K_{SP} are the half-saturation constants of the corresponding bacteria, K_{ia}, K_{ib1}, K_{ib2} are the inhibition constants for the corresponding substrates; γ_b, γ_p are the respective
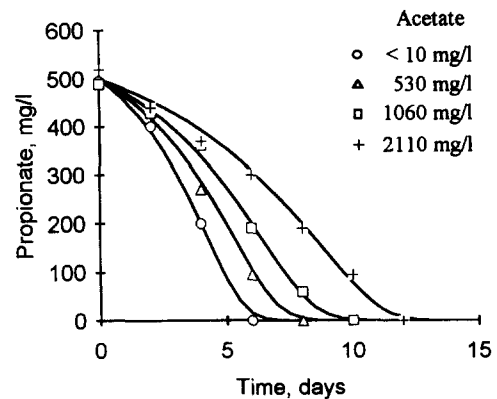

Fig. 3. Progress curves of propionic acid degradation by a synthetic medium-pregrown inoculum at different initial concentrations of acetic acid: lines—model equation (15) predictions; symbols—experimental data (Mawson *et al.*, 1991).

stoichiometric coefficients of transformation of butyrate and propionate into acetate; α_a, α_b, α_p are the respective stoichiometric coefficients of transformation of acetate, butyrate and propionate into methane. Hydrogen-utilizing methanogenic bacteria were not included into the equation system (15). It suggested that hydrogen transforms into methane immediately because in the anaerobic systems described below hydrogen content in the biogas could not be measured. The following values were taken for stoichiometric coefficients:

$$\gamma_b = 0.54 \text{ mg COD Bu/mg COD Ac,}$$

$$\gamma_p = 0.38 \text{ mg COD Pr/mg COD Ac}$$

$$\alpha_a = 0.38 \text{ mg COD Ac/ml CH}_4$$

$$\alpha_b = 0.10 \text{ mg COD Bu/ml CH}_4$$

$$\alpha_p = 0.38 \text{ mg COD Pr/ml CH}_4$$

The Monod kinetic with non-competitive inhibition functions was selected related to the sums of ionized and unionized forms of volatile fatty acids because the authors of molasses and synthetic media experimental systems described below did not show data for pH changes.

Synthetic media-pregrown inoculum

A series of batch experiments were performed at 37°C by Mawson *et al.* (1991) to determine the effect of elevated acetic acid concentrations on the degradation of propionic acid and vice versa. Effluent from a 5.0-l digester, operated as batch-fed with modified Hansson medium (Hansson, 1979) every second day, was used as the seed material. In Fig. 3 the plate curves of propionic acid utilization with different initial acetate concentrations using the equation system (15) are shown. The length of plate depends on the initial concentration of propionate degraders and the initial concentration of acetate as inhibitor. Mawson *et al.* (1991) noted that an inhibition effect could not be attributed to pH, which varied by only 0.1–0.3 units of pH. They analyzed the data using the simple logistic equation in a formal way which did not allow evaluation of the traditional product-inhibition constants.

Some parameters of the equation system (15) are summarized in Table 4.

Molasses pregrown inoculum

During the batch experiments with VFA mixtures (ratio between initial concentrations of acetate, propionate and butyrate equal to 25:35:40) two feedings were performed by Ozturk (1991). The granular sludge from a thermophilic anaerobic digester (55°C), fed on pure molasses, was used as a seed material. The liquid of the batch reactor was continuously stirred. The pH level remained fairly constant at 7.0.

The first stage of experiments lasted about 163 h after feeding. During that time the total VFA concentration decreased almost to zero. Then an additional feeding was performed with the same initial VFA mixture with an initial biomass corresponding to the end of the first stage. After that the second stage of the batch experiment began.

The equation system (15) dynamics tested on Ozturk's experimental data, with the same set of constants for both feedings, is shown in Figs 4 and 5. For the Ozturk system, which was more complicated than Augilar's systems analyzed above, similar results were obtained for two sets of values of constant (Table 5). It can be concluded that the values of maximum specific biomass growth rate, μ_m, and half-saturation coefficient, K_S, are correlated. So, a set of the values of constants which can be obtained from a complex model are not singular, in contrast to the values obtained directly from the simple eqns (11) and (12).

Comparing the values of kinetic constants summarized in Table 1 with the corresponding values in Table 5, one can say that high values of μ_m and K_S (Fig. 4) are less evident than the lower values of constants (Fig. 5). For thermophilic propionate syntrophs the maximum specific biomass growth rate proved to be significantly higher than the corresponding values for mesophilic propionate syntrophs shown in Table 1. The values of the acetate inhibition constant, K_{ia}, obtained for Mawson's and Ozturk's data (Tables 4 and 5) were comparable with the value reported by Fukuzaki *et al.* (1990b) and equal to about 800 mgCOD/l of total acetate concentration (unionized and ionized) at the pH of 7.2.

There was a significant lag time in propionate degradation after the first feeding (Figs 4 and 5). Propionate was converted to acetate only after the initial butyrate and acetate were completely degraded. The kinetics of propionate degradation presented in Fig. 4 in the period of 45 days $\leq t \leq 90$

Table 4. The initial biomass concentrations, B_0, and the constants of model equation (15) for a synthetic-medium-pregrown inoculum

Microorganisms	B_0 (mg/l)	μ_m (per day)	K_S (mg/l)	Y (mg/mg)	K_d (per day)	K_{ib} (mg/l)	K_{ia} (mg/l)
B_a	0.10	0.50	100	0.02	0	—	—
B_p	2.1	0.44	100	0.02	0	—	2000

days may be approximated by eqn (14) and at $t \geq 90$ days they may be approximated by eqn (10).

It can be concluded that there were rather high concentrations of acetoclastic methanogens and butyrate-oxidizing acetogens in the initial sludge because acetate and butyrate were the intermediate products of anaerobic molasses conversion. Some of the lag time for acetate conversion to methane and carbon dioxide is caused by butyrate inhibition. After a second feeding (Figs 4 and 5) propionate-degradation kinetics approximately follow a zero-order reaction, without delay, because of an increase in the population of the propionate-degrading bacteria after the first feeding.

According to the equation system (15), propionate degradation is inhibited by butyrate and acetate; acetate degradation is inhibited by butyrate; but butyrate degradation is not inhibited at all. All

inhibitors were considered as the total (unionized and ionized) concentrations. However, generally unionized VFA inhibition must be taken into account.

For the case studies analyzed using Monod kinetics with the sum of unionized and ionized forms of VFA as the real substrates, good coincidences between the model and the batch data were obtained. As was mentioned in the Introduction, Haldane kinetics [eqn (2)] transform into Monod kinetics (1) at $K_{in} \to \infty$ at a constant pH. An acid and alkaline correction of pH inhibition introduced into the Monod kinetics could improve the fitting of the model to experimental data.

Allowable acetate loading

The batch kinetics of volatile fatty acids modeled above were used to evaluate the constants of a
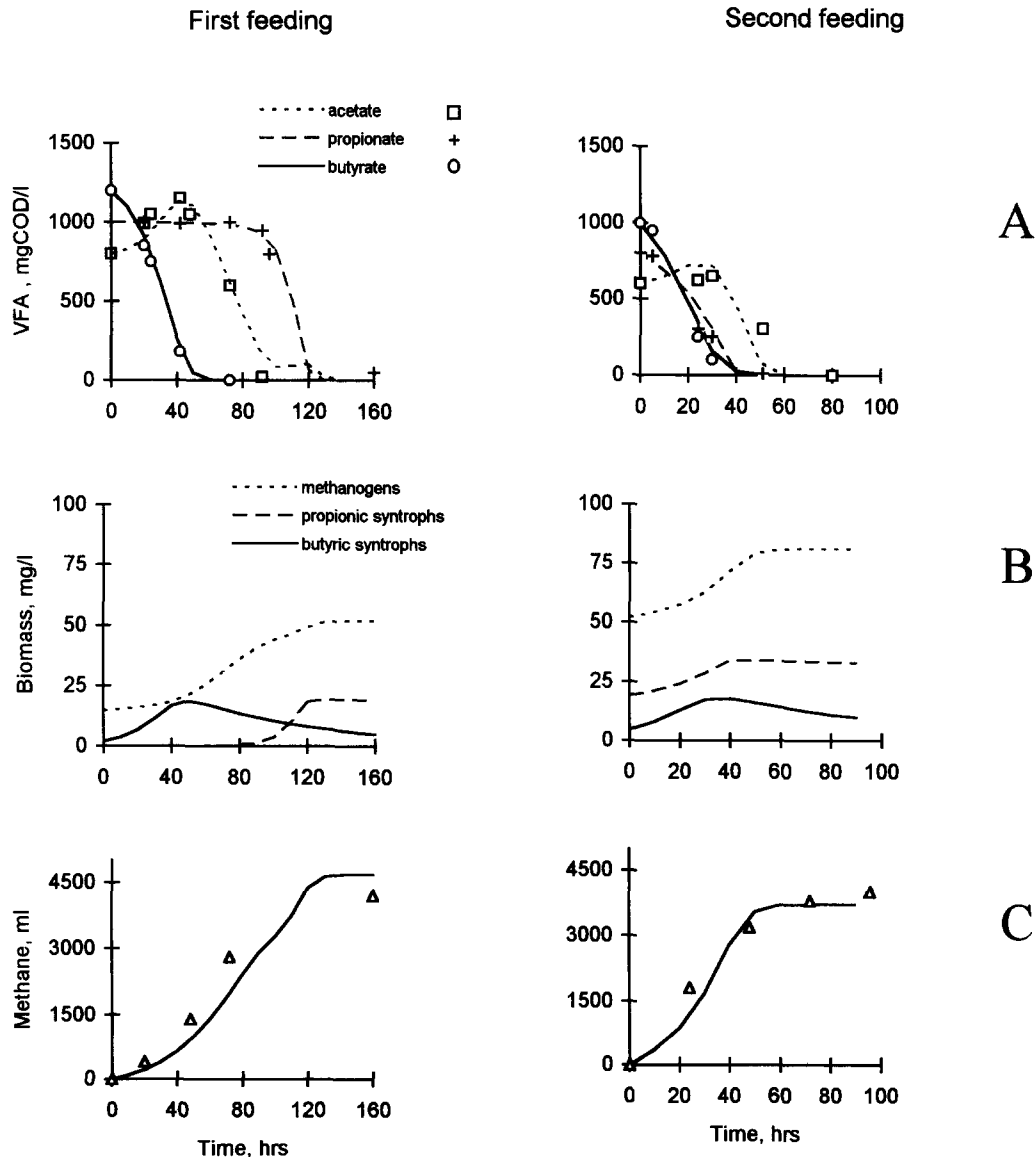

Fig. 4. Progress curves of (a) butyric, acetic and propionic acids, (b) methanogens, butyric- and propionic-syntrophs and (c) methane volume released by a molasses-pregrown inoculum after the first and second feedings: lines—model equation (15) predictions with the high values of μ_m and K_S (see Table 5); symbols—experimental data (Ozturk, 1991).

microorganism inoculum taken from continuous-flow reactors. Costello *et al.* (1991b) used only one set of biological parameters in the comparison of their simulation model with each set of experimental data for three independent case studies with various reactor types. Batch kinetics show that the model satisfactorily predicts the relative accumulation of acids· at ·moderate loading. With changes of model

parameters large improvements may be obtained in the model fit.

The influence of solids retention time, as well as organic loading, on bacterial trophic populations is well known for the continuous-flow systems. There are two morphological types of acetoclastic metha-nogens: bacilli and sarcinae (Zehnder *et al.*, 1982). *Methanothrix* is dominant at long SRT, resulting in
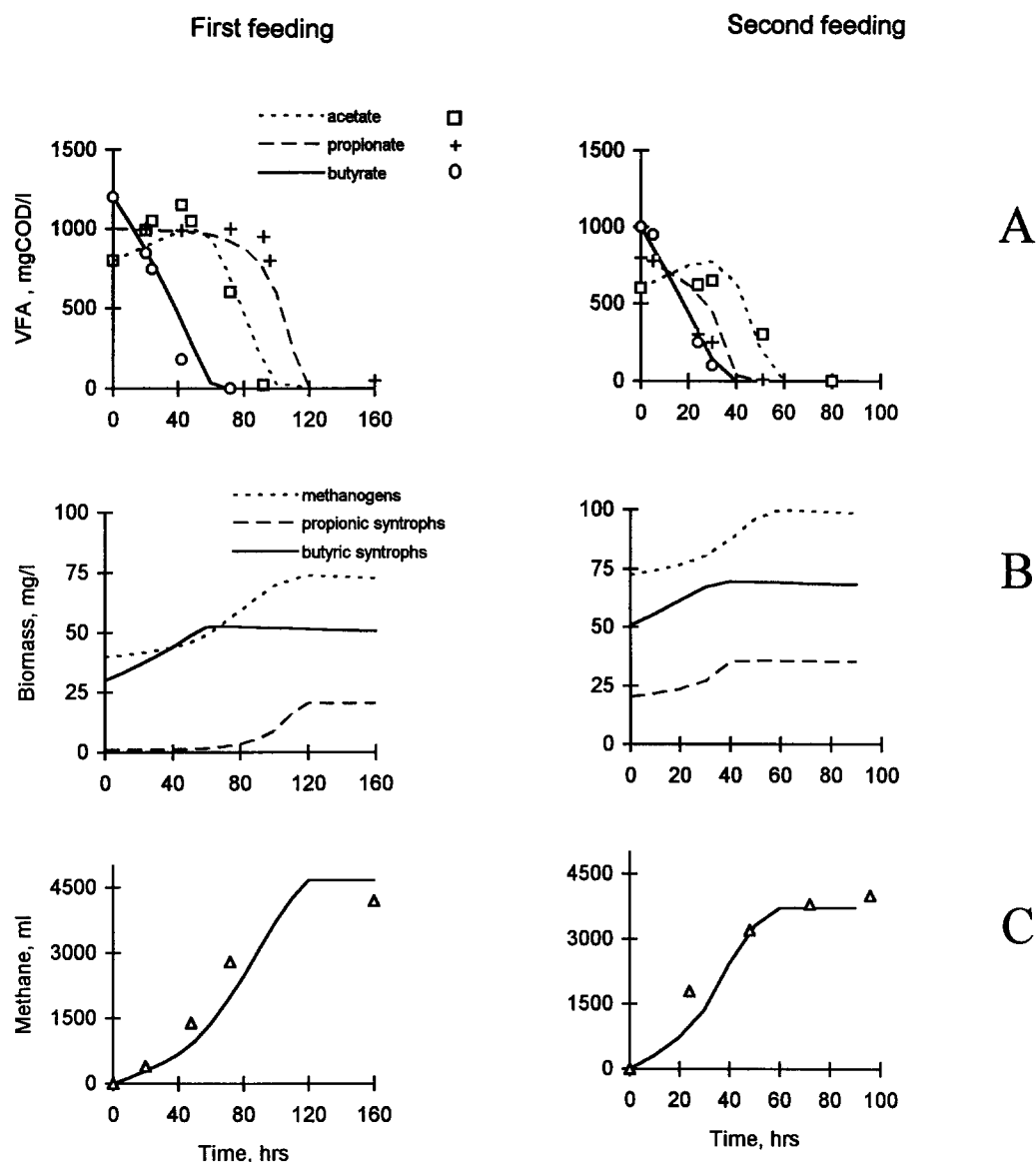

Fig. 5. Progress curves of (a) butyric, acetic and propionic acids, methanogens, (b) butyric and propionic syntrophs and (c) methane volume released by molasses pregrown inoculum after first and second feedings: lines—model (15) predictions with the low values of μ_m and K_S (see Table 5); symbols—experimental data (Ozturk, 1991).

Table 5. Two values of the initial biomass concentrations, B_0, with the constants of model equation (15) obtained for a molasses-pregrown inoculum

Microorganisms	B_0 (mg/l)	μ_m (per day)	K_S (mgCOD/l)	Y (mg/mgCOD)	K_d (per day)	K_{ib} (mgCOD/l)	K_{ia} (mgCOD/l)
B_a	15/40	0.6/0.25	300/30	0.02	0.0/0.01	300	—
B_p	0.05/1.0	4.0/1.5	300/30	0.02	0.2/0.01	200	650/1000
B_b	2.0/30	2.5/0.25	450/30	0.02	0.3/0.01	—	—

Table 6. Kinetic coefficients for mesophilic acetoclastic bacteria

Culture	pH	Temperature (°C)	μ_m (per day)	K_S (mg/l)	Reference
M. barkeri strain 227	—	36	0.6	320	Smith & Mah, 1978
M. barkeri strain 227	—	36	0.43	—	Smith & Mah, 1980
M. barkeri	—	—	0.595	965	Tretter *et al.*, 1988
M. barkeri DSM 804	6.3	37	0.19	257	Wandrey & Aivasidis, 1983
Methanothrix soehn.	—	33	0.09	30	Zehnder *et al.*, 1982
Methanothrix soehn.	7.4–7.8	37	0.17	45	Huser *et al.*, 1982

— Not given.

low values of μ_m and K_S, while *Methanosarcina* is dominant at short SRT, resulting in high values of μ_m and K_S. Kos (1993) summarized the kinetic coefficients of acetoclasic methanogens as described by the Monod [eqn (1)] and Haldane [eqn (2)] functions. Some values of μ_m and K_S for pure cultures with Monod kinetics are presented in Table 6.

A theoretical analysis of substrate consumption by bacterial cell populations with different sizes and forms was performed by Vasiliev and Vavilin (1992). It was shown that the growth rates of cocci and rods are dependent upon the substrate concentrations. Using a Monod-type approximation it was demonstrated that at low substrate concentration the rods having the low K_S and μ_m values grow much faster than cocci having the greater K_S and μ_m values. With increasing substrate concentration the cocci grow faster then the rods.

In this paper the Monod and Haldane functions, included in the ⟨METHANE⟩ model, were tested on the experimental data of Noike *et al.* (1985), where anaerobic conversion of acetate was studied in a continuous-flow system at 37°C and under a wide range of influent acetate concentrations and

Table 7. The feed composition in the acetate system of Noike *et al.* (1985)

Component	Concentration (mg/l)
$(NH_4)_2HPO_4$	700
$MgSO_4.7H_2O$	250
NH_4Cl	850
KCl	750
$MgCl_2.6H_2O$	810
$FeCl_3.6H_2O$	420
$CoCl_2.6H_2O$	18
$NaHCO_3$	6720

SRT. The chemical composition of feed solution and some parameters from the ⟨METHANE⟩ model are presented in Tables 7 and 8.

Figure 6 shows the steady-state model variables at the different influent acetate concentrations. It can be concluded that Monod and Haldane kinetics do not fit the acetate experimental data very well. The models describe the system failure at high influent acetate concentrations of about 65 g/l because of pH inhibition (Monod kinetics) or of non-ionized acetate inhibition (Haldane kinetics). As was mentioned in the Introduction the acid and alkaline pH inhibitions were either incorporated with substrate inhibition (Haldane kinetics) or by assuming some empirical-type pH inhibition function (Monod kinetics).

However, in the experimental system considered, with an increase in feed acetate concentration the effluent substrate concentration continued to increase. In fact, the effluent substrate concentration should be constant irrespective of the influent substrate concentration in Monod kinetics and should decrease slightly in Haldane kinetics. So, neither the Monod-type kinetics nor the Haldane kinetics could describe methanogenesis from acetate perfectly under wide ranges of SRT or organic-loading values.

Microscopic examination of the methanogenic bacteria (Noike *et al.*, 1985) showed shifts of dominant morphological type under different conditions. The same authors determined the half-saturation coefficient, K_S, at different SRT:14 mg/l (SRT ≥ 9.6 days), 40 mg/l (SRT = 6.5 days) and 395 mg/l (SRT = 4.5 days). It can be concluded that *Methanothrix* (with low μ_m and K_S values) is dominant at the low effluent-acetate concentration (high SRT or low substrate loading), while *Methanosarcina* (with high μ_m and K_S values) is dominant at the high effluent-

Table 8. Some parameters of the ⟨METHANE⟩ model using Monod and Haldane functions for acetate degradation

Function	μ_m (per day)	K_S (mg/l)	Y (mM/mM)	K_{ia} (g/l)	K_{pH1}	K_{pH2}[a]
Monod	0.30	200	0.015	—	3.15	2.95
Haldane	0.45	20	0.015	50	—	—

[a]For pH inhibition two constants were used. The first constant was the parameter value at which the growth rate of microorganisms was reduced to half of the maximum value. The second constant corresponded to reduction of the growth rate to one-hundredth of maximum.

acetate concentration (low SRT or high substrate loading). A high threshold value of acetate of between 15 and 130 mg/l, at which biomass ceases to grow, has been reported by Pavlostathis and Giraldo-Gomez (1991) for *Methanosarcina*.

In Fig. 6 the graphs are shown using the Monod and Haldane kinetics with correction. The following equation was used for a half-saturation coefficient as a function of influent acetate concentration, S_{inf}, and SRT:
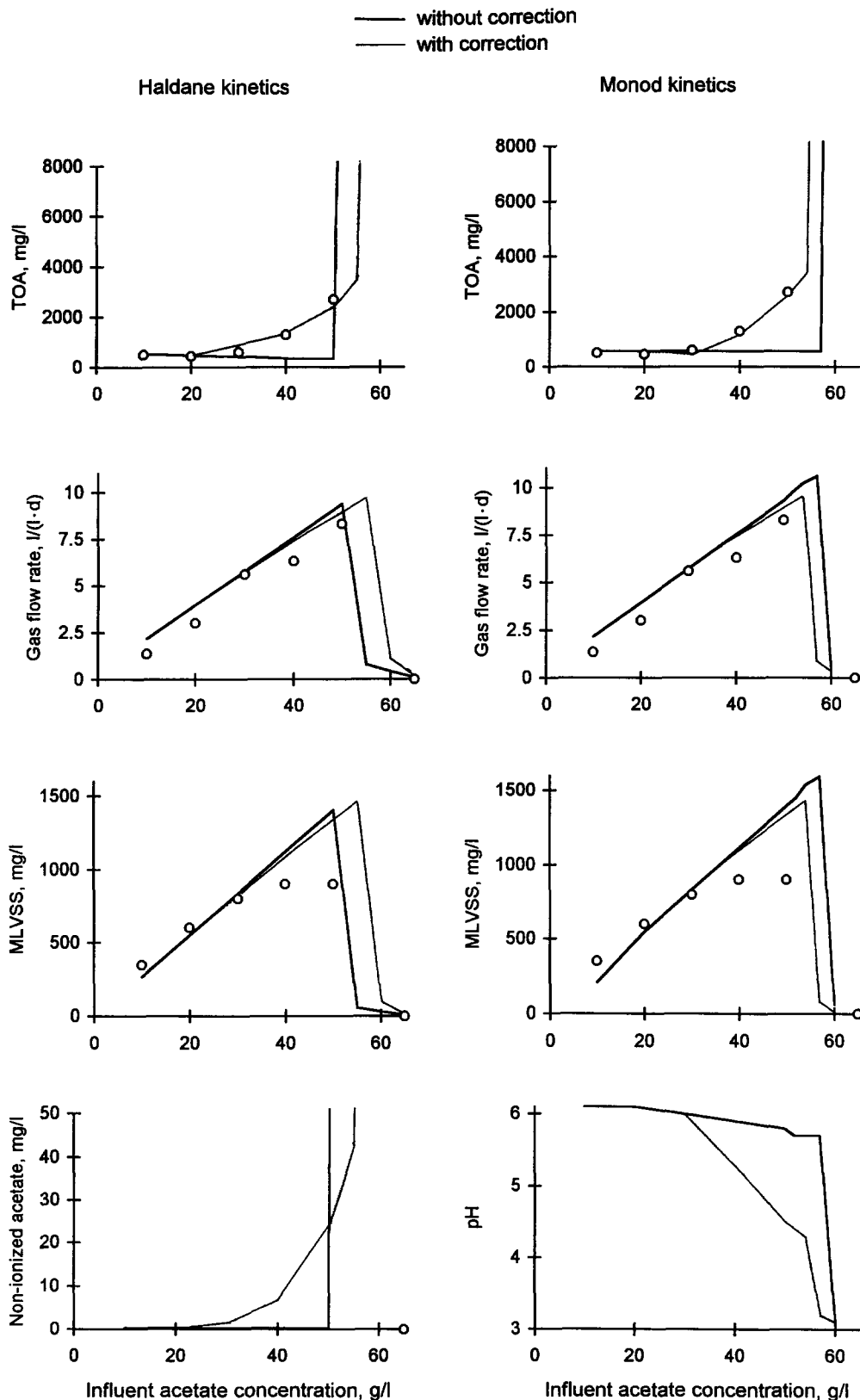

Fig. 6. Steady-state values of the different components in the reactor according to the ⟨METHANE⟩ model with a change of influent acetate concentration at SRT of 4.5 days. Experimental data (symbols) are taken from Noike *et al.* (1985). The original and corrected Monod and Haldane functions were used in the model curves.

Table 9. Some parameters of ⟨METHANE⟩ model with corrected Monod and Haldane functions for acetate degradation

Function	S_{inf} (g/l)	μ_m (per day)	n	k $\left(\dfrac{day}{mg/l}\right)^n mg/l$	K_s (mg/l)	Y (mM/mM)	K_{ia} (g/l)	K_{pH1}, $K_{pH2}{}^a$
Monod	10	0.3	—	—	200	0.015	—	3.2, 2.65
	20	0.3	—	—	200	0.015	—	3.2, 2.65
	30	0.435	3.5	1.79×10^{-11}	419[b]	0.015	—	3.2, 2.65
	40	0.435	3.5	1.79×10^{-11}	1146[b]	0.015	—	3.2, 2.65
	50	0.435	3.5	1.79×10^{-11}	2503[b]	0.015	—	3.2, 2.65
Haldane	10	0.45	—	—	20	0.015	50	—
	20	0.45	—	—	20	0.015	50	—
	30	0.585	5.5	1.33×10^{-19}	135[b]	0.015	45	—
	40	0.585	5.5	1.33×10^{-19}	657[b]	0.015	45	—
	50	0.585	5.5	1.33×10^{-19}	2243[b]	0.015	45	—

[a]For pH inhibition two constants are used. The first constant means the parameter value at which the growth rate of microorganisms is reduced to half of the maximum value. The second constant corresponds to reduction of the growth rate to one-hundredth of maximum.
[b]The values of the half-saturation constant are calculated by equation (16).

$$K_S = k \left(\frac{S_{inf}}{SRT} \right)^n \qquad (16)$$

where k and n are the constants. The corresponding parameter values for corrected Monod and Haldane kinetics with a SRT of 4.5 days are shown in Table 9. In both cases the model fits the experimental results substantially better than the uncorrected model does.

CONCLUSION

Acetogenis and acetoclastic methanogenic activities of different pregrown inocula can be evaluated from batch experiments using Monod kinetics with mutual inhibition of VFA. For a continuous-flow reactor fed on acetate the changes of K_S and μ_m values following a population shift must be taken into account for high acetate loadings. With such correction no difference was found between the Monod kinetics using the sum of unionized and ionized acetate concentrations as the real substrate and the Haldane kinetics using unionized acetate concentration as the real substrate, if an additional pH inhibition was included in the Monod equation.

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