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Modeling and Simulation of Mixed-Culture Interactions in Anaerobiosis of Inhibitory Substrate

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ABSTRACT

The model describes the anaerobiosis of 2-ethylhexanoic acid (2-EHA), which is a persistent and inhibitory organic waste. Model development was based on a biphasic degradation pathway mediated by three groups of bacteria. The model comprises seven equations with 13 constant parameters. The distinctive features include:

1. Grouping of anaerobic bacteria according to the metabolic functions;
2. Description of all essential reactions and mixed-culture interactions, namely interspecies H_2 and volatile fatty acid transfers; and
3. Correlation of 2-EHA inhibition with the concentration of unionized 2-EHA.

The validated and fine-tuned model was applied to simulate substrate utilization, intermediate and product formations, and bacterial cell synthesis.

Index Entries: Anaerobiosis; biphasic pathway; mathematical model; mixed-culture interaction; substrate inhibition.

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INTRODUCTION

Biological degradation of branched-chain fatty acids (BCFAs) has not been intensively studied because these compounds are not commonly found in municipal sewage. In recent years, BCFAs have been produced through degradation of certain industrial wastes or directly discharged in other industrial effluents. For instance, 2-methybutanoic acid (2-MBA) and 3-methylbutanoic acid (3-MBA) are produced through anaerobic degradation of amino acids, namely leucine, isoleucine, and valine (*1*). Neopentanoic acid (NPA) and 2-ethylhexanoic acid (2-EHA) are discharged by the pharmaceutical industry (*2*). These BCFAs are reported to be persistent and inhibitory to biological processes, and adversely affect treatment performance (*2*). McInerney et al. (*3,4*) reported that an anaerobic bacterial consortium that degraded straight-chain fatty acids up to eight carbons did not effectively degrade the branched-chain 2-MBA. On the other hand, recent studies by Chua et al. (*5*), Chua (*6*), and Jimena et al. (*7*) showed contrary results. BCFAs, such as 2-EHA and NPA, were found to be degradable in anaerobic reactors. Richardson et al. (*8*) isolated an anaerobic 2-MBA-degrading bacterial consortium, comprising of an obligate-syntrophic acidogen and two methanogens, but the degradation mechanism was not described. Chua et al. (*9*) described a biphasic pathway in the anaerobiosis of 2-EHA involving three groups of bacteria.

This article presents a mathematical model that describes the biphasic anaerobiosis of 2-EHA and the interactions among the groups of bacteria. The operation data of anaerobic continuous stirred-tank reactors (CSTRs) reported by Chua (*6*) are used to validate the model.

PROCESS DESCRIPTION

The anaerobic ecosystem that degrades 2-EHA comprises three morphologically distinctive bacterial genera (*6,9*). There are rods of 0.3–2.0 by 1.5–6.0 μ (Fig. 1). These organisms are consistent with the *Syntrophomonas spp.* described by McInerney et al. (*3,4*), and are the acidogens that convert 2-EHA to ethanoic acid. The second group of bacteria in the anaerobic ecosystem are cocci of 0.5–1.2 μ in diameter, which autofluoresce when excited at 420 nm (Fig. 2). The fluorescence property is attributed to the coenzyme F_{420nm} present in *Methanococcus spp.* (*10*). *Methanococcus* is therefore identified as the H_2-utilizing methanogens in the ecosystem. Finally, there are large rods with distinctive truncated or rectangular ends, which are the ethanoic acid-utilizing or acetoclastic *Methanothrix spp.* (Fig. 3).

The substrate 2-EHA is degraded through a biphasic pathway (*9*). In the acidogenic phase, 2-EHA is β-oxidized by *Syntrophomonas* to butanoic acid. This multistep reaction results in a cleavage between the α and β

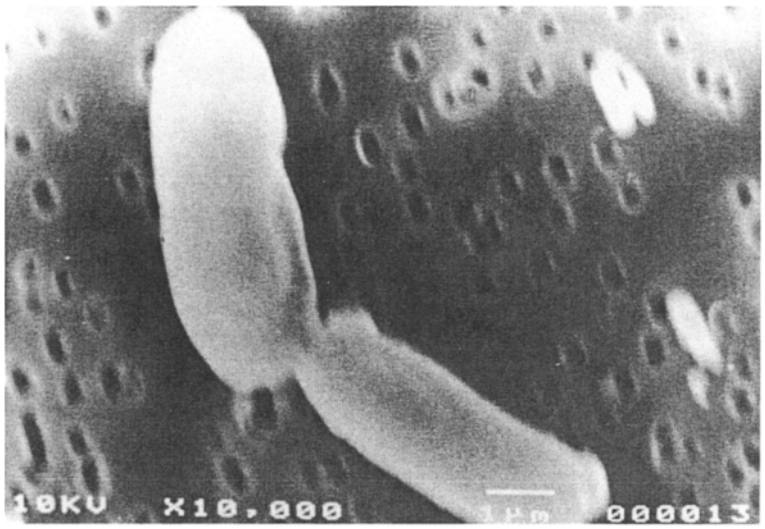


Fig. 1. Electron micrograph of *Syntrophomonas spp.*

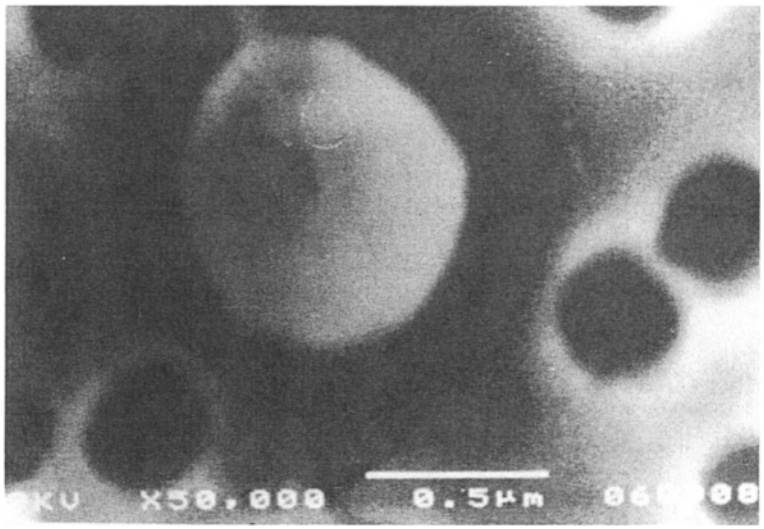


Fig. 2. Electron micrograph of *Methanococcus spp.*

carbons and removal of two-carbon acetate groups from the carboxylic end of the carbon chain as summarized in Fig. 4. Hydrogen is generated in the β-oxidation reaction. Butanoic acid is then similarly β-oxidized to ethanoic acid with concomitant H_2 production. The overall sequential β-oxidation of 2-EHA to ethanoic acid is shown in Fig. 5. In the methanogenic phase, ethanoic acid is decarboxylated by *Methanothrix* to CH_4 and CO_2, whereas H_2 is utilized by *Methanococcus* to reduce CO_2 to CH_4. The complete biphasic degradation pathway is summarized in Fig. 6.

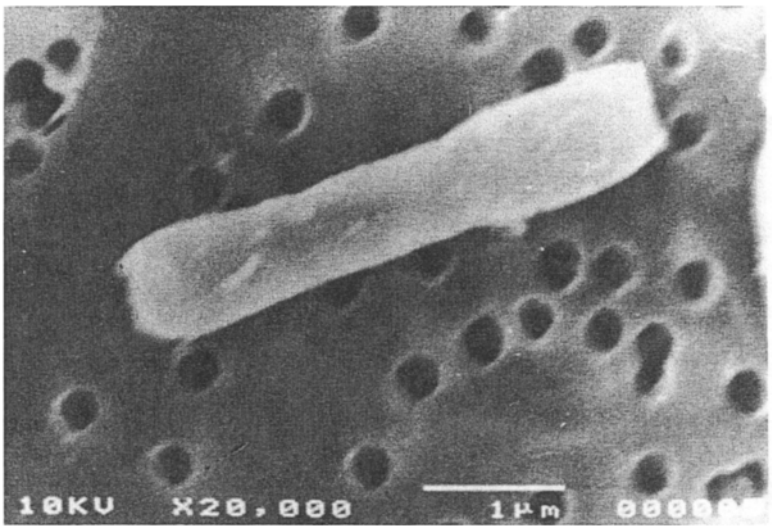


Fig. 3. Electron micrograph of *Methanothrix spp.*

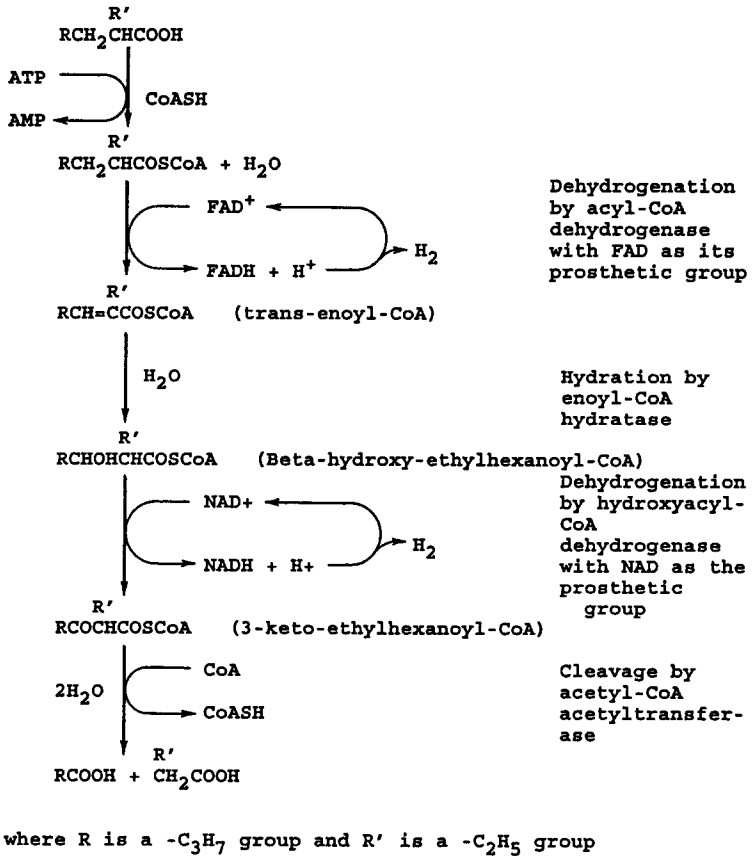


Fig. 4. β-Oxidation of 2-EHA.

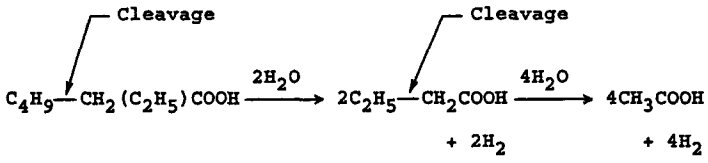


Fig. 5. Mechanism of sequential conversion from 2-EHA to ethanoic acid.

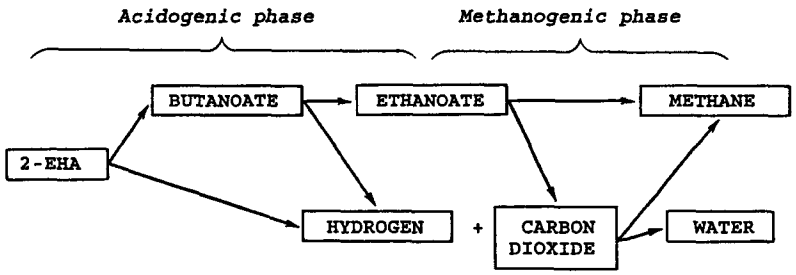


Fig. 6. Bi-phasic pathway of anaerobiosis of 2-EHA.

The acidogenic and methanogenic phases are linked by mixed-culture interactions. Interaction takes the form of a coupling of hydrogen in interspecies hydrogen transfer between the acidogens and the H_2-utilizing methanogens. Acidogens are frequently referred to in the literature as H_2-producing bacteria because their only means of regenerating the reduced coenzymes, nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH), is by oxidizing these compounds through H_2 evolution (Fig. 4). A typical acidogenic reaction on butanoate, with H_2 evolution, is shown below:

$$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2 \qquad \text{(A-1)}$$

This reaction is endodermic (48.1 kJ/mol) (*11*). The reaction is possible only if it is coupled with an energy yielding H_2-utilizing reaction that maintains a low partial pressure of H_2. Therefore, acidogens are obligate syntrophs. The H_2-utilizing methanogens obtain energy for growth by utilizing H_2 for the reduction of CO_2 to form CH_4 as shown in Eq. (A-2) (*12,13*).

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \qquad \text{(A-2)}$$

This reaction yields an energy of 135.6 kJ/mol. The H_2-utilizing methanogens have a low saturation coefficient K_s for H_2 ($5 \times 10^{-6}M$). They can, therefore, maintain the H_2 partial pressure below 10^{-4} atm in a healthy anaerobic ecosystem and induce the acidogens to produce the "non-reduced" ethanoate, which then becomes the substrate for the acetoclastic (ethanoic acid-utilizing) methanogens.

Mixed-culture interaction is also in the form of interspecies transfer of volatile fatty acid (VFA), namely ethanoic acid, between the acidogens

and the acetoclastic methanogens. Ethanoic acid is the precursor for 75% of the total methane production in anaerobiosis (*14,15*). If the ethanoic acid produced by the acidogens is not removed by the methanogens, acid accumulation will result in souring of the anaerobic ecosystem and eventual failure of the anaerobiosis process. Ethanoate is utilized in an energy-yielding, decarboxylation reaction, in which the methyl group is reduced to CH_4, whereas the carboxyl group is oxidized to CO_2, as in Eq. (A-3) (*16*).

$$*CH_3COO^- + H_2O \rightarrow *CH_4 + HCO_3^- \quad \text{(A-3)}$$

However, the standard change in free energy (−31 kJ/mol) was barely sufficient to form 1 mol of ATP, which is equivalent to 30.6 kJ (*17*), explaining the relatively slow growth rate of acetoclastic methanogens. Interactions between the groups of bacteria in the form of interspecies H_2 and VFA transfer, and maintenance of a balanced mixed population are important for a stable anaerobiosis process.

MODEL DEVELOPMENT

The general mass balance used throughout the model is as follows:

{Rate of accumulation} = {Rate of input}
− {Rate of output}
+/− {Rate of reaction}

Acidogenic Phase

A mass balance on 2-EHA is shown as follows:

$$(dS_1 / dt) = D(S_{10} - S_1) - \grave{I}_1 X_1 / Y_1 - \grave{I}_1 X_1 Y_2 / R_1 \quad (1)$$

where S_1 = effluent concentration of 2-EHA, mg/L, t = time, d, D = dilution rate, d^{-1}, S_{10} = influent concentration of 2-EHA, 8200 mg/L, $\grave{I}_1$ = specific growth rate of *Syntrophomonas*, d^{-1}, X_1 = effluent concentration of *Syntrophomonas*, mg VSS/L, Y_1 = growth yield of *Syntrophomonas* on 2-EHA, mg/mg, Y_2 = butanoic acid yield by *Syntrophomonas*, mg/mg, and R_1 = stoichiometric ratio of 2-EHA-butanoate conversion.

A similar mass balance on butanoic acid gives Eq. (2).

$$(dS_4 / dt) = \grave{I}_1 X_1 / Y_2 - \grave{I}_1 X_1 Y_3 / R_2 - DS_4 \quad (2)$$

where S_4 = effluent concentration of butanoic acid, mg/L, Y_3 = ethanoic acid yield by *Syntrophomonas*, mg/mg, and R_2 = stoichiometry of butanoate-ethanoate conversion.

In Eqs. (1) and (2), ethanoic and butanoic acid productions were assumed to be associated with the growth of *Syntrophomonas*. Substrate

*C = labeled carbon

consumption to support cell viability and activities, such as cell mobility, enzyme turnover, osmotic work, nutrient storage, and other maintenance functions, is usually as low as on the order of 10^{-2} mg substrate/mg cell/d (*18*), and has not been taken into account in these equations. A mass balance on acidogenic cell mass is shown below:

$$(dX_1 / dt) = I_1X_1 - K_{d1}X_1 - DX_1 \quad (3)$$

where K_{d1} = specific decay rate of *Syntrophomonas*, d^{-1}. The concentrations of ethanoic and butanoic acids in the anaerobic ecosystem are usually below their respective inhibitory concentrations. On the other hand, residual concentration of 2-EHA reaches and exceeds the inhibitory level (*9*). Therefore, 2-EHA is assumed to be the inhibiting substrate for *Syntrophomonas* in the model. However, it is not certain whether inhibition is owing to high 2-EHA concentrations or low pH, or even by the combined effect of these two interrelated factors. Therefore, the unionized 2-EHA is taken as the inhibitor because it is a function of both pH and 2-EHA concentration. 2-EHA dissociates according to the ionic equilibrium:

$$US_1 \overset{K}{\rightarrow} H^+ + S_1^- \quad (3A)$$

where K = dissociation constant of 2-EHA.

The fraction of unionized 2-EHA, US_1, is, therefore, given as follows:

$$US_1 = S_1 [H^+ / (H^+ + K)) = S_1 (10^{-pH}/(10^{-pH} + K)] \quad (4)$$

The specific growth rate of *Syntrophomonas*, $\grave{I}_1$, is describd by the substrate inhibition function:

$$\grave{I}_1 = \grave{I}_{M1} / (1 + K_{S1}/S_1 + US_1/K_{I1}) \quad (5)$$

where $\grave{I}_{M1}$ = maximum specific growth rate of *Syntrophomonas*, d^{-1}, K_{S1} = saturation constant of 2-EHA, mg/L, K_{I1} = inhibition constant of 2-EHA on *Syntrophomonas*, mg/L, and US_1 = concentration of unionized 2-EHA, mg/L.

The production rate of molecular hydrogen in the ecosystem, in mL/L/d, is calculated from the stoichiometry of the β-oxidation reactions.

$$Q_H = (22.4/88)\grave{I}_1X_1Y_2 + (22.4/60)\grave{I}_1X_1Y_3 \quad (6)$$

The numerical coefficients in Eq. (6) convert the mass of VFA production to the equivalent volume of hydrogen gas.

Methanogenic Phase

In the methanogenic phase, the mass balances on ethanoate and *Methanothrix* cell mass, with similar assumptions used in the acidogenic phase, results in the following equations:

$$(dS_2 / dt) = \grave{I}_1X_1Y_3 - \grave{I}_2X_2Y_4/R_3 - DS_2 - \grave{I}_2X_2/Y_5 \quad (7)$$

$$(dX_2 / dt) = \grave{I}_1 X_2 - K_{d2} X_2 - D X_2 \quad (8)$$

where S_2 = effluent concentration of ethanoic acid, mg/L, $\grave{I}_2$ = specific growth rate of *Methanothrix*, d^{-1}, X_2 = effluent concentration of *Methanothrix*, mg VSS/L, Y_4 = methane yield by *Methanothrix*, mg/mg, R_3 = stoichiometric ratio of ethanoate-CH_4 conversion, Y_5 = growth yield of *Methanothrix* on ethanoate, mg/mg, and K_{d2} = specific decay rate of *Methanothrix*, d^{-1}. There is no accumulation of VFAs in the anaerobic ecosystem, which suggests that the methanogens are not inhibited by VFAs and 2-EHA. Therefore, the specific growth rate of *Methanothrix* is expressed by a saturation function in the form of the Monod equation:

$$\grave{I}_2 = \grave{I}_{M2} S_2 / (K_{S2} + S_2) \quad (9)$$

where $\grave{I}_{M2}$ = maximum specific growth rate of *Methanothrix*, d^{-1} and K_{S2} = saturation constant of ethanoic acid, mg/L. The *Methanococcus* cell mass in the ecosystem only accounts for a small fraction of the total cell mass and is, therefore, not described by the model. The production rate of CH_4, in mL/L/d, is the sum of production from decarboxylation of ethanoate and H_2 reduction of CO_2:

$$Q_M = (22.4/16)\grave{I}_2 X_2 Y_4 + Q_H/4 \quad (10)$$

where Q_M = specific production rate of methane, mL/L/d.

Steady-state solutions and simplification of Eq. (1) to (10) result in a set of seven equations, with 13 constant parameters. For the convenient of validation, these equations are arranged to express S_1, X_1, S_2, X_2, S_4, and Q_M as functions of D, and U as a function of pH, as follows:

$$S_1 = (+ \{[1 - \grave{I}_{M1}/(K_{d1} + D)]2 - 4UK_{S1}/K_{I1}\}^{0.5} / (2U/K_{I1}) \quad (11)$$

$$X_1 = [D(S_{10} - S_1) / (K_{d1} + D)(1/Y_1 + Y_2/R_1)] \quad (12)$$

$$S_2 = [K_{S2}(K_{d2} + D) / \grave{I}_{M2} - (K_{d2} + D)] \quad (13)$$

$$X_2 = [(K_{d1} + D) X_1 Y_3 - DS_2 / (K_{d2} + D)(1/Y_5 + Y_4/Y_5)] \quad (14)$$

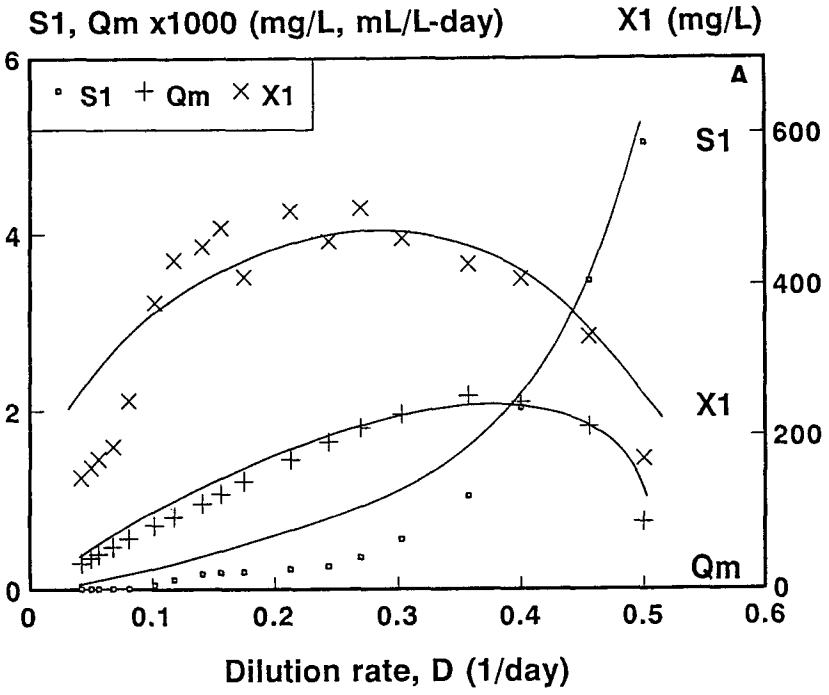
$$S_4 = [X_1 (K_{d1} + D)/D](Y_2 + Y_3/R_2) \quad (15)$$

$$Q_m = (22.4/16)(K_{d2} + D)X_2 Y_4 + (22.4/352((K_{d2} + D)X_1 Y_2 + (22.4/240)(K_{d1} + D)X_1 Y_3 \quad (16)$$

$$U = 10^{-pH} / (10^{-pH} + K) \quad (17)$$

Model Validation

Figure 7 compares the predictions of the model and operation data from CSTRs reported by Chua (*6*). The constant parameters were substituted with values taken from the literature, which were obtained through independent experiments. General agreement between the model



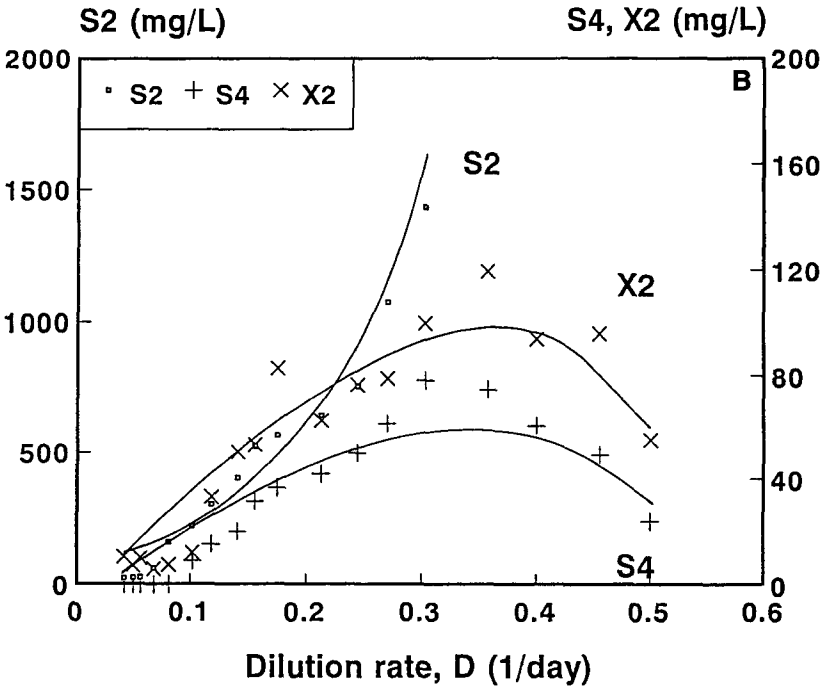


Fig. 7. Model simulation (curves) vs CSTR data (markers) of the anaerobiosis of 2-EHA.

Table 1
Refined Parameters and Prediction Errors

K_{S1}	782 mg/L	K_{S2}	696 mg/L	Y_1	0.21 mg/mg
K_{d1}	0.08 d^{-1}	K_{d2}	0.02 d^{-1}	Y_5	0.002 mg/mg
K_{I1}	997 mg/L	K	1.31×10^{-5}	Y_3	72.34 mg/mg
I_{m1}	0.70 d^{-1}	I_{m2}	0.58 d^{-1}	Y_2	53.27 mg/mg
				Y_4	2.07 mg/mg

Variable	Sum of square error	% Error[a]	Variable	Sum of square error	% Error
S_1	1,794,504	6.3	X_2	42,400	29.6
X_1	80,929	13.4	S_4	38,061	36.9
S_2	69,450	4.3	Q_M	1,016,272	11.0

[a] Average prediction error for each variable, calculated by:

% error = Abs. (Prediction – CSTR Data) / CSTR Data × 100.

predictions and the operation data validated the model. The constant parameters of the model were refined by a computer-aided least-square curve-fitting technique. The refined constant parameters and relative errors of the model predictions are tabulated in Table 1. The model allowed reasonably accurate predictions of the concentrations of 2-EHA, ethanoic acid, and acidogenic cell mass in the anaerobic ecosystem and CH_4 production rate with < 15% error. Larger errors were observed in the concentrations of methanogenic cell mass and butanoic acid, which were attributed to the inaccuracies of analytical methods because these concentrations were usually at very low levels.

The substrate inhibition function, which has been used in many models (*19–23*) to describe the growth of anaerobic bacteria under the inhibitory effects of toxic substrates, was shown to be applicable to *Syntrophomonas* with 2-EHA as the substrate. The inhibition constant, K_{I1}, of 997 mg/L is an order higher than the 40.0–58.7 mg/L used by Stephanopoulos (*23*) to model VFA inhibition on different groups of bacteria in the anaerobic population. This indicated that the inhibitory effect of 2-EHA on the acidogens was mild compared to that of VFA observed in other anaerobic ecosystems. The commonly observed inhibition of methanogenic bacteria by VFA accumulation (*24*) did not occur in this ecosystem with 2-EHA as the substrate. The Monod saturation function was sufficient for describing the growth of *Methanothrix* where VFA concentrations were maintained at low levels.

The maximum specific growth rates of acidogens and methanogens, 0.70 and 0.58 d^{-1}, respectively, were higher than the dilution rate of 0.5 d^{-1} when the 2-EHA removal efficiency in the continuous stirred-tank reactors was observed to drop drastically. This agreed with the observa-

tion that ethanoic acid was at a very low level because methanogens were not washed out at the dilution rate of 0.5 d^{-1}.

The only exception to the general agreements between the model predictions and the operation data was that the turning point and the descending trend of ethanoic acid at dilution rates higher than 0.3 d^{-1} were not predicted. However, this discrepancy was not significant in practical applications, because ethanoic acid only accounted for <30% of the total residual organic content. Therefore, it did not justify the use of a different equation to describe the descending trend of ethanoic acid at high dilution rates, which would have added complexity to the solution of the model.

CONCLUSION

The mathematical model developed in this article was based on a biphasic degradation pathway of 2-EHA. It incorporated a substrate inhibition function to account for the effects of unionized 2-EHA on growth and VFA production by *Syntrophomonas*. The model was able to describe the main reactions in the anaerobiosis of 2-EHA and mixed-culture interactions in an anaerobic ecosystem. The model could predict the CH_4 production rate and concentrations of 2-EHA, acidogenic cell mass and ethanoic acid with <15% error. Although the turning point and descending tend of ethanoic acid at dilution rates higher than 0.3 d^{-1} were not predicted by the model, the discrepancy was not significant in practical applications because ethanoic acid only accounted for <30% of the total residual organic content.

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