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Analysis and application of ADM1 for anaerobic methane production

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Abstract An anaerobic model for the serum bottle test was developed and analyzed with sensitivities of stoichiometric and kinetic parameters to the components in order to establish a basis for appropriate application of the model. Anaerobic glucose degradation in a serum bottle was selected as an example. The anaerobic model was developed based on the anaerobic digestion model no. 1 (ADM1), which had five processes with 17 kinetic and stoichiometric parameters. Sensitivity analysis showed that the yield of product on the substrate (f) has high sensitivities to model components, and that the methane concentration was the most sensitive component. Important parameters including yield of product on the substrate (f), yield of biomass on the substrate (Y), and half-saturation values (K) were estimated using genetic algorithms, which optimized the parameters with experimental results. The Monod maximum specific uptake rate (k) was, however, so strongly associated with the concentration of biomass, that values could not be estimated individually. Simulation with estimated parameters showed good agreement with experimental results in the case of methane production. However, there were some differences in acetate and propionate concentrations.

Keywords ADM1 · Sensitivity analysis · Genetic algorithms · Parameter estimation

Introduction

Anaerobic digestion model no. 1 (ADM1) was the integrated anaerobic model developed by the IWA Task Group for Mathematical Modeling of Anaerobic Digestion Processes in 2002 [1]. It consists of a number of processes to simulate all possible reactions occurring in anaerobic sludge including not only biological reactions, such as disintegration, hydrolysis of suspended solid, uptake (growth) and decay of microorganisms, but also physico-chemical reactions, including ion association/dissociation and liquid–gas transfer. In total, 19 processes, 24 components, and 56 relative stoichiometric and kinetic parameters were assumed for biological processes, and also, additional processes and parameters were determined for physico-chemical processes. Since a mechanistic model, such as the activated sludge model (ASM) series [2] and ADM1, has such delicate structures, the biological mechanisms are expressed well. However, it has a critical disadvantage that many parameters are difficult or impossible to measure [3, 4]. Furthermore, in the case of anaerobic digestion, practical application is very limited due to not only the complexity of processes and components, but also the lack of experience.

This research focused on how this anaerobic model should be analyzed and simulated for efficient application in anaerobic digestion processes. As an illustrative example of the analysis and evaluation of the application of this model, the anaerobic serum bottle test was considered, as its simplicity can give basic information about substrate degradation, methane production, and their mathematical expressions.

The serum bottle test model was constructed using ADM1 and the analysis of the model was based upon sensitivity analysis and parameter estimation. Sensitivity analysis is used to ascertain how a given model output depends upon the input parameters [5, 6] and it can be used as a tool for evaluating the importance and priority of parameters and reducing the amount of parameters to be estimated for effective model calibration [7].

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Genetic algorithms (GAs), one of the stochastic optimization algorithms, which was at first introduced by Holland in 1975 [8], was used for the parameter estimation [4, 9]. Because GAs have advantages in solving nonlinear and complex problems, they have been widely used in many applications to produce a global optimal solution. Recently, GAs have been applied in environmental engineering, including estimation of water quality parameters, calibration of rainfall–runoff models [10], ground–water management problems [11], and the optimization of water distribution networks [12].

In this study, in order to demonstrate and evaluate the performance of ADM1, the anaerobic model for glucose degradation in a batch-type bottle reactor was constructed based on ADM1, and the sensitivities of kinetic and stoichiometric parameters to simulation results were analyzed. With the results of the sensitivity analysis, important parameters in the model implementation were suggested, and their values were estimated using GAs and experimental results [13].

Materials and methods

Development of a kinetic model for the serum bottle test

The dynamic model used in this study describes the anaerobic batch-type experiments with glucose as the main substrate, as the degradation mechanisms of glucose can give basic information about the processes and kinetic parameters.

The COD flow assumed in ADM1 is rather complex. The decay of microorganisms and the regeneration cycle are strongly interrelated. The decay processes of all microorganisms result in the production of X_{ch} , X_{pro} , and X_{ji} , which can be used as substrates after disintegration and hydrolysis, and this regeneration approach makes model analysis more complex [14]. However, in this model, the decay process could be ignored for the simplicity purposes, and the simulation results can be compared with the net methane production by measuring the endogenous methane production and subtracting it from the total methane production. Figure 1 shows the COD flow in the model and the process numbers when the glucose (sugar) was used as the main substrate.

For the simple application, additional assumptions were used:

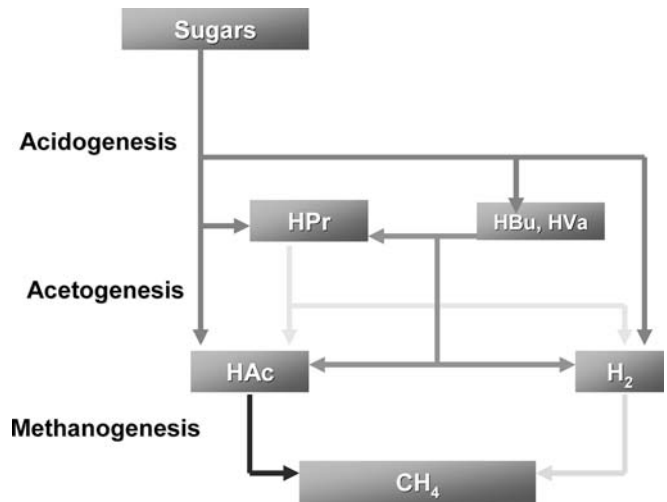


Fig. 1 Interrelated COD flow in ADM1

- Mesophilic conditions (35°C) were maintained
- No inhibition by pH and free ammonia
- Methane was gasified as soon as it was produced (i.e., gas–liquid transfer process was ignored)
- Hydrogen was quickly converted to methane

The matrix form used here is presented in Table 1. It includes processes, components, and stoichiometric and kinetic parameters when glucose was used as the main substrate. The processes used in this model were described by Bastone et al. [1]:

$$\text{Process 5, uptake of sugars: } k_{m, su} \frac{S_{su}}{K_S + S_{su}} X_{su}$$

Process 9, uptake of butyrate:

$$k_{m, c4} \frac{S_{bu}}{K_S + S_{bu}} X_{c4} \frac{1}{1 + S_{va}/S_{bu}}$$

$$\text{Process 10, uptake of propionate: } k_{m, pro} \frac{S_{pro}}{K_S + S_{pro}} X_{pro}$$

$$\text{Process 11, uptake of acetate: } k_{m, ac} \frac{S_{ac}}{K_S + S_{ac}} X_{ac}$$

$$\text{Process 12, uptake of hydrogen: } k_{m, h2} \frac{S_{h2}}{K_S + S_{h2}} X_{h2}$$

Table 1 Components and processes for the serum bottle test model: glucose as the substrate

Process ^a	Components										
	S_{su}	S_{bu}	S_{pro}	S_{ac}	S_{h2}	S_{ch4}	X_{su}	X_{c4}	X_{pro}	X_{ac}	X_{h2}
5	-1						Y_{su}				
9		$(1 - Y_{su})f_{bu, su}$						Y_{c4}			
10			$(1 - Y_{su})f_{pro, su}$						Y_{pro}		
11				$(1 - Y_{c4})0.8$						Y_{ac}	
12					$(1 - Y_{pro})0.43$						Y_{h2}

^aProcesses were numbered according to the order of ADM1

When the acetate was only used as a substrate, the model could be simplified greatly. Only X_{ac} (acetate degrader) and acetate fermentation to methane gas was considered, so only the “uptake of acetate” process was used. The stoichiometric coefficients within the matrix mean that Y_{ac} mol of new biomass and $(1 - Y_{ac})$ mol of methane gas were produced when 1 mol of acetate was degraded. When propionate was used as the main substrate, two kinds of microorganisms, including X_{pro} (propionate degrader) and X_{h2} (hydrogen degrader), and two components, including S_{pro} (propionate) and S_{h2} (soluble hydrogen), should be added. In this case, 1 mol of propionate was degraded by X_{pro} , resulting in $0.57 \cdot (1 - Y_{pro})$ mol of acetate and $0.43 \cdot (1 - Y_{pro})$ mol of hydrogen. Also, methane was produced from both the acetate and the hydrogen fermentation. When glucose was used as the main substrate, the equations became more complex, comprising five processes and 11 components. In this case, glucose was fermented into butyrate, propionate, acetate, and hydrogen and, subsequently, butyrate was degraded to acetate and hydrogen. In this case, $f_{bu, su}$, $f_{pro, su}$, $f_{ac, su}$, and $f_{h2, su}$, yields of butyrate, propionate, acetate, and hydrogen on glucose, respectively, were additionally assumed.

Sensitivity analysis

For the dynamic sensitivity analysis for the batch-type experimental model, the average of absolute differences between simulation results with prior determined parameters values (suggested by IWA [1]) and with parameters with a relative change of target parameter was used as the sensitivity index, as presented below in Eq. 1.

Table 2 shows suggested parameter values and their variability.

Table 2 Values for kinetic and stoichiometric parameters used in this model

Parameter	Suggested value	Unit	Variability ^a	Range (%)
Y_{su}	0.1	–	1	70–130
Y_{c4}	0.06	–	1	70–130
Y_{pro}	0.04	–	1	70–130
Y_{ac}	0.05	–	1	70–130
Y_{h2}	0.06	–	1	70–130
$f_{bu, su}$	0.13	–	4	10–200
$f_{pro, su}$	0.27	–	4	10–200
$f_{ac, su}$	0.41	–	4	10–200
$f_{h2, su}$	0.19	–	4	10–200
$k_{m, su}$	1.25	h^{-1}	2	10–200
$K_{m, c4}$	0.833	h^{-1}	1	70–130
$k_{m, pro}$	0.542	h^{-1}	2	10–200
$k_{m, ac}$	0.333	h^{-1}	3	20–400
$K_{s, su}$	500	$mg\ l^{-1}$	1	70–130
$K_{s, c4}$	200	$mg\ l^{-1}$	1	70–130
$K_{s, pro}$	100	$mg\ l^{-1}$	2	10–200
$K_{s, ac}$	150	$mg\ l^{-1}$	3	20–400

^aVariability: 1=varies within 30%, 2=varies within a factor of 100%, 3=varies within a factor of 300%

$$\text{Sensitivity index} = \frac{\sum |C_{STD}(t) - C_{SENS}(t)|}{N} \quad (1)$$

where N is the number of data (simulation time), and C_{STD} and C_{SENS} are the simulation results with the suggested parameter values and the parameters with a relative change of target parameter, respectively.

The sensitivity analysis of stoichiometric and kinetic parameters for five components was carried out by changing the value of a target parameter from 10% to 400% with respect to their suggested values [1].

In a dynamic sensitivity analysis, the number of data can have an effect on the RMSE. Thus, in this study, to ensure the end point, the simulation was stopped when the concentrations no longer changed in three time-steps.

Serum bottle test

Biochemical methane potential (BMP) tests of acetate, propionate, and glucose degradation were conducted for evaluating the applicability of the model, as well as estimating the kinetic and stoichiometric parameters [15]. Sludge from an anaerobic culture acclimated to glucose was used as an inoculum. The composition of the three kinds of substrates and the mineral salts medium is shown in Table 3. The serum bottles were incubated in a rotary shaker at 35°C. Gas production was measured using a glass syringe. The time interval of gas sampling was determined by the head pressure of the serum bottle, which was always maintained within to 2 atmospheric pressures. At the same time, 1-ml samples from the mixture were taken in order to analyze the concentrations of volatile fatty acids (VFAs). The net methane production was obtained by subtracting the methane produced in the control inoculum from that in the study serum bottle, and by adjusting it to standard temperature and pressure. The gas composition was analyzed using a gas chromatograph (Gow Mac series 580) and the VFAs were quantified by a high-performance liquid chromatography (SpectraSYSTEM P2000).

Parameter estimation

Only the methane concentration was used for estimating the parameters. A fitness function used in the GAs minimized the average of the absolute differences between the experimental results and the simulated results, as presented in the following equation:

$$\text{Fitness function} = \text{Minimize} \left(\frac{\sum |C_{EXP}(t) - C_{SIM}(t)|}{N} \right) \quad (2)$$

where N is the number of data (simulation time), and C_{EXP} and C_{SIM} are the experimental results and the simulation results, respectively.

Table 3 Composition of each bottle for the BMP test

	Bottle: glucose	Bottle: propionate	Bottle: acetate	Bottle: control
Volatile suspended solids (VSS) concentration of seed sludge		4,250 (mgVSS l ⁻¹)		
Main substrate	Glucose 500 (mgCOD l ⁻¹)	Propionate 468 (mgCOD l ⁻¹)	Acetate 456 (mgCOD l ⁻¹)	–
Alkalinity		1,000 (mgCaCO ₃ l ⁻¹)		
Mineral salts		NH ₄ Cl 0.53 (g l ⁻¹) CaCl ₂ ·2H ₂ O 75 (mg l ⁻¹) MgCl ₆ ·H ₂ O 100 (mg l ⁻¹) FeCl ₂ ·4H ₂ O 20 (mg l ⁻¹)		
Trace metals		MnCl ₂ ·4H ₂ O 0.5 (mg l ⁻¹) H ₃ BO ₃ 0.05 (mg l ⁻¹) ZnCl ₂ 0.05 (mg l ⁻¹) CuCl ₂ 0.03 (mg l ⁻¹) NaMoO ₄ ·2H ₂ O 0.01 (mg l ⁻¹) CoCl ₂ ·6H ₂ O 0.5 (mg l ⁻¹) NiCl ₂ ·6H ₂ O 0.05 (mg l ⁻¹) Na ₂ SeO ₃ 0.05 (mg l ⁻¹)		

Table 4 Parameters for the GA used in this study

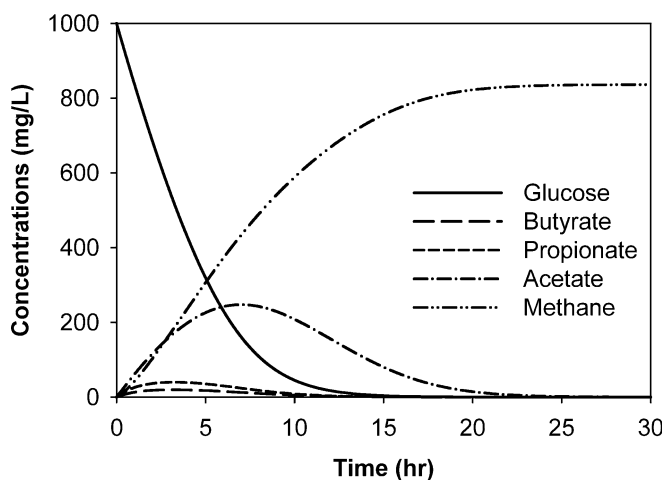
Number of generations	200
Population size	100
Probability of crossover	0.80
Probability of mutation	0.01
Number of crossover points	2
Selection strategy	<i>Elitism + Russian Roulette</i>

The GA was coded using Matlab version 6.0 (Math Works, 1999) and the parameters for it are presented in Table 4.

Results and discussion

Kinetic model for the serum bottle test

Typical simulation results of the serum bottle model using glucose as the main substrate are presented in Fig. 2. The initial concentration of glucose was

**Fig. 2** Typical simulation results of anaerobic glucose degradation

1,000 mg COD l⁻¹ and the concentrations of each microorganism, including sugar degrader, valerate and butyrate degrader, propionate degrader, acetate degrader, and hydrogen degrader, were 200 mgCOD l⁻¹ each. For the kinetic and stoichiometric parameters, the values suggested by IWA Task Group were used.

As shown in Fig. 2, the concentration of glucose sharply decreased and methane was produced, while acetate was produced and degraded simultaneously. A small amount of butyrate and propionate was also produced, but they disappeared quickly.

Sensitivity analysis

In this study, the kinetic and stoichiometric parameters were considered, but no analysis of the initial components was included. The sensitivity analysis results are presented in Table 5 by using arbitrary values. The values indicate the sensitivities at the maximum and minimum ranges according to their variety.

Among all kinetic and stoichiometric parameters, $f_{\text{product, substrate}}$ (yield of product on substrate) values showed high sensitivities to almost components. $k_{\text{m, process}}$ (maximum specific uptake rate) and $Y_{\text{substrate}}$ (yield of biomass on substrate) were parameters that are not so important and $K_{\text{S, process}}$ was negligible, except $K_{\text{S, ac}}$ sensitivity to acetate and methane concentrations. Methane concentration was the most sensitive among the five components. And while acetate and glucose had high sensitivities, propionate and butyrate showed relatively low sensitivities.

It was found that the sensitivities of components are most dependent on the number of processes related to them. For example, glucose was the main substrate and its concentration was controlled by the “uptake of sugars” process only. This means that the glucose concentration is dependent on parameters that are used

Table 5 Sensitivity of the kinetic and stoichiometric parameters to components

	Glucose		Butyrate		Propionate		Acetate		Methane	
	30%	130%	30%	130%	30%	130%	30%	130%	30%	130%
Y_{su}	0	0	0.00150	0.00150	0.00356	0.00350	0.0342	0.0334	0.118	0.119
Y_{c4}	0	0	0	0	0	0	0.00578	0.00577	0.0164	0.0164
Y_{pro}	0	0	0	0	0	0	0.00573	0.00572	0.0236	0.0236
Y_{ac}	0	0	0	0	0	0	0	0	0.155	0.155
Y_{h2}	0	0	0	0	0	0	0	0	0.103	0.103
$k_{m, c4}$	0	0	0.0239	0.0132	0	0	0.0125	0.00699	0.0125	0.00700
$K_{s, su}$	0.131	0.118	0.00440	0.00339	0.00982	0.00737	0.0536	0.0451	0.0639	0.0628
$K_{s, c4}$	0	0	0.0161	0.0153	0	0	0.00854	0.00800	0.00864	0.00809
	10%	200%	10%	200%	10%	200%	10%	200%	10%	200%
$f_{bu, su}$	0	0	0.0506	0.0645	0	0	0.172	0.211	0.5201	0.5611
$f_{pro, su}$	0	0	0	0	0.1011	0.186	0.254	0.321	1.151	1.22
$f_{ac, su}$	0	0	0	0	0	0	0.894	1.57	2.70	2.63
$f_{h2, su}$	0	0	0	0	0	0	0	0	2.86	3.18
$k_{m, su}$	2.73	0.486	0.0296	0.0206	0.059	0.0480	0.457	0.204	2.06	0.222
$k_{m, pro}$	0	0	0	0	0.813	0.0615	0.206	0.0244	0.621	0.0384
$K_{s, pro}$	0	0	0	0	0.0985	0.0898	0.0384	0.0339	0.0633	0.0560
	20%	400%	20%	400%	20%	400%	20%	400%	20%	400%
$k_{m, ac}$	0	0	0	0	0	0	3.25	1.09	3.09	1.04
$K_{m, ac}$	0	0	0	0	0	0	0.758	1.40	0.720	1.33

by the “uptake of sugar” process ($K_{s, su}$ and $k_{m, su}$). Especially, $k_{m, su}$ showed a higher sensitivity value (2.73) than $K_{s, su}$ (0.131) at low ranges (30%) of the suggested value. Butyrate is related to the “uptake of sugars” and “uptake of butyrate” processes and propionate is connected to the “uptake of sugars,” “uptake of valerate,” and “uptake of butyrate” processes. Acetate is related to most of processes and is very sensitive to almost all parameters without the “uptake of hydrogen” process. Methane production has the most complex mechanisms as it is interlinked to all processes and almost every parameter showed a sensitivity to the methane concentration. High sensitivities means that the methane concentration could be used for parameter estimation.

Sensitivity values of the kinetic and stoichiometric parameters for the methane concentration with various changes are presented in Fig. 3.

The sensitivities of f , Y , and K displayed asymmetric characteristics, as shown in the figure. $f_{bu, su}$ and $f_{pro, su}$ were the most sensitive for all the ranges examined. The Monod maximum specific uptake rates showed singular shapes. In low range, the sensitivities increased sharply, while in high range they increased gradually. It was suggested that the F/M ratio in the serum bottle was the effecting factor. The reactor maintained the relative microorganism-rich condition and the increase of the uptake rate showed less sensitivity to methane production. In low range, however, the uptake rate could have effects on the concentration of the substrate and, thus, showed the high sensitivities.

In this case, $k_{m, su}$ was the most important parameter. Y_{ac} , Y_{c4} , and Y_{h2} showed higher sensitivities than the other yield parameters. In the case of half-saturation values, $K_{s, ac}$ was the only important parameter.

Parameter estimation

Using results of the serum bottle test with the anaerobic model, the parameters were optimized using a GA. Among five component concentrations, only methane concentration, which was found most to be sensitive on almost all the parameters, was used for optimization.

The results of the serum bottle test and optimization when acetate was used as the main substrate are presented in Fig. 5a. Acetate quickly disappeared in 43 h and methane was produced up until 80 h.

Y_{ac} could be calculated with the initial concentration of acetate and the final concentration of methane. The other parameter and the initial concentration of X_{ac} were estimated using methane production in acetate degradation using GAs.

However, $k_{m, ac}$ and X_{ac} were not successfully estimated. Combinations of $k_{m, ac}$ and X_{ac} , which had different values, showed a good accuracy in methane production and acetate degradation. It was thought that the linear relationship between $k_{m, ac}$ and X_{ac} makes them difficult to be estimated successfully. To demonstrate the identifiability between these two parameters, simulations were carried out and it was revealed that several combinations of k and X_{ac} showed high fitnesses, as seen in Fig. 4, and that the global optimum could not be estimated. Figure 4a–c showed that different combinations could result in very similar simulation results and with great accuracy. However, the values of $k_{m, ac} \times X_{ac}$ were almost identical, suggesting that it was more effective to estimate the combination of k and X for each process.

The optimized parameters and their values are presented in Tables 6 and 7.

Figure 5 shows a comparison of simulation and experimental results for anaerobic acetate, propionate,

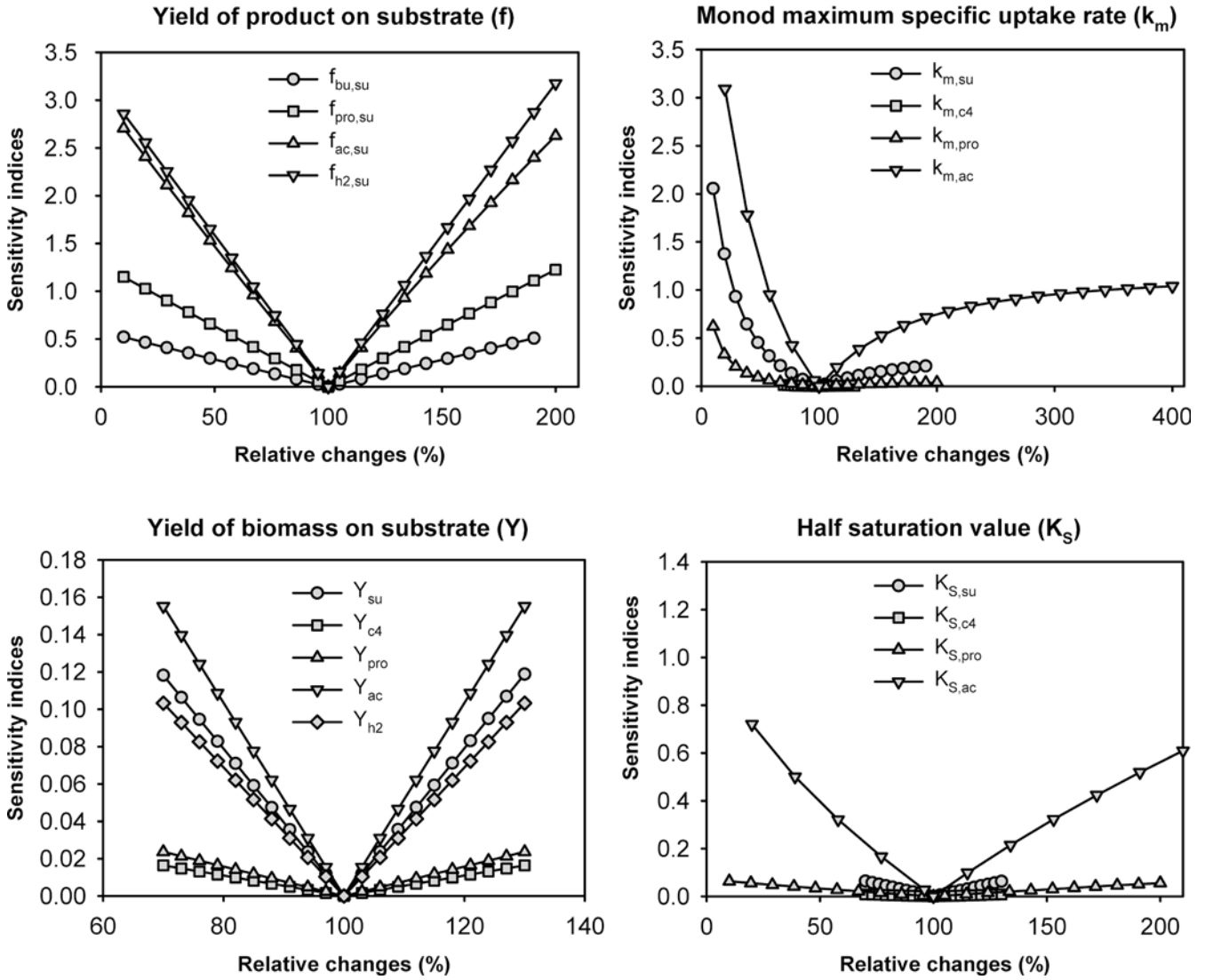


Fig. 3 Sensitivities of **a** yield coefficient (f), **b** Monod maximum specific uptake rate (k_m), **c** yield of biomass (Y) on the methane concentration, and **d** half-saturation value (K) on methane concentration

and glucose fermentation. The simulation results with optimized parameters showed good agreement with the experimental data in methane concentration. However, there is a small difference between the acetate concentrations. Methane production continued after the depletion of acetate. It was assumed that acetate was absorbed on the surface of microorganisms or stored as the cell internal materials. It was also possible that the gas-liquid transfer and separation of methane took some time, which would explain the time gap between methane production and acetate utilization.

When propionate was used as the main substrate, acetate was produced and disappeared as quickly as the propionate was consumed (Fig. 5b). Methane production was well simulated. However, the propionate decrease showed a small difference but the acetate showed a big difference in the estimations. There was

also a time delay in methane production after the depletion of both the propionate and the acetate. It was suggested that the difference of acetate concentrations between the simulated and the measured data were from the error of estimating the yield coefficient. In this test, the parameters related to the acetate degradation (Y_{h2} , $K_{S,ac}$) and the concentration of X_{ac} were used with the estimated value in the previous batch test. And the other parameters (Y_{pro} , $K_{S,pro}$) except Y_{h2} and the concentration of X_{pro} were estimated with methane production in propionate degradation using GAs. Y_{h2} was calculated with the final concentration of methane (S_{ch4}), the initial concentration of propionate (S_{pro}), and the estimated value of Y_{pro} and Y_{ac} through Eq. 4 in the Appendix, which is at the end of this paper. The estimated and calculated parameters and the concentration of X_{pro} are presented in Tables 6 and 7.

When glucose was used as the main substrate, propionate and acetate were produced with glucose degradation (Fig. 5c). There was also a time delay between methane production and depletion of glucose, acetate,

Fitnesses of X_{ac} and $k_{m,ac}$ combination

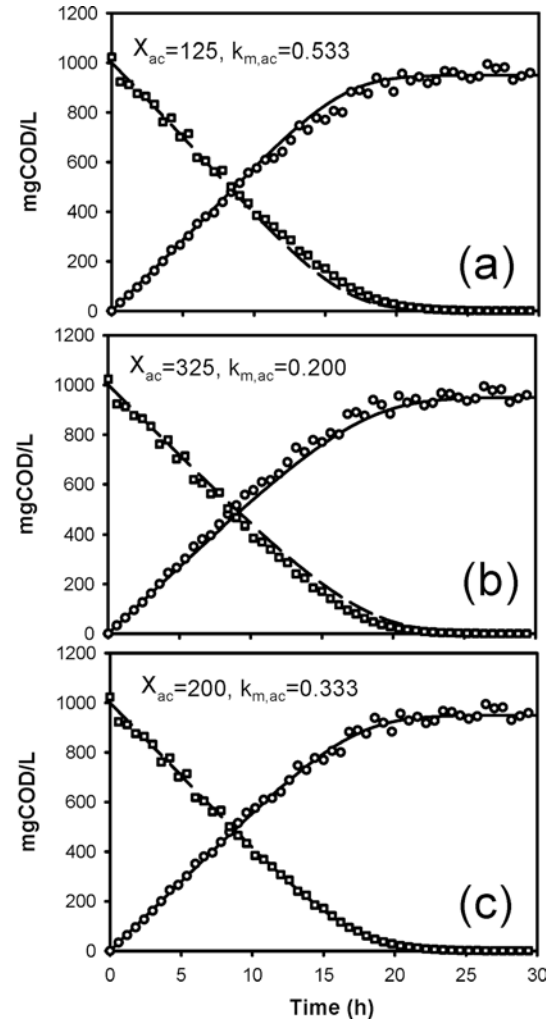
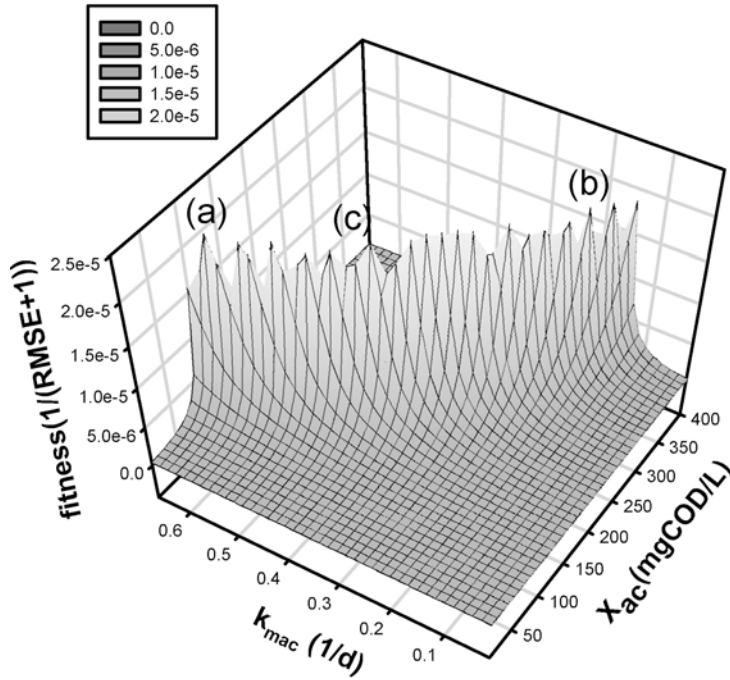


Fig. 4a–c Fitnesses values of combinations of X_{ac} and $k_{m,ac}$, which have high fitness values and **b–d** their simulation results with estimated parameters

and propionate. In this test, the parameters were also estimated and calculated in a similar manner as with the case of propionate. The parameters related to acetate degradation (Y_{h2} , $K_{S,ac}$), propionate degradation (Y_{pro} , $K_{S,pro}$), and the concentrations of X_{ac} and X_{pro} were used with the previously estimated value. And the other parameters (Y_{su} , $f_{ac,su}$, $f_{pro,su}$, $f_{bu,su}$) except Y_{c4} and the

concentration of X_{su} were estimated with methane production in propionate degradation using GAs. Y_{c4} was calculated using the initial concentration of glucose (S_{su}), the final concentration of methane (S_{ch4}), and the estimated values of parameters through Eq. 4 in the [Appendix](#). The estimated and calculated parameters and the concentration of X_{pro} are presented in Tables 6 and 7.

Table 6 Estimated kinetic and stoichiometric parameter values

Parameter	Unit	Estimated value
$K_{s,ac}$	mg l ⁻¹	259
$K_{s,pro}$	mg l ⁻¹	582
Y_{ac}	–	0.100
Y_{h2}	–	0.0282
Y_{pro}	–	0.0520
Y_{c4}	–	0.0193
Y_{su}	–	0.0500
$f_{ac,su}$	–	0.202
$f_{pro,su}$	–	0.540
$f_{bu,su}$	–	0.111

Conclusion

A model for the anaerobic serum bottle test was developed using the anaerobic digestion model no. 1 (ADM1) and various simulations were carried out. We highlight the following points:

Table 7 Estimated concentrations of the initial component

Biomass	Estimated value (COD mg l ⁻¹)
X_{ac}	47.0
X_{pro}	49.2
X_{c4}	79.2
X_{su}	156

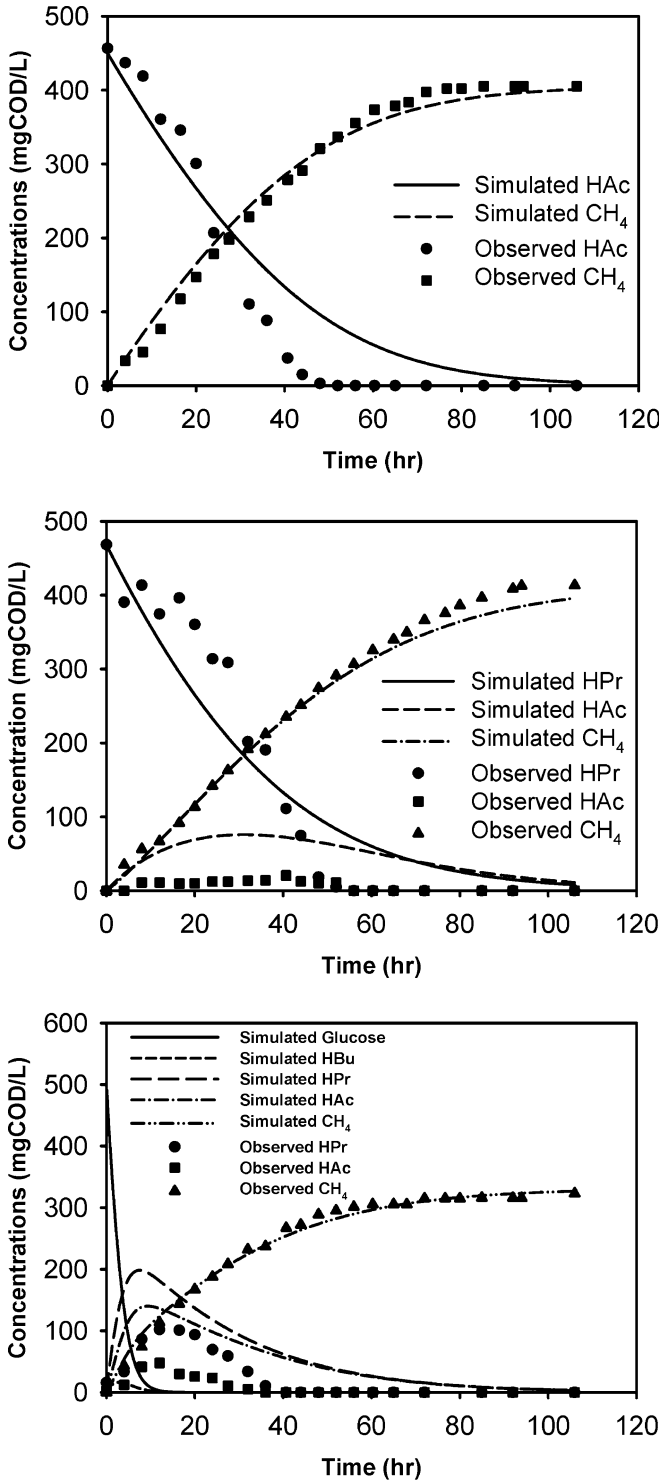


Fig. 5a–c Comparison of simulation and experimental results of anaerobic a acetate, b propionate, and c glucose fermentation

– The batch reaction of glucose degradation was analyzed by performing sensitivity analysis. In most cases, f (yield of product on the substrate) showed high sensitivities to model components, and methane concentration was the most sensitive components, which was related to all processes used in the model.

- A genetic algorithm was used for the model parameter estimation, and the important parameters (yield of product on the substrate, yield of biomass on the substrate, and half-saturation values) were optimized using experimental results. The results are presented in Table 6.
- The Monod maximum specific uptake rate was so strongly associated with the concentration of biomass, that the values could not be estimated individually. In this study, the combination of the biomass and the maximum specific uptake rate was estimated as presented in Table 7.
- Simulations with the estimated parameters showed good agreement with experimental results in the case of methane production. However, there were some differences between the acetate and propionate concentrations.

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Appendix

Parameter estimation

Propionate

In the case of using propionate as the main substrate, one yield can be calculated using the other yield and the final concentration of the components in the serum bottle test. The equations described below show the deviation of Y_{h_2} .

The yield of acetate (H_{ac}) on propionate from Table 1: $0.57(1 - Y_{pro})$

The yield of hydrogen (H_2): $0.43(1 - Y_{pro})$

So, if the initial concentration of propionate is S_{pro} , the concentrations of acetate and hydrogen converted by propionate degraders are:

$$H_{ac}: 0.57(1 - Y_{pro}) \times S_{pro}$$

$$H_2: 0.43(1 - Y_{pro}) \times S_{pro}$$

And then, the yield of methane on acetate: $(1 - Y_{ac})$

The yield of methane on hydrogen: $(1 - Y_{h_2})$

So, the concentration of methane (S_{ch_4}), which is converted by acetate and hydrogen upgraders is:

$$\underbrace{\{0.57(1 - Y_{pro}) \times S_{pro}\} \times (1 - Y_{ac})}_{\text{by acetate upgraders}} + \underbrace{\{0.43(1 - Y_{pro}) \times S_{pro}\} \times (1 - Y_{h_2})}_{\text{by hydrogen upgraders}}$$

Rewriting and rearranging, the final concentration of methane is:

$$S_{ch_4} = \{0.57(1 - Y_{pro})(1 - Y_{ac}) + 0.43(1 - Y_{pro})(1 - Y_{h_2})\} S_{pro}$$

Therefore, Y_{h_2} can be derived as follows:

$$Y_{h2} = 1 - \frac{1}{0.43} \left\{ \frac{S_{ch4}}{(1 - Y_{pro})S_{pro}} - 0.57(1 - Y_{ac}) \right\} \quad (3)$$

Glucose

Y_{c4} can be derived by a procedure similar to the derivation of Y_{h2} .

If the initial concentration of glucose is S_{su} , the concentrations of acetate and hydrogen, which are finally converted, are:

H_{ac} :

$$\begin{aligned} & \underbrace{0.8(1 - Y_{c4})(1 - Y_{su})f_{bu, su}S_{su}}_{\text{by glucoseandbutyrateupgraders}} \\ & + \underbrace{0.57(1 - Y_{pro})(1 - Y_{su})f_{pro, su}S_{su}}_{\text{by glucoseandpropionateupgraders}} \\ & + \underbrace{(1 - Y_{su})f_{ac, su}S_{su}}_{\text{by glucoseupgraders}} \end{aligned}$$

H_2 :

$$\begin{aligned} & \underbrace{0.2(1 - Y_{c4})(1 - Y_{su})f_{bu, su}S_{su}}_{\text{by glucoseandbutyrateupgraders}} \\ & + \underbrace{0.43(1 - Y_{pro})(1 - Y_{su})f_{pro, su}S_{su}}_{\text{by glucoseandpropionateupgraders}} \\ & + \underbrace{(1 - Y_{su})f_{h2, su}S_{su}}_{\text{by glucoseupgraders}} \end{aligned}$$

So, the concentration of methane (S_{ch4}) converted by acetate and hydrogen upgraders is:

$$S_{ch4} = \left[\begin{aligned} & \{0.8(1 - Y_{c4})(1 - Y_{su})f_{bu, su} + 0.57(1 - Y_{pro})(1 - Y_{su})f_{pro, su} + (1 - Y_{su})f_{ac, su}\}(1 - Y_{ac}) \\ & + \{0.2(1 - Y_{c4})(1 - Y_{su})f_{bu, su} + 0.43(1 - Y_{pro})(1 - Y_{su})f_{pro, su} + (1 - Y_{su})f_{h2, su}\}(1 - Y_{h2}) \end{aligned} \right] S_{su}$$

Therefore, Y_{c4} can be derived as follows:

$$Y_{c4} = 1 - \frac{\left[\frac{S_{ch4}}{S_{su}(1 - Y_{su})} - \{f_{ac, su} + 0.57f_{pro, su}(1 - Y_{pro})\}(1 - Y_{ac}) - \{f_{h2, su} + 0.43f_{pro, su}(1 - Y_{pro})\}(1 - Y_{h2}) \right]}{\{0.8f_{bu, su}(1 - Y_{ac}) + 0.2f_{bu, su}(1 - Y_{h2})\}} \quad (4)$$

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