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Parameter Identification of Thermophilic Anaerobic Degradation of Valerate

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Abstract

The considered mathematical model of the decomposition of valerate presents three unknown kinetic parameters, two unknown stoichiometric coefficients, and three unknown initial concentrations for biomass. Applying a structural identifiability study, we concluded that it is necessary to perform simultaneous batch experiments with different initial conditions for estimating these parameters. Four simultaneous batch experiments were conducted at 55°C, characterized by four different initial acetate concentrations. Product inhibition of valerate degradation by acetate was considered. Practical identification was done optimizing the sum of the multiple determination coefficients for all measured state variables and for all experiments simultaneously. The estimated values of kinetic parameters and stoichiometric coefficients were characterized by the parameter correlation matrix, the confidence interval, and the student's *t*-test at 5% significance level with positive results except for the saturation constant, for which more experiments for improving its identifiability should be conducted. In this article, we discuss kinetic parameter estimation methods.

Index Entries: Anaerobic; mathematical modeling; identifiability; parameter estimation; optimization; valerate.

Introduction

As in other wastewater treatment processes, anaerobic digestion models have become a major tool to increase the understanding of the

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underlying biodegradation mechanisms; to communicate using it as a common ground; in the design of treatment plants, control, and operating strategies; and for training operators and process engineers *(1)*. The aim of universality and of describing the general process with a set of differential equations, in which each subset or each individual equation describes a given subprocess, explains the evolution of the generation of dynamic and structured models. A joint effort to create a unified language and to propose a general structured model has resulted in the anaerobic digestion model IWA-Anaerobic Digestion Model No. 1 (ADM1) *(2)*.

A good mathematical model needs accurate and significant parameter values in order to be useful. Although the issue of parameter estimation methods is important, it is often neglected. Whereas the application of a systematized methodology for model identification and parameter significance evaluation has been a progressive and constructive activity for aerobic activated sludge processes *(3,4)*, the application for anaerobic systems is still scarce *(5,6)*. This rule can be synthesized with the following steps:

1. Set up the mathematical model and define the calibration problem.
2. Create a preliminary experimental design approach.
3. Conduct a structural identifiability study.
4. Create an experimental design.
5. Conduct practical identification and statistical characterization of estimated parameter values.
6. Return to step 4 or 1 if step 5 provides low significance levels for calculated parameters or if the model's structure cannot explain experimental behavior, respectively.

Identification of parameters describing the anaerobic decomposition of valerate has not received special attention in the literature, although it is an important intermediate during anaerobic degradation of proteins *(7)*. Butyrate and valerate are thought to be degraded by the same organism *(2,8,9)*, and, hence, the ADM1 model proposes that all kinetic parameters for valerate and butyrate be lumped. This assumption has not yet been proven.

The objective of the present work was to apply the general identification rule to the anaerobic degradation model of valerate in thermophilic range, using batch experiments. Fractionation of valerate into *n*- and isoforms as a function of partial pressure of hydrogen *(10)* was not considered and they are lumped together in the present work.

Set Up of Mathematical Model and Definition of Calibration Problem

Dynamic and structured biokinetic models are usually defined by n state variables, C_i, $i = 1, 2, \ldots n$, following n nonlinear differential equa-

tions, and involving *m* processes. The set of ordinary differential equations is represented by

$$\frac{dC_i}{dt} = \sum_{j=1}^{m} \nu_{ij}\rho_j \qquad i = 1, 2, \ldots n, \qquad j = 1, 2, \ldots m \tag{1}$$

in which ν_{ij}, $i = 1, 2, \ldots n$, $j = 1, 2, \ldots m$, are the yield coefficients; and ρ_j is the *j*th component of the *m* processes rates vector. First-order, Monod, or other appropriate kinetics, modified by an inhibition expression, usually express these rates. In the case in which process *j* is the growth of the microorganism population *j*, with *r* as the substrate and *s* as inhibitor, the rate could be expressed as

$$\rho_j = \mu_{mj} \frac{C_r}{(K_S)_{jr} + C_r} \frac{(K_{in})_{js}}{(K_{in})_{js} + C_s} C_j \qquad r, s \neq j \tag{2}$$

in which μ_{mj} is the maximum growth rate of microorganisms *j* with a concentration C_j; $(K_S)_{jr}$ is the saturation constant of population *j* for the substrate *r* with a concentration C_r; and $(K_{in})_{js}$ is the inhibition constant of population *j* owing to substrate *s* with a concentration C_s, considering the inhibition process expressed as noncompetitive reversible inhibition. The set of differential equations is completed with the initial conditions for the *n* state variables. *S* and *X* notations are usually used to indicate substrates and microorganisms, respectively. In the present work all state variables are named *C* in order to simplify notation.

In the previous general model expression, calibration consists of the calculation of some unknown kinetic parameters, μ_{mj}, $(K_S)_{jr}$, or $(K_{in})_{js}$ for some *j* processes, and for the *r* or *s* substrates, and/or some unknown yield coefficients ν_{ij}, taking into account the measured values obtained for some state variables. Additionally, some initial conditions could also be unknown, such as initial biomass concentration. For the general problem formulation, the three kinds of unknown parameters are grouped and named θ_k, $k = 1, 2, \ldots p$, with *p* being the total number of unknown parameters.

Stoichiometry of Anaerobic Degradation of Valerate

The valerate-degrading acetogenic step follows the β-oxidation mechanism *(8)*, producing 1 mol of acetate and 1 mol of propionate from 1 mol of valerate. If the bacterial population degrading valerate and butyrate is considered the same *(8)*, the carbon-utilizing mechanisms could be considered similar. Based on the butyrate-degrading acetogenic step from ref. *11*, in which the carbon used for biomass synthesis is assumed to be taken from acetate, the stoichiometry of valerate degradation can be expressed in a general form as follows:

$$\begin{gathered} C_5H_{10}O_2 + aNH_3 + bCO_2 + dH_2O \rightarrow \\ aC_5H_7NO_2 + cC_2H_4O_2 + C_3H_6O_2 + eCH_4 \end{gathered} \tag{3}$$

Table 1
Yield Coefficients Matrix[a]

State variable C_i, i = 1, 2, … 8	Process j, j = 1, 2, … 6					
	1	2	3[b]	4	5	6
1 Acetate degraders	1			–1		
2 Propionate degraders		1			–1	
3 Valerate degraders			1			–1
4 Acetate	–24.1352	8.0057	$\frac{60\theta_5}{113\theta_4}$			
5 Propionate		–10.5658	$\frac{74}{113\theta_4}$			
6 Valerate			$\frac{-102}{113\theta_4}$			
7 N-NH_3	–0.1504	–0.1504	–0.1504			
8 CH_4	6.0821	1.5087	$\frac{16A}{113\theta_4}$			

[a]Units are given as g of C_i/g of biomass.
[b]$A = -2.5\theta_4 - \theta_5 + 1.5$.

Balancing Eq. 3, two unknown coefficients are enough to calculate the rest of coefficients,

$$\begin{aligned} b &= 2.5a + c - 0.5 \\ d &= -3a + 1 \\ e &= -2.5a - c + 1.5 \end{aligned} \tag{4}$$

Coefficients a and c will be the θ_4 and θ_5 parameters to be estimated. Yield coefficients calculated from this stoichiometry are shown in Table 1. Yield coefficients for propionate and acetate anaerobic decomposition are taken from ref. *11*.

Reaction Rates

The model is expressed as in Eq. 1, using eight state variables defined in Table 1, and 6 processes with reaction rates defined in Table 2. Associated kinetic parameters for acetate and propionate degradation (Table 3) are adopted from ref. *11*. Reaction rate for valerate degraders considers valerate as unique substrate and acetate as inhibitor, with maximum growth constant μ_{m3} as unknown parameter θ_1, saturation constant $(K_S)_{36}$ as unknown parameter θ_2, and inhibition constant owing to acetate $(K_{in})_{34}$ as θ_3. Inhibition by acetate is assumed since acetate is an inhibitor of butyrate *(12)*, and it has been observed in preliminary experiments. Inhibition by hydrogen is not considered in the present work, but it would be taken into account if partial pressure of hydrogen were high enough to observe an

Table 2
Rates Vector Considered in Model

Process j	Rates vector ρ_j, $j = 1, 2, \ldots 6$
Acetate degraders growth	$\rho_1 = \mu_{m1} \dfrac{C_4}{(K_S)_{14} + C_4} \dfrac{(K_{in})_{17}}{(K_{in})_{17} + C_7} C_1$
Propionate degraders growth	$\rho_2 = \mu_{m2} \dfrac{C_5}{(K_S)_{25} + C_5} \dfrac{(K_{in})_{24}}{(K_{in})_{24} + C_4} C_2$
Valerate degraders growth	$\rho_3 = \mu_{m3} \dfrac{C_6}{(K_S)_{36} + C_6} \dfrac{(K_{in})_{34}}{(K_{in})_{34} + C_4} C_3$
Acetate degraders decay	$\rho_4 = k_{d1} \cdot C_1$
Propionate degraders decay	$\rho_5 = k_{d2} \cdot C_2$
Valerate degraders decay	$\rho_6 = k_{d3} \cdot C_3$

Table 3
Kinetic Parameters Used and Identification of Unknown Parameters

	Kinetic parameter[a]			
Microorganisms group	μ_m (d^{-1})	K_s (g /L)	K_i (g/L)	k_d (d^{-1})
Acetate degraders	0.6	0.12 (Ac)	0.26 (NH_3)	0.03
Propionate degraders	0.54	0.259 (Pr)	0.96 (Ac)	0.027
Valerate degraders	θ_1	θ_2 (Val)	θ_3 (Ac)	$0.05 \cdot \theta_1$

[a]Ac, acetate; Pr, propionate; Val, valerate.

accumulation of propionate or valerate not explained by the inhibition owing to acetate. Decay rate constant for valerate degraders k_{d3} is assumed to be 5% of the maximum growth rate for this population. This value is in accordance with data found in reviews about kinetic parameter values for acetate, propionate, and butyrate degraders *(2,13)*, and it has been assumed for mathematical simulations in refs. *11* and *14* with very satisfactory results.

In batch experiments using digested sludge or manure as inoculum, the initial concentration of every bacterial population is not known. Therefore, three more unknown parameters must be added: θ_6, initial concentration of acetate degraders; θ_7, initial concentration of propionate degraders; and θ_8, initial concentration of valerate degraders. The present problem presents a p value of 8.

Preliminary Experimental Design Approach

Experiments for calibration must be designed in order to have an observable and identifiable system. For steady-state models, the product $\mu_{mj} \cdot C_j$ usually can be identified, but not separately, owing to the steadiness of the population jth. For this reason, a change in C_j evolution must be forced out by introducing a pulse or a step in an appropriate feeding C_s component.

By using batch experiments, it is possible to ensure time evolution on all state variables with the appropriate experimental design, assisting in parameter identifiability. If batch experiments are performed in small vials, repetitions can be programmed at the same time, ensuring the same values for the known initial conditions. It is also possible to perform simultaneous experiments with equal unknown initial conditions for some variables and different unknown initial conditions for others. In the present work, the experimental methodology is based on simultaneous batch experiments.

Structural Identifiability Study

Prior to a formulation of an algorithm for approximation of unknown parameters, structural identifiability must be studied. The concept of structural identifiability is related to the possibility of giving a unique value to each parameter of a mathematical model from precise and noiseless experimental data *(15)*. For nonlinear models, there are a few methods for this study and they usually become extremely complex. When the output function is considered the vector components that are the measured state variables of the model, one of the available methods for the identifiability study consists of expanding in Taylor series the output function around the initial time. The principle of the method consists of expressing the measurement function and its successive derivatives as a function of the model parameters, and trying to obtain a system of independent equations for calculating the parameters *(16)*. For the present problem, the output function is defined by the state variables with an experimentally measurable evolution: acetate, propionate and valerate concentrations and methane production:

$$[C_4, C_5, C_6, C_8]^T \tag{5}$$

First and second derivatives of the four function components provide eight equations for a given batch experiment. A second simultaneous batch experiment with different initial conditions will provide another eight equations. If initial concentration of biomass is considered to be variable between simultaneous experiments, three more unknown parameters are added for every experiment.

There are several possible batch experimental designs, defined by simultaneous batch tests with different initial condition combinations for acetate, valerate, and biomass concentrations. With a simple batch experiment, with given initial values, only six independent equations are obtained and the eight unknown parameters cannot be identified. Using two simultaneous batch tests with different initial concentrations of acetate or valerate, and keeping the initial inoculum concentration constant, the 16 equations obtained provide 8 independent equations for calculating the eight parameters univocally.

Considering that different initial concentrations of acetate will facilitate the calculation of K_{in}, producing different time evolution of valerate

output responses, thus facilitating the calculation of K_S, the experimental design will be based on simultaneous batch tests with different initial concentrations of acetate within a wide range to ensure different valerate profiles, while trying to keep microorganism concentration constant. It is rather difficult to ensure this experimentally, but the identifiability study, described next, ensures that it is also possible to estimate univocally different initial biomass concentrations for this experimental design.

The identifiability procedure applied for this experimental design was as follows: We named $(C_i)'_z$ as the first derivative and $(C_i)''_z$ as the second derivative of the output function component i, $i = 4, 5, 6, 8$, for the batch z, $z = 1, 2$; θ_9, θ_{10}, θ_{11} as the initial microorganism concentration for the three population concentrations in the second batch experiment; and θ_{12} as the product $\theta_1 \cdot \theta_8$ and θ_{13} as the product $\theta_1 \cdot \theta_9$, that appear only separated in the second derivative of C_6. We obtained 11 independent equations by deriving one unknown parameter at every equation and substituting it in the following one, with the order indicated from Eqs. 6 to 16:

$$(C_6)'_1 \rightarrow \theta_3 = g_1(\theta_2, \theta_4, \theta_{12}) \quad (6)$$

$$(C_5)'_1 \rightarrow \theta_7 = g_2(\theta_2, \theta_4, \theta_{12}) \quad (7)$$

$$(C_4)'_1 \rightarrow \theta_6 = g_3(\theta_2, \theta_4, \theta_{12}) \quad (8)$$

$$(C_8)'_1 \rightarrow \theta_2 = g_4(\theta_4, \theta_5) \quad (9)$$

$$(C_6)''_1 \rightarrow \theta_1 = g_5(\theta_4, \theta_5, \theta_{12}) \quad (10)$$

$$(C_5)''_1 \rightarrow \theta_{12} = g_6(\theta_4, \theta_5) \quad (11)$$

$$(C_8)''_1 \rightarrow \theta_4 = g_7(\theta_5) \quad (12)$$

$$(C_6)'_2 \rightarrow \theta_{13} = g_8(\theta_5) \quad (13)$$

$$(C_5)'_2 \rightarrow \theta_{10} = g_9 \quad (14)$$

$$(C_4)'_2 \rightarrow \theta_9 = g_{10}(\theta_5) \quad (15)$$

$$(C_8)''_2 \rightarrow \theta_5 = g_{11} \quad (16)$$

The system can be solved by substituting every calculated parameter from Eq. 16, with θ_5 expressed independently of other parameters, to Eq. 6 backward. This result ensures that the system will be identifiable with a minimum of two simultaneous batch experiments with different initial acetate concentrations, regardless of different initial microorganism concentrations.

Practical Identification and Statistical Characterization of Estimated Parameter Values

The general calibration problem can be expressed as how to calculate θ_k, $k = 1, 2, \ldots p$, that minimizes a performance index function, such as the

sum of the squares of deviations between experimental and predicted values:

$$F(\theta_1, \theta_2, \ldots, \theta_p) = \sum_{i=1}^{n} \sum_{t=t_1}^{t_e} w_i \left\| (C_i)_t^P - (C_i)_t^E \right\|^2 \quad (17)$$

in which $(C_i)_t^P$ is the C_i value predicted by the model at time t and $(C_i)_t^E$ is the C_i measured experimental value at time t, considering experimental values at times $t_1, t_2, \ldots t_e$, for the state variables C_i belonging to the output function $i = 4, 5, 6, 8$. The relative importance of every variable is expressed by the use of appropriate weighting coefficients w_i *(4)*. In the present work, these coefficients are defined as

$$w_i = \frac{1}{\sum_{t=t_1}^{t_e} \left\| (C_i)_t^E - (\overline{C}_i)^E \right\|^2} \quad (18)$$

with $(\overline{C}_i)^E$ as the average of experimental values obtained for the measured *i*th component of the output function for a given experiment.

By using Eqs. 17 and 18, the performance index function F is the sum of the reciprocals of all obtained coefficients of multiple determination $(1 - R^2)$ of the model fitting, for every measured state variable. Therefore, F is tending to zero for the best global fitting and to minimize F is equivalent to maximize the sum of R^2 values. Available methods for optimizing function F and parameter estimation are discussed in ref. *1*. Depending on the experimental data obtained, F can be nonunimodal, and in this case, random search methods can approach the global optimum *(17)*.

The best parameter estimates will be characterized by the correlation matrix, calculated as in ref. *18*, and by the confidence interval (CI) and by student's *t*-test as in ref. *5*. A previous study of the Fisher Matrix Information *(4)*, obtained by evaluating the Jacobian matrix at the best estimated parameter values, can guide the experimental design, in order to decide, e.g., when it is more interesting to sample in order to obtain data allowing significant parameter calculation. Unfortunately, these values are not previously known. Taking into account that low variations in initial biomass concentration can produce large variations in output function, and high values in the corresponding Jacobian matrix elements, it is concluded that a dense sampling during the first hours of the experiment will help the identifiability of initial biomass concentration.

Materials and Methods

Experimental Design

Degradation of valerate was tested in 116-mL vials, containing 36 mL of BA medium *(19)* supplemented with 0.1 g/L of yeast extract, vitamins,

and 0.5 g/L of cysteine at four initial acetate concentration levels (0, 42, 88, and 168 m*M* acetate). Four milliliters of inoculum from a full-scale reactor operated on cattle manure with a 15-d hydraulic retention time at 55°C was added, after some days of rest in order to minimize volatile fatty acids (VFA) concentration in the inoculum. Initial concentration of valerate was 10 m*M* for all vials. Initial concentration of ammonia was 0.94 g of $N\text{-}NH_4$/L. The experiment was performed three times and the duration was 27 d. The 12 vials were closed with butyl rubber stoppers and sealed with aluminum crimps, after the displacement of air with a mixture of N_2:CO_2 gas (80:20). The vials were placed in a 55°C incubator and shaken vigorously by hand once a day. VFA and methane were analyzed according to Sorensen et al. *(20)*.

Mathematical and Computing Methods

For minimizing function *F*, Eqs. 17 and 18, and estimating unknown parameter values, two general optimization methods (random direct search and gradient methods) were combined, and the algorithm applied is shown in Fig. 1. The random direct search method applied was the Luus and Jaakola (LJ) method *(21)*. This method is based on using a number of randomly chosen test points over some region of feasible parameter values and contracting the region after every iteration, always starting the iteration with the best point found from the previous iteration as the center of the region. The estimated parameters by the previous method were used as first guess for a second optimization step, using the Polak-Ribière (PR) gradient conjugate method *(22)*. Convergence was increased by applying the steepest descent method *(22)* every *p*th iteration. Gradient values used in the PR method and Jacobian matrix, for deriving the parameter correlation matrix, were estimated numerically using second-order central differences operator. Convexity was ensured by estimating numerically the second derivative at the first guess point in the PR method. Maximum iteration number was fixed at 2500. Runge-Kutta-Fehlberg adaptive method of fifth order *(23)* was used to approximate solutions to the set of ordinary differential equations.

In the LJ procedure, two passes were used, the number of random evaluation of function *F* was 3000, and the contraction factor at the end of every iteration was chosen as 0.96. The process was repeated three times, with different collections of random numbers.

Results and Discussion

During the experiment, butyrate appeared in all vials at low-level concentrations (data not shown), with higher concentrations around d 8 and decreasing to zero at the end of the experiment. It is assumed that all butyrate detected was produced from the decomposition of the inoculum during the experiment. Since the initial concentration of butyrate was zero in all replicates and experiments, its decomposition and products evolution cannot be predicted and therefore not fitted. This phenomenon will

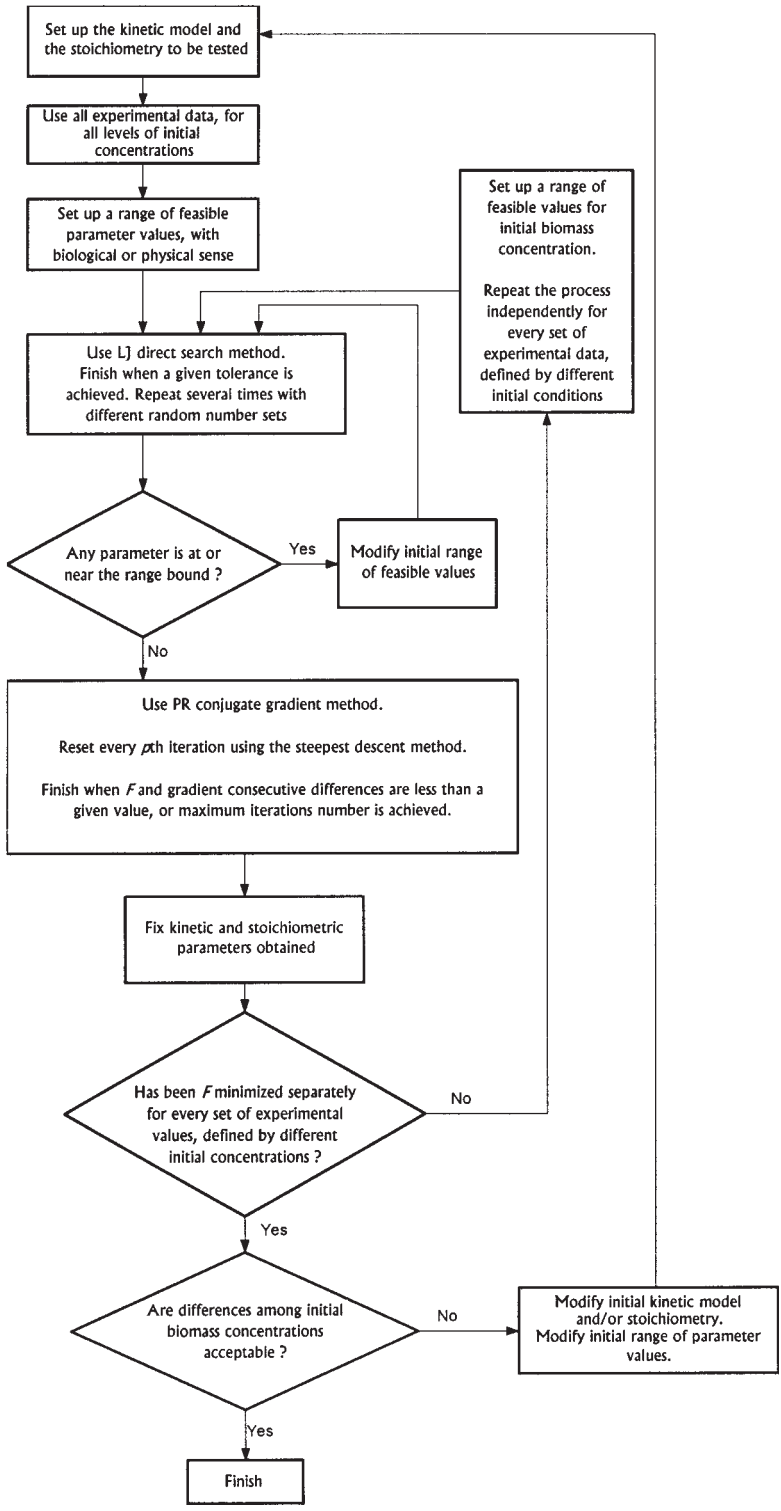

Fig. 1. Algorithm applied for practical identification procedure.

introduce noise in the data, and, therefore, it can produce a low determination coefficient for vials with the lowest initial concentration of acetate, for acetate and methane evolution.

Preliminary numerical experiments showed that function *F* was wrinkled and with many local minima. Whereas the LJ module was very fast, the PR module was very slow owing to this functional behavior, and maximum iteration number was always reached. The results are given in Table 4 (calculated values for initial concentration of biomass are not shown). Obtained kinetic parameters were not significantly different from those used for butyrate degraders in refs. *11* and *14*, differing only in the value of the acetate inhibition constant.

Acetate evolution for the experiment at an initial acetate concentration of 0 m*M* is the unique state variable, whose tendency cannot be predicted by the model (see Fig. 2). The model cannot explain acetate values obtained after d 15, and the determination coefficient, R^2, is not shown because of its nonsignificance. For the other measured variables, the obtained fittings are very good and with high R^2 values (indicated in Figs. 2–5).

As can be also appreciated in Figs. 3–5, values for acetate concentration after d 15 of the experiment cannot be predicted, but the high initial value of acetate for these experiments (more than 40 m*M* acetate) avoids noise data disturbance to the fitting and to parameter calculation. Nevertheless, noncontrolled decomposition of the inoculum produced higher methane production than predicted at the final days for all experiments, slightly decreasing determination coefficients for methane.

The evolution of valerate is very well predicted for the four different initial condition experiments and presents the highest R^2 values, 0.95 and 0.98, in all fitted output function components. The progressive delay in degradation, as initial acetate concentration was increased, is very well predicted by the use of the noncompetitive inhibition form by acetate in the model, in the same way as was previously used for butyrate *(11)*. Inhibition by hydrogen was not observed. The equations and parameters used to define the dynamics of acetate and propionate, taken from ref. *11*, showed their validity, providing high determination coefficients for the present experimental data. The lowest correlation obtained, 0.7, was for propionate at an initial concentration of 168 m*M* acetate (Fig. 5), and as can be seen, it was owing to experimental values far out of the predicted range at d 25 and 27 for one vial, which could be considered an outlier.

With the best estimates of the unknown coefficients, the stoichiometry of valerate degradation can be expressed as follows:

$$\begin{aligned} C_5H_{10}O_2 + 0.0653\ NH_3 + 0.5545\ CO_2 + 0.8041\ H_2O \rightarrow \\ 0.0653\ C_5H_7NO_2 + 0.8912\ C_2H_4O_2 + C_3H_6O_2 + 0.4455\ CH_4 \end{aligned} \quad (19)$$

Yield coefficients calculated from Eq. 19 are 0.072 g of biomass/g of valerate, 0.52 g of acetate/g of valerate and 0.73 g of propionate/g of valerate.

Table 4
Estimated Parameter Values, Correlation Matrix, CIs, and Student's *t*-Test for the Best Global Fitting[a]

Correlation matrix	μ_{m3} (θ_1)	$(K_S)_{36}$ (θ_2)	$(K_{in})_{34}$ (θ_3)	Biomass coefficient *a* (θ_4)	Ac coefficient *c* (θ_5)
θ_1	1.00				
θ_2	0.93	1.00			
θ_3	0.41	0.53	1.00		
θ_4	0.87	0.69	0.53	1.00	
θ_5	–0.13	–0.04	–0.26	–0.24	1.00
Parameter values	0.676 d^{-1}	0.176 g of Val/L	0.405 g of Ac/L	0.0653	0.8912
Standard deviation	0.180	0.156	0.120	0.022	0.051
CI (95%)	±0.353	±0.306	±0.235	±0.043	±0.1001
Student's *t*-test value	3.757	1.126	3.377	2.438	17.475
Student's *t*-test probability (%)	99.98	73.93	99.92	98.50	100.00

[a]Val, valerate; Ac, acetate.

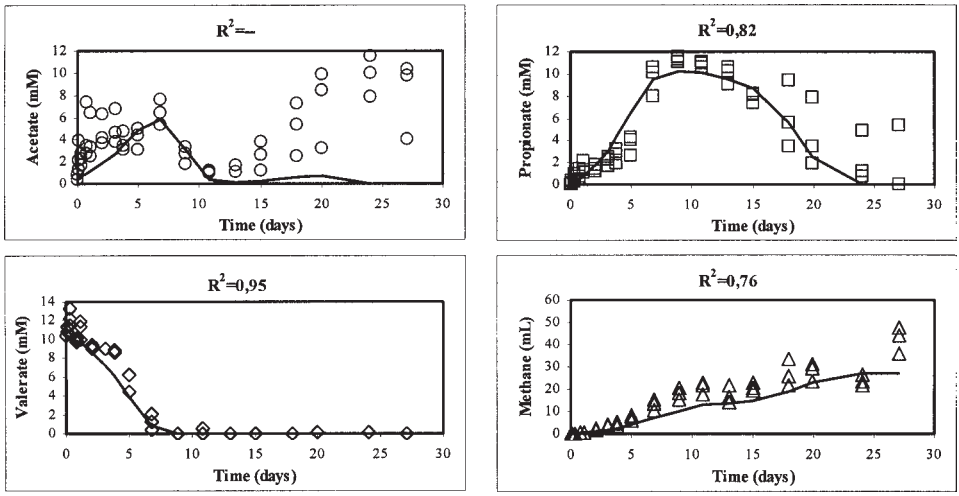

Fig. 2. Experimental data and model prediction for initial concentration of 0 m*M* acetate.

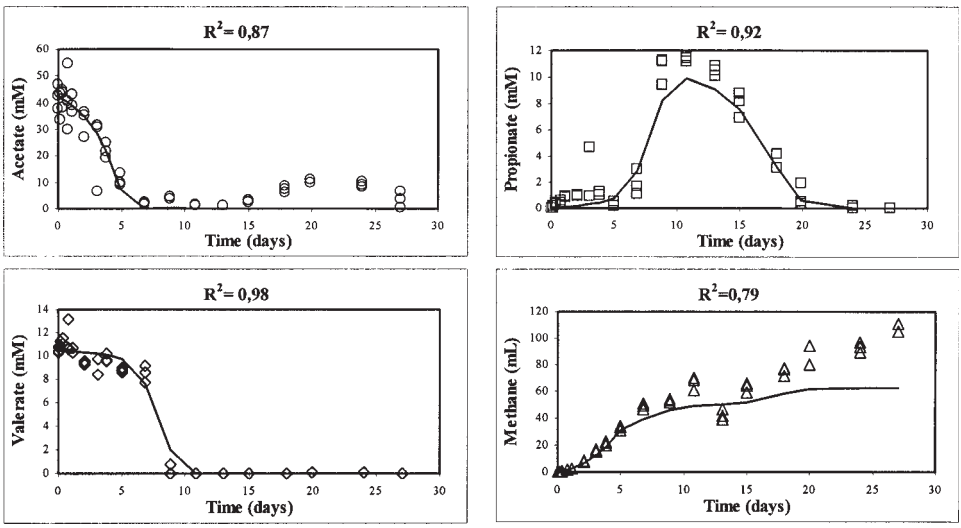

Fig. 3. Experimental data and model prediction for initial concentration of 42 m*M* acetate.

Other relations between coefficients of Eq. 3 were tested but always provided unknown parameter values in the CI ranges calculated in Table 4 and with lower R^2 values. The optimization process was repeated considering coefficient *c* in Eq. 3 to be 1, resulting in very low R^2 values for the best global fitting.

Parameter correlation matrix and 95% CIs were calculated and student's *t*-test was applied to the estimated set of parameters (shown in Table 4). It was considered that calculated initial concentration of biomass

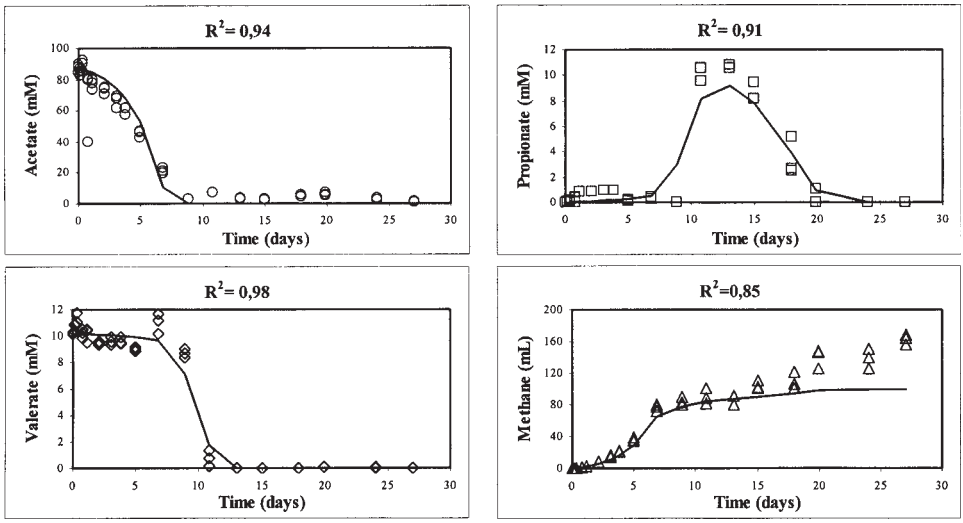

Fig. 4. Experimental data and model prediction for initial concentration of 88 m*M* acetate.

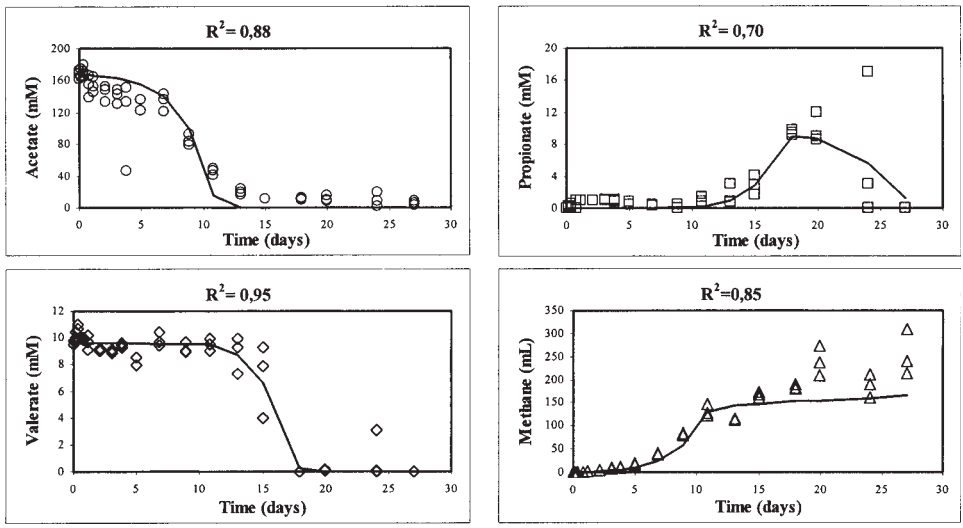

Fig. 5. Experimental data and model prediction for initial concentration of 168 m*M* acetate.

is a constant value for every simultaneous experiment conducted, defined by estimated values (data not shown), and not a model parameter. For this analysis, data and model predictions obtained after d 18 were rejected and, hence, degrees of freedom decreased, in order not to consider outliers.

The correlation matrix shows low values, and hence a positive result, except for θ_2 (K_S) and not as much for the coefficient θ_4. The student's *t*-test applied to θ_4 presents a satisfactory result with a significance level of 0.05,

and only K_S fails the student's *t*-test with this level of significance. This result can guide new experiments for improving K_S identifiability. It would be interesting to perform new experiments ensuring initial values for valerate in the CI range that actually will present experimental and mathematical difficulties owing to this low-level value (about 1.7 m*M* valerate), which could be easily affected by experimental data noise. In general, it is very difficult to identify K_S owing to Monod function properties, and usually μ_{max} and K_S parameters are strongly correlated, as has been pointed out by Vanrolleghem and Keesman *(1)*.

Although the student's *t*-test has proven the significance of the estimated parameters, with the limitations found for K_S, and although the multiple determination coefficients present high values, the obtained CIs are wide, owing to an appreciable dispersion of experimental data, as can be observed in Figs. 2–5. To ensure better calibration results, it is necessary to improve sampling and measurement procedures in batch experiments.

Conclusion

Simultaneous batch experiments provide an interesting tool for calibrating biokinetic models. Parameter identifiability depends on experimental design, and, hence, it is necessary to perform a structural identifiability study for designing adequate simultaneous experiments.

The estimated values of kinetic parameters for the valerate degrader population in the thermophilic range were 0.676 d^{-1} for the maximum growth rate (μ_m), 0.176 g of valerate/L for the saturation constant (K_S), and 0.405 g of acetate/L for the constant of acetate inhibition (K_{im}), considering Monod kinetics and a noncompetitive inhibition form. These values are not significantly different from kinetic values for butyrate anaerobic degradation found in the literature. All calculated kinetic parameters and stoichiometric coefficients presented a high statistical significance level except for the saturation constant K_S, for which it would be necessary to perform new experiments for improving its identifiability.

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