

MATHEMATICAL MODELLING OF COMPETITION BETWEEN SULPHATE REDUCTION AND METHANOGENESIS IN ANAEROBIC REACTORS

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(Received 21 October 1996; revised version received 9 January 1998; accepted 11 January 1998)

Abstract

A structured mathematical model of competition between sulphate reduction and methanogenesis in anaerobic reactors has been developed. This model includes multiple-reaction stoichiometry, microbial growth kinetics, conventional material balances for an ideally mixed reactor, liquid gas interactions and liquid phase equilibrium chemistry. The model agrees well with existing experimental studies of this competition in anaerobic reactors. Hypothetical computer simulations are presented to illustrate the influence of hydraulic retention time, SO_4^{2-} : COD ratio, initial proportion of sulphate reducing and methanogenic bacteria in seed sludge, sludge retention, hydrogen sulphide inhibition and other factors on the outcome of this competition.
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Key words: mathematical modelling, sulphate reduction, methanogenesis, substrate competition, sulphide inhibition.

NOMENCLATURE

Ac acetate
 AB acetogenic bacteria
 AcRR acetate removal rate (g acetate-COD/g VSS-day)
b bacterial decay rate constant (day^{-1})
 COD chemical oxygen demand
 CRIT criterion for evaluation of outcome of competition between SRB and MB (AB) (dimensionless)
 CSTR continuous stirred tank reactor
 ER efficiency of retention of biomass in reactor (dimensionless)
 FB fermentative bacteria
G gas volumetric flow rate from the reactor ($l day^{-1}$)
 H Henry coefficient ($atm \cdot l/g COD (g, g S, mol)$)
 HRT hydraulic retention time (days)
k conversion factor (g COD/g biomass)

k_{1a} mass transfer coefficient (day^{-1})
K Monod saturation constant for sulphate (g/l)
K_{d(b)} dissociation constant
K_i inhibition constant by undissociated hydrogen sulphide (g S/l)
K_S Monod saturation constant for organic substrates and hydrogen (g COD/l)
m conversion factor to molar concentration (g COD (g, g S)/mol)
M mass transfer rate to the gas phase (g COD (g, g S, mol)/l-day)
 MB methanogenic bacteria
N total ammonia concentration (mol/l)
 ORR organic removal rate (g COD/g VSS-day)
P partial pressure of substrate in gaseous form (atm)
P total phosphate concentration (mol/l)
 Pr propionate
S substrate concentration in liquid phase (g COD/l, g/l for sulphate, g S/l for sulphide, or mol/l for soluble CO₂ and its ionised form)
 SLR sludge loading rate (g COD/g VSS-day)
 SRB sulphate-reducing bacteria
r_i net biological production rate of substrate *i* (g COD (g, g S, mol)/l-day)
R_j decay rate of bacteria *j* (g/l-day)
 UASB upflow anaerobic sludge blanket
V_s specific volume of gas (l/g COD (g S, mol))
V_G volume of reactor gas phase (l)
V_R volume of reactor liquid phase (l)
 VFA volatile fatty acids
 VSS volatile suspended solids (biomass)
X bacterial concentration (g VSS/l)
Y bacterial yield (g VSS/g COD consumed)
μ specific growth rate (day^{-1})
μ_m maximum specific growth rate (day^{-1})
Subscripts
 * undissociated
 G gas
 e effluent
i substrate *i*
j bacteria *j*
n SRB *n*
 L liquid
 i influent
 t total

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INTRODUCTION

In anaerobic reactors treating sulphate-containing waste waters, both sulphate reduction and methanogenesis can be the final step in the degradation process, because SRB are capable of using many of the intermediates formed during methanogenesis. In general, substrate competition in such systems is possible on three levels: competition between SRB and FB for sugars and amino acids; competition between SRB and AB for VFA and ethanol; and competition between SRB and MB for acetate and hydrogen.

Though thermodynamic data are often used to predict the outcome of bacterial substrate competition, it is not quite correct, because biological processes are not always in a thermodynamic equilibrium. Therefore it is better to use kinetic data for this prediction. From this point of view, it is evident that the competition of the first level is won by the very fast growing FB and sulphate reduction with sugars and amino acids playing a unimportant role (Widdel, 1988). Therefore, it will not be considered in the development of the mathematical model described below. However, Monod-kinetics data of SRB, AB and MB (Table 1) for growth on VFA and hydrogen indicate that SRB should be able to out-compete AB and MB. This prediction has been confirmed experimentally for hydrogen (Alphenaar *et al.*, 1993; Mulder, 1984; Rinzema *et al.*, 1986; van Houten *et al.*, 1994) and for propionate (Alphenaar *et al.*, 1993). For utilisation of acetate in anaerobic reactors the situation is different. Various researchers have observed that during the break-

down of sulphate-containing waste water, SRB indeed can successfully compete with MB for acetate (Alphenaar *et al.*, 1993; Rinzema and Schultz, 1987; Visser, 1995) whereas other results indicate that the latter is preferentially degraded to methane (Hoeks *et al.*, 1984; Mulder, 1984; O'Flaherty and Colleran, 1995; Rinzema and Lettinga, 1988). To explain the differences found, besides pure bacterial kinetics, other factors influencing the outcome of competition between SRB and MB should be taken into account. These factors include the $\text{SO}_4^{2-} : \text{COD}$ ratio, type of seed sludge, sludge retention, hydrogen sulphide inhibition, pH, nutrient limitation, etc. (Visser, 1995).

The possibility of controlling the competition is important for practical application of anaerobic treatment processes. Under classical anaerobic (methanogenic) treatment of sulphate containing waste waters, the activity of SRB should be minimised because of well known problems caused by hydrogen sulphide (direct inhibition actions on methanogenesis, corrosion of equipment, malodour and necessity of post-treatment (sulphide removal) of effluents). On the other hand, biological sulphate reduction with a complete suppression of methanogenesis followed by conversion of sulphide into elemental sulphur has been proposed (van Houten *et al.*, 1994; van Houten *et al.*, 1995; Stucki *et al.*, 1993) as an option for removing sulphate from inorganic waste water and reutilisation of sulphur.

This paper will first develop a structured mathematical model of competition between sulphate reduction and methanogenesis in anaerobic reactors with biomass retention. Second, the resultant model

Table 1. Kinetics of SRB, AB and MB for growth on propionate, acetate and hydrogen

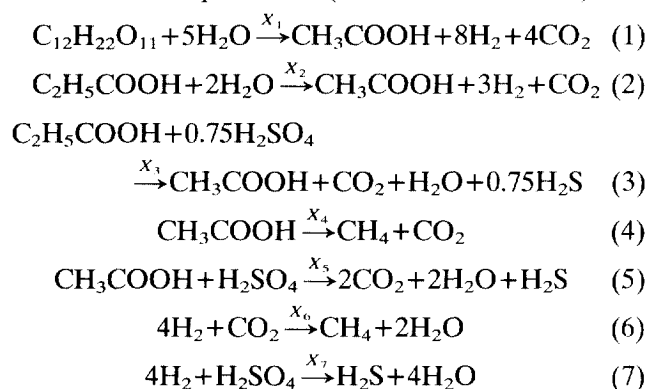
	T (°C)	μ_m (day ⁻¹)	K_s (g COD/l)	Y (g VSS/g COD)	Reference
Propionate-degrading AB:					
<i>Syntrophobacter wolinii</i>	35	0.1–0.2			Boone and Bryant (1980)
Enrichment	33	0.16	0.246	0.025	Gujer and Zehnder (1983)
Propionate-degrading SRB:					
<i>Desulfolobus propionicus</i>	30	0.89			Stams <i>et al.</i> (1984)
Acetotrophic MB:					
<i>Methanosarcina barkeri</i>	37	0.21	0.258		Wandrey and Aivasidis (1983)
<i>Methanobacterium hungatei</i>	37	0.11	0.028	0.023	Huser (1981)
<i>Methanothermobacter thermoautotrophicus</i>	30	0.24	0.409	0.051	Lawrence and McCarty (1969)
Acetotrophic SRB:					
<i>Desulfobacter postgatei</i>	28	1.03	0.015	0.04	Brandis-Heep <i>et al.</i> (1983)
<i>Desulfomaculum acetoxidans</i>	36	0.55		0.098	Widdel and Pfennig (1977)
<i>Desulfonema limicola</i>	30	0.55			Widdel (1980)
Mixed culture	31	0.51	0.006		Middleton and Lawrence (1977)
Hydrogenotrophic MB:					
<i>Methanobacterium formicicum</i>		2.0	3.2×10^{-5}	0.05	Schauer and Ferry (1980)
<i>Methanobacterium hungatei</i>	37	1.2	1.1×10^{-4}	0.0125	Robinson and Tiedje (1984)
<i>Methanobacterium</i> sp.	30			0.0375	Lupton and Zeikus (1984)
Hydrogenotrophic SRB:					
<i>Desulfovibrio vulgaris</i>	35	5.52		0.063–0.078	Badziong and Thauer (1978)
<i>Desulfovibrio vulgaris</i>	30		6.4×10^{-5}	0.0688	Lupton and Zeikus (1984)
<i>Desulfovibrio</i> sp.	37	1.37	5.3×10^{-5}	0.053	Robinson and Tiedje (1984)
<i>Desulfovibrio gigas</i>	35	1.37		0.109–0.125	Brandis and Thauer (1981)

is calibrated to existing laboratory studies of this process. Finally, several scenarios are presented to determine the influence of hydraulic retention time, SO_4^{2-} : COD ratio, initial proportion of SRB and MB in seed sludge, sludge retention, hydrogen sulphide inhibition and influent pH on outcome of this competition.

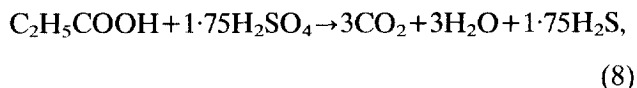
MODEL DESCRIPTION

Stoichiometry

The present model simulates the anaerobic treatment of soluble organic waste waters containing sulphate concentrations comparable with COD concentrations. Since sugars and volatile fatty acids are typical components of the organic part of these wastes, we chose sucrose, propionate, acetate and sulphate as influent substrates in the variant of our model discussed below. The general reaction sequence by which the chosen influent substrates are transformed by the different groups of anaerobic bacteria can be presented (in molar coefficients) as:



Though oxidation of propionate by SRB can proceed not only incompletely to acetate (reaction (3)) but also completely to CO_2 :



we did not include this reaction in the stoichiometric schemes (1–7) to avoid an excessive complexity of the mathematical model. Furthermore reaction (8) is the sum of reactions (3) and (5) from the perspective of mass balance in the system.

Thus, according to the accepted stoichiometric schemes (1–7), the conversion process is carried out by seven groups of microorganisms: the group X_1 contains all FB; X_2 , all propionate-degrading AB; X_3 , all acetogenic SRB; X_4 , all acetotrophic MB; X_5 , all acetotrophic SRB; X_6 , all hydrogenotrophic MB; and X_7 , all hydrogenotrophic SRB. For simplicity, the groups of microorganisms X_1 – X_7 will be referred to as bacteria X_1 – X_7 .

Since the concentrations of organic contaminants are usually expressed in g COD/l, sulphates in g/l and sulphides in g S/l, the stoichiometric schemes (1–7) can be presented on this basis as the following (components with zero COD are omitted; for sulphate reduction: consumption of 1 g COD stoichiometrically corresponds to consumption of 1.5 g sulphate and evolution of 0.5 g sulphide (as S)):

Table 2. Conversion factors used in the model

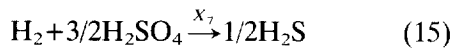
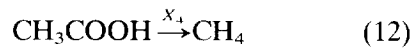
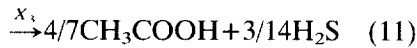
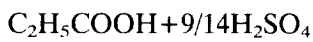
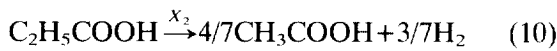
Conversion factor	Value	Basis
k (g COD/g biomass)	1.222	$\text{C}_5\text{H}_8\text{O}_3\text{N}$
m_1 (g COD/mol)	342	$\text{C}_{12}\text{H}_{22}\text{O}_{11}$
m_2 (g COD/mol)	112	$\text{C}_2\text{H}_5\text{COOH}$
m_3 (g COD/mol)	64	CH_3COOH
m_4 (g COD/mol)	16	H_2
m_5 (g/mol)	96	SO_4^{2-}
m_6 (g S/mol)	32	S

Table 3. Details of experimental study of Alphenaar et al. (1993) used in model calibration

Parameter	Case	
	1	2
Reactor type	UASB+CSTR with recycle	UASB with recycle
Working reactor volume (l)	6.1	1.1
Recirculation factor	10	10
Mixing regime	Close to CSTR	Close to CSTR
Temperature (°C)	30	30
Seed sludge	80% granular+20% sulphate adapted	
HRT at steady-state, days 50–150 (days)	1.675	0.2875
Upward velocity (m/h)	0.65	0.65
SLR (g COD/g VSS day)	gradual increase from 0.25 to ≈ 1	
Start-up (days 0–50)	1.01	0.98
Steady-state (average)		
Mineral medium content (g/l)	NH_4Cl , 1.044; KCl , 0.27; KH_2PO_4 , 0.169; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.15; influent pH – 6.8	
Influent sulphate (added as Na_2SO_4) (g/l)	5	5
Influent COD (g/l)	2.5	2.5
COD content	acetate : propionate : sucrose, 5 : 4 : 1 (as COD)	
Duration of experiment (days)	150	160

Table 4. Physico-chemical parameters (for 30°C) used in the model (values were taken or recalculated from Rabinovich and Havin, 1977).

Parameter	Value	Parameter	Value
K_{b,NH_3} (mol/l)	6.3×10^{-5}	K_w (mol/l)	1.46×10^{-14}
K_{a1,H_3PO_4} (mol/l)	7.6×10^{-3}	k_{La} (day ⁻¹)	100
K_{a2,H_3PO_4} (mol/l)	6.46×10^{-8}	H_{H_2} (atm l/g COD)	82.3
K_{a2,H_3PO_4} (mol/l)	4.2×10^{-13}	H_{H_2S} (atm l/g S)	0.343
K_{a,C_2H_3COOH} (mol/l)	1.33×10^{-5}	H_{CH_4} (atm l/g COD)	12.67
K_{a,CH_3COOH} (mol/l)	1.75×10^{-5}	H_{CO_2} (atm l/mol)	33.66
K_{a1,H_2S} (mol/l)	10^{-7}	V_{s,H_2} (l/g COD)	1.554
K_{a2,H_2S} (mol/l)	10^{-14}	V_{s,H_2S} (l/g S)	0.777
K_{a1,CO_2} (mol/l)	4.71×10^{-7}	V_{s,CH_4} (l/g COD)	0.388
K_{a2,CO_2} (mol/l)	5.13×10^{-11}	V_{s,CO_2} (l/mol)	24.862



Since carbon dioxide has zero COD, its stoichiometric relationships in the model are expressed on the basis of schemes (1–7), i.e. in mol/l (see Appendix).

Kinetics

The kinetics are based on the following assumptions and considerations.

1. Growth of biomass proceeds according to Monod kinetics with simultaneous inhibition by undissociated H_2S . Instead of using true Monod kinetics it is assumed that reaction kinetics for carbon dioxide (reaction (6)) is of zero order in its concentration because carbon dioxide is usually present in significant concentrations in anaerobic reactors. A dual substrate form of the Monod equation is postulated for SRB to account for their growth limitations under the treatment of sulphate-deficient waste waters.
2. The direct effect of pH on the growth rates is not included to avoid an excessive complexity of the model.
3. Undissociated H_2S inhibition proceeds according to first order inhibition kinetics (Levenspiel,

Table 5. Bacterial parameters used in the model

Bacterial groups	μ_m (day ⁻¹)	K_s (g COD/l)	K (g/l)	K_I (g S/l)	Y (g VSS/g COD)	b (day ⁻¹)	ER
X_1	8.0	0.028	—	0.55	0.043	0.056	0.983
X_2	0.16	0.247	—	0.215	0.018	0.018	0.983
X_3	0.81	0.295	0.0074	0.285	0.035	0.018	0.979
X_4	0.24	0.056	—	0.285	0.026	0.0155	0.983
X_5	0.51	0.024	0.0192	0.285	0.041	0.025	0.979
X_6	1.0	0.00013	—	0.215	0.018	0.05	0.983
X_7	5.0	0.00005	0.0009	0.55	0.077	0.03	0.979

Table 6. Model versus experiment (average values over days 50–150)

Parameters	Case 1		Case 2	
	Experiment ^a	Model	Experiment ^a	Model
HRT (days)	1.675	1.675	0.2875	0.2875
Y_t (g VSS/g COD)	0.041	0.043	0.041	0.042
SLR (g COD/g VSS day)	1.01	0.99	0.98	1.010
RR (g COD/g VSS day)	0.79	0.78	0.73	0.80
AcRR (g COD/g VSS day)	0.67	0.63	0.61	0.67

^aAlphenaar *et al.* (1993).

1980) for all bacteria. Because not much actual information about inhibition kinetics is available, this is a reasonable first approximation. Thus, a specific growth rate equation for FB, AB and MB has a form:

$$\mu_j = \mu_{m,j} S_i (1 - H_2 S^* / K_{L,j}) / (K_{S,j} + S_i); \quad (16)$$

for SRB bacteria:

$$\mu_j = \frac{\mu_{m,j} S_i [SO_4^{2-}] (1 - H_2 S^* / K_{L,j})}{((K_{S,j} + S_i)(K_n + SO_4^{2-}))} \quad (17)$$

4. All product formations are directly coupled to biomass production due to the dissimilatory nature of sulphate reduction and methanogenesis.
5. Bacterial decay is described by first order kinetics (Byers, 1985; Costello *et al.*, 1991; Mosey, 1983):

$$R_j = -b_j X_j \quad (18)$$

6. Substrate consumption for maintenance is incorporated in the overall biomass yield.
7. Sulphate consumption for biomass growth is negligible.

8. All reactions are effectively rate controlled, i.e. the effects of diffusional limitations of biomass aggregates are constant and incorporated into the kinetic term. This was shown by Denac *et al.* (1988) to be a reasonable assumption given that diffusional gradients were not important in the calculation of the bulk concentrations of reactor components.

By definition, this model also assumes that all bacteria present in the reactor are active. Hence, any relationship between the simulated concentration of bacteria and reported concentrations of VSS would have to account for the levels of biological inactivity in an operating system.

Liquid phase equilibrium chemistry

For calculation of pH values and concentrations of undissociated forms S^* during the process we used the approaches proposed previously (Kalyuzhnyi *et al.*, 1986; Rytov *et al.*, 1992; Angelidaki *et al.*, 1993). In summary, the pH value is calculated from the ionic balance equation, which includes all the

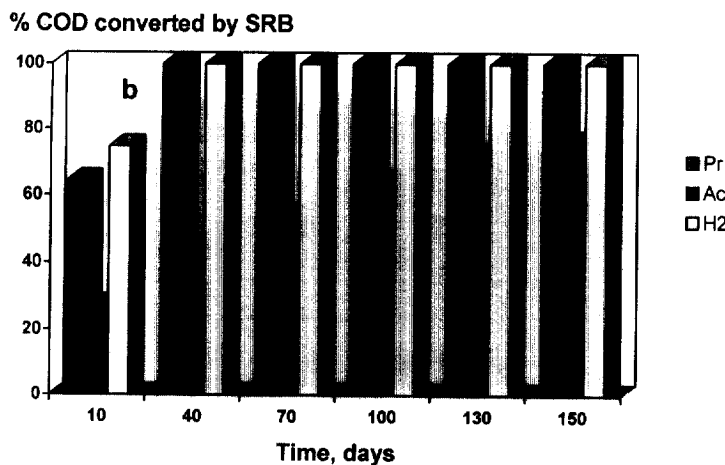
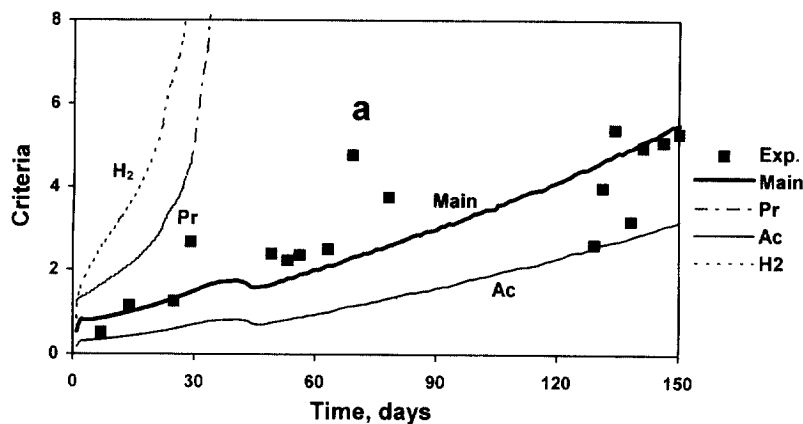


Fig. 1. Model versus experiment. Case with steady-state HRT of 1.675 days. (a) Main and specific criteria (eqns 25–28): points, experimental data of Alphenaar *et al.* (1993); lines, model. (b) Modelling results about percentage of electron donors used by SRB.

ionised compounds in the liquid phase (see Appendix).

Material balances

Liquid phase

The model was developed for an ideally mixed reactor using the dynamic rate equations for a CSTR. The flowrate of biomass through the reactor was considered to be different from the liquid flowrate. This was to account for the retention of biomass by high-rate anaerobic processes such as UASB-reactors, upflow filter etc.

For a constant-volume reactor with no concentration gradients, a general material balance for influent substrates (sucrose, propionate, acetate and sulphate) can be written as

$$dS_i/dt = (S^o - S_i)/HRT + r_i \quad (19)$$

For liquid concentrations of carbon dioxide, hydrogen, methane and hydrogen sulphide, scheme (19) should contain an additional term describing its escape into the gas phase, i.e.

$$dS_i/dt = (S^o - S_i)/HRT + r_i - M_i \quad (20)$$

where

$$M_i = k_L a (S_i^* - p_i / H_i) \quad (21)$$

(for hydrogen and methane $S_i^* = S_i$).

The general mass balance equation used to describe the behaviour of each bacteria in the reactor is presented below (influent does not contain biomass):

$$dX_j/dt = \mu_j X_j - (1 - ER_j) X_j / HRT - b X_j \quad (22)$$

where ER_j characterises the efficiency of the retention of bacteria X_j in the reactor (the same approach was used by Bolle *et al.*, 1986, for modelling the UASB-reactor). Thus, the assumption is made with this model that the biomass retained in the reactor by wall growth, adhesion to support media and agglomeration can be effectively accounted for by choosing an appropriate ER . Hence, the consideration of the dynamics of aggregation and dispersion of biomass from support media or granulating (flocculating) particles is beyond the scope of the current model.

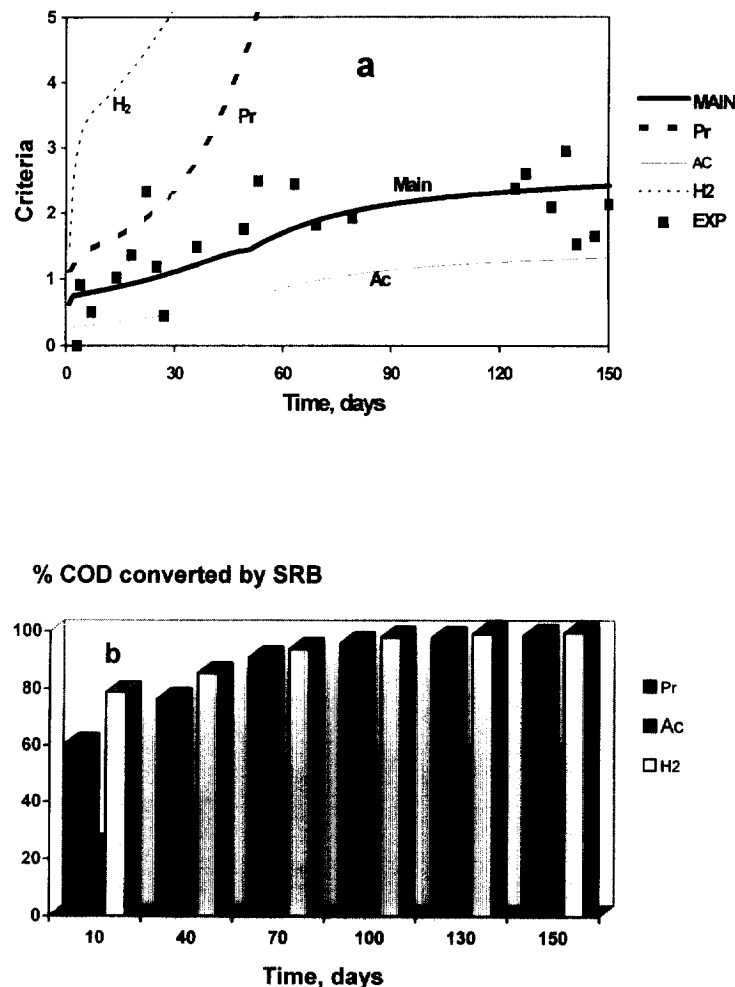


Fig. 2. Model versus experiment. Case with steady-state HRT of 0.2875 days. (a) Main and specific criteria (eqns 25–28): points, experimental data of Alphenaar *et al.* (1993); lines, model. (b) Modelling results about percentage of electron donors used by SRB.

Gas phase

The partial pressure in the gas volume is calculated by a component balance around the gas phase:

$$dp_i/dt = (M_i V_{si} V_R p_i - G p_i) / V_G \quad (23)$$

where $p_t = \sum p_i$ (p_t was accepted to be equal to 1 atm). A total balance gives the gas volumetric flow rate from the reactor:

$$G = V_R \sum (M_i V_{si}) \quad (24)$$

With the assumptions formulated above, the resulting material balances are summarised in the Appendix. Conversion factors used are given in Table 2.

The criteria for evaluation of outcome of competition between SRB and MB or AB

The ratio of COD converted by SRB relative to that converted by MB was used as a main criterion for evaluation of outcome of competition between SRB and MB:

$$\begin{aligned} \text{CRIT} &= \text{COD}_{\text{SRB}} / \text{COD}_{\text{MB}} \\ &= 2(\text{SO}_4^- - \text{SO}_4) / 3r_{\text{CH}_4} \text{HRT} \end{aligned} \quad (25)$$

Also three specific criteria were introduced to evaluate an outcome of competition between SRB and MB or AB for specific substrates:

$$\begin{aligned} \text{CRIT}_{\text{Pr}} &= \text{Pr} - \text{COD}_{\text{SRB}} / \text{Pr} - \text{COD}_{\text{AB}} \\ &= 2r_{\text{H}_2\text{S}-\text{Pr}} / r_{\text{H}_2-\text{Pr}} \text{ (for propionate)} \end{aligned} \quad (26)$$

$$\begin{aligned} \text{CRIT}_{\text{Ac}} &= \text{Ac} - \text{COD}_{\text{SRB}} / \text{Ac} - \text{COD}_{\text{MB}} \\ &= 2r_{\text{H}_2\text{S}-\text{Ac}} / r_{\text{CH}_4-\text{Ac}} \text{ (for acetate)} \end{aligned} \quad (27)$$

$$\begin{aligned} \text{CRIT}_{\text{H}_2} &= \text{H}_2 - \text{COD}_{\text{SRB}} / \text{H}_2 - \text{COD}_{\text{MB}} \\ &= 2r_{\text{H}_2\text{S}-\text{H}_2} / r_{\text{CH}_4-\text{H}_2} \text{ (for hydrogen)} \end{aligned} \quad (28)$$

EXPERIMENTAL SYSTEMS CONSIDERED

Results of the experimental study of Alphenaar *et al.* (1993) that considered the competition between sulphate reduction and methanogenesis in UASB reactors with recycle treating synthetic sulphate-containing waste water, were used to calibrate the structured model above. Salient features of experimental protocol are given in Table 3. Briefly, the same UASB reactors with the same feed content and the same feeding regime were used for both cases, only for case 1, the UASB reactor was placed

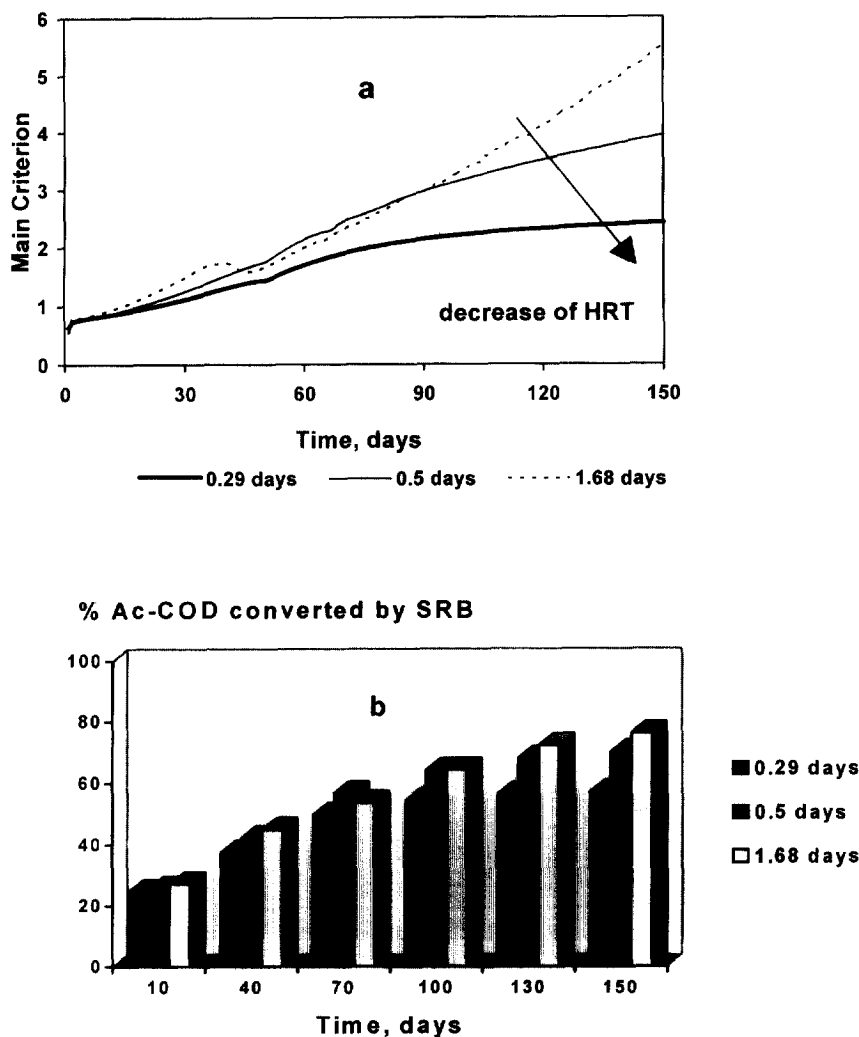


Fig. 3. Modelling results under variation of HRT. (a) Main criteria. (b) Percentage of Ac-COD used by SRB.

in series with CSTR to increase the HRT. Effluent recycling was applied in the UASB/CSTR system as well as in the single UASB system (flow 40 l day⁻¹) in order to increase the upflow velocity. The results showed that hydrogen, generated during the anaerobic mineralisation process, and propionate were converted by SRB. However, acetate was converted by both SRB and MB. The fraction of acetate used by SRB relative to MB increased with time, resulting in a predominance of SRB, especially at relative long HRT (1.625 days).

COMPUTATIONAL METHODS

Simulations were performed on an IBM-compatible personal computer (processor Pentium-133) by numeric integration of the differential eqns (A23)–(A36), (A37) and (A41)–(A45) with an automatic selection of time step by a computer program based on a Runge–Kutta (fifth order)

technique (Millne, 1955). On each step of the numeric integration, pH values were calculated by solution of eqn (A8) using an iteration technique (Korn and Korn, 1968) with automatic selection of the roots according to the physical sense. The computer program was written by authors in Fortran-77 in a generalised form, where a variable number of steps, organisms, compounds, substrates and inoculum data could be specified through an input file. The program created an output data file in a format suitable for graphic processing.

MODEL PARAMETERS AND INITIAL CONDITIONS

The physico-chemical model parameters were directly taken from the literature (Table 4). Numerous preliminary simulations were undertaken to determine the most appropriate set of bacterial model parameters. Consequently, the values of these

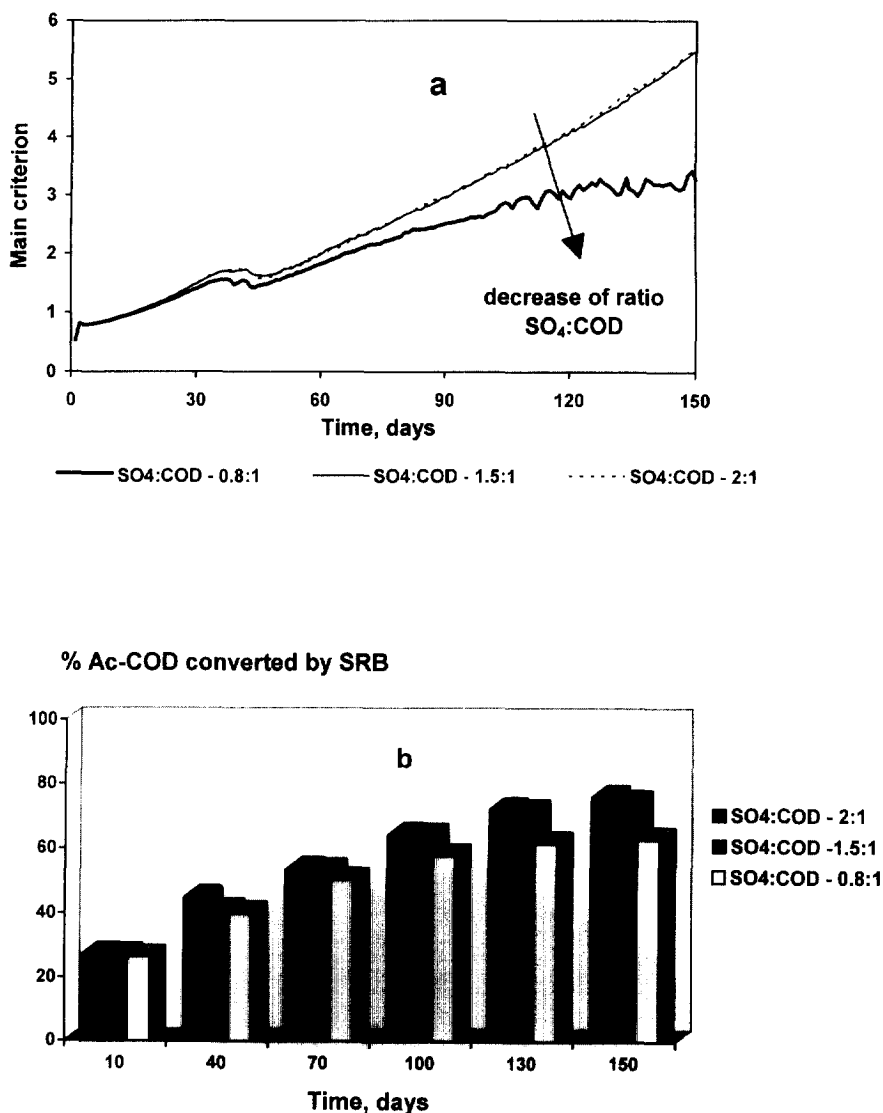


Fig. 4. Modelling results under variation of SO₄²⁻ : COD ratio (HRT = 1.675 days). (a) Main criteria. (b) Percentage of Ac-COD used by SRB.

parameters have been chosen in a range consistent with the experimental study of Alphenaar *et al.* (1993) and/or values reported in the literature (references of Table 1; Bolle *et al.*, 1986; Bryers, 1985; Costello *et al.*, 1991; Denac *et al.*, 1988; Kalyuzhnyi, 1997; Mosey, 1983; Stucki *et al.*, 1993; van Houten *et al.*, 1994; Vavilin *et al.*, 1994a,b; Visser, 1995). The bacterial parameters used in the model are presented in Table 5.

Although the seed sludge quantity, which was supposed to be the same for both the experimental cases (Table 3), was not given in Alphenaar *et al.* (1993), it was estimated from the experimental data as 9–10 g VSS per reactor. Taking into account that the seed sludge was methanogenic rather than sulphidogenic (Table 3), the distribution of quantity of individual bacteria in the seed sludge was arbitrarily fixed as follows (g VSS per reactor):

$$X_1 = 0.79; X_2 = 1.40; X_3 = 0.67; X_4 = 3.11; X_5 = 0.31; \\ X_6 = 2.20; X_7 = 1.04. \quad (29)$$

These values were chosen to be consistent with the experiments mentioned above (the sensitivity of the model to the relative distribution of SRB and MB in the seed sludge is discussed below, see Fig. 5). Possible incongruity between the sum of these

biomass concentrations and the experimental VSS concentration can be attributed to the presence of other bacteria in the inoculum and the biological inactivity of some part of the VSS.

Since the mode of increasing of SLR during the reactor start-up (Table 3) was also not presented in Alphenaar's paper, a linear decrease of HRT during days 0–50 to its steady-state value was introduced in the model to provide a gradual increase of SLR from 0.25 to ≈ 1 g COD/g VSS-day for both the experimental cases.

RESULTS AND DISCUSSION

The results of calibration of the model are presented in Table 6 and Figs 1 and 2. It is seen that with a steady-state HRT of 1.675 days, predictions agree well with reported integrated parameters such as SLR, ORR, AcRR and Y (Table 6) as well as with the reported increase in ratio of the total COD converted by SRB relative to that converted by MB (main criterion) during the experiment (Fig. 1(a)). However, the three primary electron donors (hydrogen, propionate and acetate) for SRB impact this ratio differently. At the end of the experiment, nearly 100% of hydrogen and propionate and only

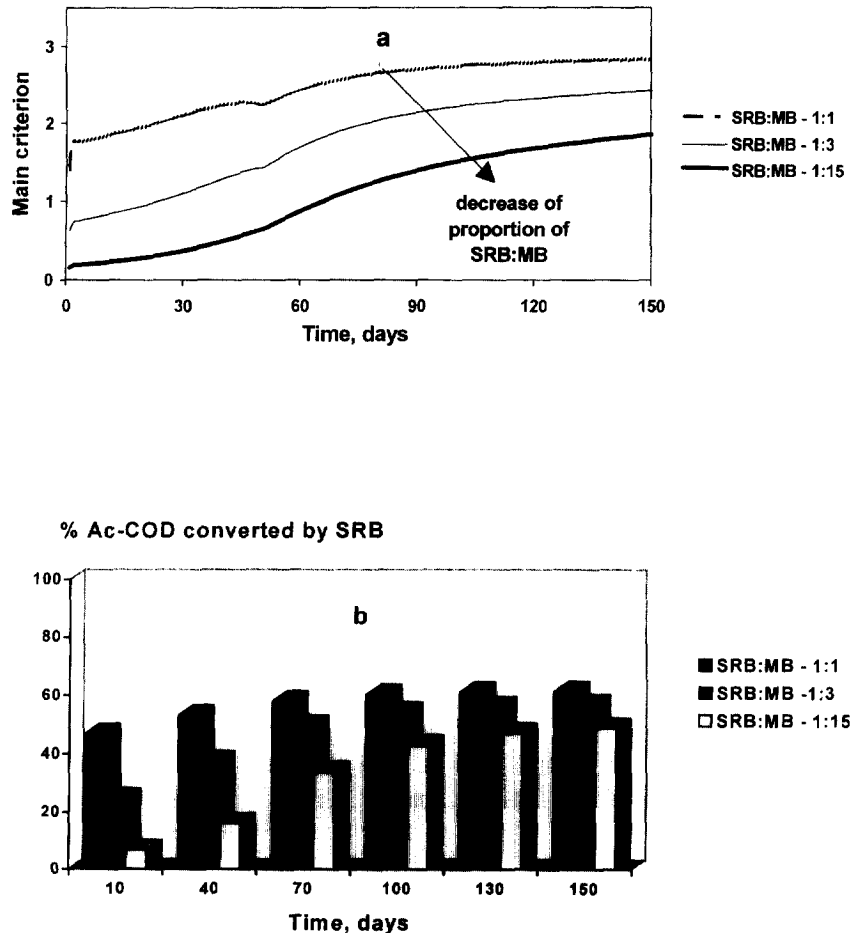


Fig. 5. Modelling results under variation of initial proportion of SRB/MB in the seed sludge (HRT = 0.2875 days). (a) Main criteria. (b) Percentage of Ac-COD used by SRB.

70% of acetate are converted by SRB (Fig. 1(b)), which agrees with previous data (Alphenaar *et al.*, 1993; Hoeks *et al.*, 1984; Mulder, 1984; Rinzema *et al.*, 1986; Rinzema and Lettinga, 1988; Rinzema and Schultz, 1987; van Houten *et al.*, 1994). Thus, the model predicts that MB and AB almost fail to compete for hydrogen and propionate, but can compete effectively for acetate.

Satisfactory agreement between model and experiment has also been obtained for a steady-state HRT of 0.2875 days (Table 6, Fig. 2(a)). Similar tendencies in the percentage of electron donors used by SRB were also observed for this case (Fig. 2(b)). Thus, since the competition between SRB and MB proceeded, in general, mainly for acetate, special attention in the following modelling scenarios (Figs 3–7) was focused on the influence of different factors on the outcome of this competition.

The modelling results for variation of steady-state HRT are shown in Fig. 3. It can be seen that the six-fold decrease of HRT nearly did not influence the main criterion (Fig. 3(a)) and percentage of Ac-COD used by SRB (Fig. 3(b)) in the short-term experiments (up to 2.5 months). Further continua-

tion of the experiments (i.e. long-term experiments) led to a substantial decrease in both parameters mentioned above (Fig. 3(a) and (b)). This was due to increased wash-out of the relatively slow growing acetotrophic SRB under shorter HRT.

An important factor which is not always considered under analysis of the outcome of competition for acetate between SRB and MB is the $\text{SO}_4^{2-}:\text{COD}$ ratio. The corresponding modelling results (Fig. 4) indicated that the decrease of this ratio below the proportion 1:1 led to decreasing both the main criterion (Fig. 4(a)) and percentage of Ac-COD used by SRB (Fig. 4(b)). The explanation may be that under conditions of deficiency of sulphate, the latter is consumed by fast growing acetogenic and hydrogenotrophic SRB rather than slow growing acetotrophic SRB (see Table 1 Table 5). Hence, sulphate deficiency leads to minimal utilisation of acetate as an electron donor for SRB, and acetate is mainly used by MB.

The next important factor for the investigated system is the initial proportion of SRB/MB in the seed sludge. From modelling results (Fig. 5), it can be seen that a decrease of this proportion led to

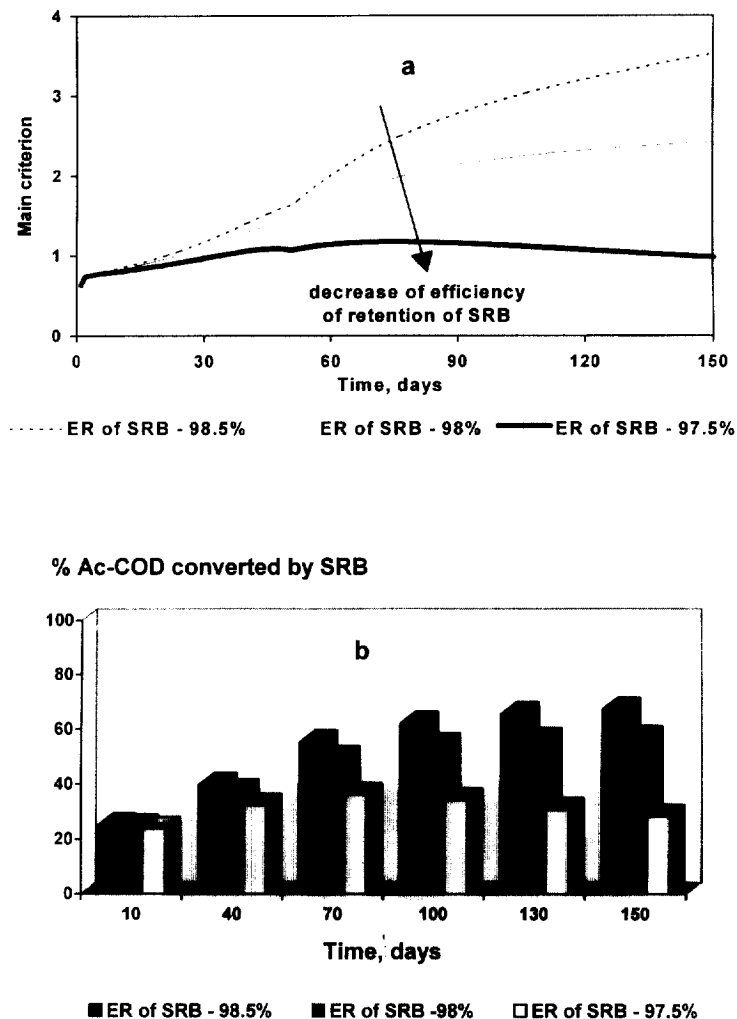


Fig. 6. Modelling results under variation of efficiency of retention of SRB (HRT = 0.2875 days). (a) Main criteria. (b) Percentage of Ac-COD used by SRB.

dramatic reduction in both the main criterion (Fig. 5(a)) and the percentage of Ac-COD used by SRB (Fig. 5(b)) particularly at the beginning of the experiment. In the long-term experiments, the differences were not so pronounced due to better growth properties of SRB (see Table 1Table 5). Thus, if the seed sludge is precultivated under sulphate-limiting conditions it may require consider-

able time before SRB can become predominant. Therefore, duration of the experiment can be a factor.

The modelling results under variation of efficiency of retention of SRB are presented in Fig. 6. It is quite obvious that decrease in ER of SRB led to a substantial decrease of both the main criterion (Fig. 6(a)) and percentage of Ac-COD

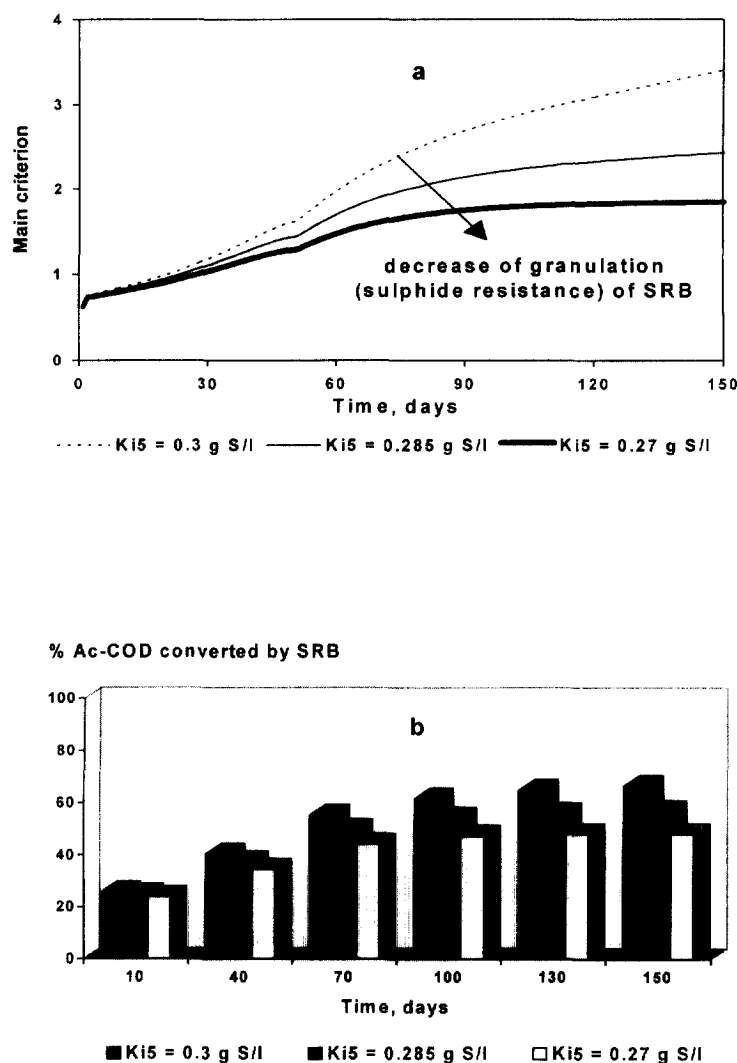


Fig. 7. Modelling results under variation of sulphide resistance of SRB (HRT = 0.2675 days). (a) Main criteria. (b) Percentage of Ac-COD used by SRB.

Table 7. Summary of influence of different factors on outcome of competition between SRB and MB

Factor	COD _{SRB} /COD _{MB} (main criterion)
HRT decrease	Weak decrease in short-term experiments Moderate decrease in long-term experiments
SO ₄ ²⁻ : COD decrease	No influence under SO ₄ ²⁻ : COD > 1 Substantial decrease under SO ₄ ²⁻ : COD < 1
SRB/MB decrease in seed sludge	Sharp decrease in short-term experiments Moderate decrease in long-term experiments
SRB retention efficiency decrease	Substantial decrease
SRB sludge quality (sulphide resistance) decrease	Moderate decrease
Influent pH decrease	No influence up to buffer capacity of influent (0.5 M as phosphate)

used by SRB (Fig. 6(b)). This was due to accelerated wash-out of SRB in comparison with MB. The poor attachment ability of SRB was manifested by Isa *et al.* (1986a,b) who concluded from their experiments that SRB are washed out of the reactor, providing acetotrophic MB with a sufficient advantage. However, the experiments of Alphenaar *et al.* (1993) did not reveal clear differences with respect to attachment ability between SRB (including acetotrophic SRB) and MB in UASB reactors. Moreover these authors have observed the predominance of acetotrophic SRB over MB in both flocculent and granular sludges in long-term experiments. Therefore, ER of SRB was chosen in our model to be only slightly less than ER of MB (Table 5).

Since minimal information about inhibition kinetics is available, one can suppose that sulphide toxicity is different for AB, MB and SRB, and this factor can potentially play a determining role in outcome of long-term competition between methanogenesis and sulphate reduction (Kalyuzhnyi *et al.*, 1997). It should be noted in considering this that sulphide resistance depends on the state (disperse or granular/biofilm) of the sludge. Therefore, the granular sludge demonstrates an elevated sulphide resistance in comparison with the dispersed one associated with methanogenic activity (Rinzema, 1989; Visser, 1995). It is evident that bacteria growing in a biofilm (e.g. granular sludge) might be more protected against undissociated H₂S, due to the existence of a pH and sulphide gradient in the granule/biofilm. From modelling results (Fig. 7) under the variation of sludge quality (or sulphide resistance) of acetotrophic SRB, it is seen that decrease (or increase) in granulation extent of acetotrophic SRB in comparison with acetotrophic MB led to a sharp decrease (or increase) in both the main criterion (Fig. 7(a)) and percentage of Ac-COD used by SRB (Fig. 7(b)). Thus, if the seed sludge contains MB mainly in a granular form and SRB mainly in a dispersed form, seed sludge can be a factor in favouring predominance of methanogenesis versus sulphate reduction.

Modelling results for a variation of influent pH (data not shown) demonstrated a very minimal influence of this factor (up to moderate buffer capacity of influent, e.g. 0.5 M as phosphate) on the main criterion because intensively forming carbon dioxide and hydrogen sulphide create a sufficient level of buffer capacity in the reactor medium.

Summarising the modelling data regarding the influence of different factors on the outcome of competition between SRB and MB (Table 7), the following can be said. An HRT decrease led to a minimal decrease in the main criterion in short-term experiments (up to 2.5 months) and a moderate decrease for longer runs. SO₄²⁻ : COD decrease has no influence under SO₄²⁻ : COD > 1, but a further decrease in this ratio led to a decrease in the main criterion. The SRB/MB proportion in the seed

sludge has a critical influence on the main criterion during the start-up period (first 2–3 months) but further continuation of the run led to a progressive elimination of this influence. A decrease in retention efficiency of SRB indicated a tendency for a substantial decrease in the main criterion. Sulphide resistance of SRB is very important for UASB systems, especially for the conversion of acetate. Variation of influent pH had practically no influence (in a reasonable range of buffer capacity) on the main criterion.

ACKNOWLEDGEMENTS

The work was partially supported by the Russian Ministry of Science and Technology and by The Netherlands Organisation for Scientific Research (NWO).

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APPENDIX

Liquid phase equilibrium chemistry

Ionic balance equation (in mol/l):

$$[\text{H}^+] + [\text{NH}_4^+] + [\text{K}^+] + [\text{Na}^+] + 2[\text{Mg}^{2+}] = [\text{OH}^-] + [\text{Cl}^-] + [\text{H}_2\text{PO}_4^-] + 2[\text{HPO}_4^{2-}] + 3[\text{PO}_4^{3-}] + [\text{C}_2\text{H}_5\text{COO}^-] \\ + [\text{CH}_3\text{COO}^-] + 2[\text{SO}_4^{2-}] + [\text{HS}^-] + 2[\text{S}^{2-}] + [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] \quad (\text{A1})$$

The concentrations of K^+ , Na^+ , Mg^{2+} , and Cl^- are constant during the conversion process, whereas the concentrations of other ions are the subject of dissociation equilibria or microbiological transformations:

$$[\text{NH}_4^+] = N_t / (1 + K_w / K_{b,\text{NH}_3} [\text{H}^+]) \quad (\text{A2})$$

$$[\text{OH}^-] = K_w / [\text{H}^+] \quad (\text{A3})$$

$$[\text{H}_2\text{PO}_4^-] = P_t / ([\text{H}^+] / K_{a1,\text{H}_3\text{PO}_4} + 1 + K_{a2,\text{H}_3\text{PO}_4} / [\text{H}^+] + K_{a2,\text{H}_3\text{PO}_4} K_{a3,\text{H}_3\text{PO}_4} / [\text{H}^+]^2) \quad (\text{A4})$$

$$[\text{HPO}_4^{2-}] = P_t / ([\text{H}^+]^2 / (K_{a1,\text{H}_3\text{PO}_4} K_{a2,\text{H}_3\text{PO}_4}) + [\text{H}^+] / K_{a2,\text{H}_3\text{PO}_4} + 1 + K_{a3,\text{H}_3\text{PO}_4} / [\text{H}^+]) \quad (\text{A5})$$

$$[\text{PO}_4^{3-}] = P_t / ([\text{H}^+]^3 / (K_{a1,\text{H}_3\text{PO}_4} K_{a2,\text{H}_3\text{PO}_4} K_{a3,\text{H}_3\text{PO}_4}) + [\text{H}^+]^2 / (K_{a2,\text{H}_3\text{PO}_4} K_{a3,\text{H}_3\text{PO}_4}) + [\text{H}^+] / K_{a3,\text{H}_3\text{PO}_4} + 1) \quad (\text{A6})$$

$$[\text{C}_2\text{H}_5\text{COO}^-] = S_2 / (m_2 (1 + [\text{H}^+] / K_{a,\text{C}_2\text{H}_5\text{COOH}})) \quad (\text{A7})$$

$$[\text{CH}_3\text{COO}^-] = S_3 / (m_3 (1 + [\text{H}^+] / K_{a,\text{CH}_3\text{COOH}})) \quad (\text{A8})$$

$$[\text{SO}_4^{2-}] = S_5 / m_5 \quad (\text{A9})$$

$$S_6^* = S_6 / (m_6 (1 + K_{a1,\text{H}_2\text{S}} / [\text{H}^+] + K_{a1,\text{H}_2\text{S}} K_{a2,\text{H}_2\text{S}} / [\text{H}^+]^2)) \quad (\text{A10})$$

$$[\text{HS}^-] = S_6 / (m_6 ([\text{H}^+] / K_{a1,\text{H}_2\text{S}} + 1 + K_{a2,\text{H}_2\text{S}} / [\text{H}^+])) \quad (\text{A11})$$

$$[\text{S}^{2-}] = S_6 / (m_6 ([\text{H}^+]^2 / K_{a1,\text{H}_2\text{S}} K_{a2,\text{H}_2\text{S}} + [\text{H}^+] / K_{a2,\text{H}_2\text{S}} + 1)) \quad (\text{A12})$$

$$S_8^* = S_8 / (1 + K_{a1,\text{CO}_2} / [\text{H}^+] + K_{a1,\text{CO}_2} K_{a2,\text{CO}_2} / [\text{H}^+]^2) \quad (\text{A13})$$

$$[\text{HCO}_3^-] = S_8 / ([\text{H}^+] / K_{a1,\text{CO}_2} + 1 + K_{a2,\text{CO}_2} / [\text{H}^+]) \quad (\text{A14})$$

$$[\text{CO}_3^{2-}] = S_8 / ([\text{H}^+]^2 / K_{a1,\text{CO}_2} K_{a2,\text{CO}_2} + [\text{H}^+] / K_{a2,\text{CO}_2} + 1) \quad (\text{A15})$$

The substitution of eqns (A2)–(A9), (A11), (A12), (A14) and (A15) into eqn (A1) leads to an algebraic equation of high degree on $[\text{H}^+]$ which can be solved by numerical methods.

Material balances

Liquid phase

FB, X_1 (g/l):

$$dX_1/dt = \mu_1 X_1 - (1 - ER_1) X_1 / \text{HRT} - bX_1 \quad (\text{A16})$$

Propionate-degrading AB, X_2 (g/l):

$$dX_2/dt = \mu_2 X_2 - (1 - ER_2) X_2 / \text{HRT} - bX_2 \quad (\text{A17})$$

Acetogenic SRB, X_3 (g/l):

$$dX_3/dt = \mu_3 X_3 - (1 - ER_3) X_3 / \text{HRT} - bX_3 \quad (\text{A18})$$

Acetotrophic MB, X_4 (g/l):

$$dX_4/dt = \mu_4 X_4 - (1 - ER_4) X_4 / HRT - b X_4 \quad (A19)$$

Acetotrophic SRB, X_5 (g/l):

$$dX_5/dt = \mu_5 X_5 - (1 - ER_5) X_5 / HRT - b X_5 \quad (A20)$$

Hydrogenotrophic MB, X_6 (g/l):

$$dX_6/dt = \mu_6 X_6 - (1 - ER_6) X_6 / HRT - b X_6 \quad (A21)$$

Hydrogenotrophic SRB, X_7 (g/l):

$$dX_7/dt = \mu_7 X_7 - (1 - ER_7) X_7 / HRT - b X_7 \quad (A22)$$

Sucrose, S_1 (g COD/l):

$$dS_1/dt = (S_1^0 - S_1) / HRT - \mu_1 X_1 / Y_1 \quad (A23)$$

Propionate, S_2 (g COD/l):

$$dS_2/dt = (S_2^0 - S_2) / HRT - \mu_2 X_2 / Y_2 - \mu_3 X_3 / Y_3 \quad (A24)$$

Acetate, S_3 (g COD/l):

$$dS_3/dt = (S_3^0 - S_3) / HRT + (2/3) \mu_1 X_1 (1 - kY_1) / Y_1 + (4/7) \mu_2 X_2 (1 - kY_2) / Y_2 + (4/7) \mu_3 X_3 (1 - kY_3) / Y_3 \\ - \mu_4 X_4 / Y_4 - \mu_5 X_5 / Y_5 \quad (A25)$$

Hydrogen in the liquid phase, S_4 (g COD/l):

$$dS_4/dt = (S_4^0 - S_4) / HRT + (1/3) \mu_1 X_1 (1 - kY_1) / Y_1 + (3/7) \mu_2 X_2 (1 - kY_2) / Y_2 - \mu_6 X_6 / Y_6 - \mu_7 X_7 / Y_7 \\ - k_L a (S_4 - p_4 / H_4) \quad (A26)$$

Sulphate, S_5 (g/l):

$$dS_5/dt = (S_5^0 S_5) / HRT - (9/14) \mu_3 X_3 (1 - kY_3) / Y_3 - (3/2) \mu_5 X_5 (1 - kY_5) / Y_5 - (3/2) \mu_7 X_7 (1 - kY_7) / Y_7 \quad (A27)$$

Total hydrogen sulphide in the liquid phase, S_6 (g S/l):

$$dS_6/dt = (S_6^0 - S_6) / HRT - (3/14) \mu_3 X_3 (1 - kY_3) / Y_3 - (1/2) \mu_5 X_5 (1 - kY_5) / Y_5 - (1/2) \mu_7 X_7 (1 - kY_7) / Y_7 \\ - k_L a (S_6^* - p_6 / H_6) \quad (A28)$$

Methane in the liquid phase, S_7 (g COD/l):

$$dS_7/dt = \mu_4 X_4 (1 - kY_4) / Y_4 + \mu_6 X_6 (1 - kY_6) / Y_6 - k_L a (S_7 - p_7 / H_7) \quad (A29)$$

Total carbon dioxide in the liquid phase, S_8 (mol/l):

$$dS_8/dt = (S_8^0 - S_8) / HRT + (4/m_1) \mu_1 X_1 (1 - kY_1) / Y_1 + (1/m_2) \mu_2 X_2 (1 - kY_2) / Y_2 + (1/m_2) \mu_3 X_3 (1 - kY_3) / Y_3 \\ + (1/m_3) \mu_4 X_4 (1 - kY_4) / Y_4 + (2/m_3) \mu_5 X_5 (1 - kY_5) / Y_5 - \mu_6 X_6 / (4m_4 Y_6) - \mu_7 X_7 / (2m_4 Y_7) \\ - k_L a (S_8^* - p_8 / H_8) \quad (A30)$$

Gas phase

Hydrogen, p_4 (atm):

$$dp_4/dt = (k_L a (S_4 - p_4 / H_4) V_{s4} V_{R p_t} - G p_4) / V_G \quad (A31)$$

Hydrogen sulphide, p_6 (atm):

$$dp_6/dt = (k_L a (S_6^* - p_6 / H_6) V_{s6} V_{R p_t} - G p_6) / V_G \quad (A32)$$

Methane, p_7 (atm):

$$dp_7/dt = (k_L a (S_7 - p_7 / H_7) V_{s7} V_{R p_t} - G p_7) / V_G \quad (A33)$$

Carbon dioxide, p_8 (atm):

$$dp_8/dt = (k_L a (S_8^* - p_8 / H_8) V_{s8} V_{R p_t} - G p_8) / V_G \quad (A34)$$

Gas volumetric flow rate from the reactor (l/l·day):

$$G = V_R k_L a ((S_4 - p_4 / H_4) V_{s4} + (S_6^* - p_6 / H_6) V_{s6} + (S_7 - p_7 / H_7) V_{s7} + (S_8^* - p_8 / H_8) V_{s8}) \quad (A35)$$

Rate expressions

Specific growth rate of FB:

$$\mu_1 = \mu_{m,1} S_1 (1 - S_6^*/K_{1,1}) / (K_{S,1} + S_1) \quad (\text{A36})$$

Specific growth rate of propionate-degrading AB:

$$\mu_2 = \mu_{m,2} S_2 (1 - S_6^*/K_{1,2}) / (K_{S,2} + S_2) \quad (\text{A37})$$

Specific growth rate of acetogenic SRB:

$$\mu_3 = \mu_{m,3} S_2 S_5 (1 - S_6^*/K_{1,3}) / ((K_{S,3} + S_2)(K_1 + S_5)) \quad (\text{A38})$$

Specific growth rate of acetotrophic MB:

$$\mu_4 = \mu_{m,4} S_3 (1 - S_6^*/K_{1,4}) / (K_{S,4} + S_3) \quad (\text{A39})$$

Specific growth rate of acetotrophic SRB:

$$\mu_5 = \mu_{m,5} S_3 S_5 (1 - S_6^*/K_{1,5}) / ((K_{S,5} + S_3)(K_2 + S_5)) \quad (\text{A40})$$

Specific growth rate of hydrogenotrophic MB:

$$\mu_6 = \mu_{m,6} S_4 (1 - S_6^*/K_{1,6}) / (K_{S,6} + S_4) \quad (\text{A41})$$

Specific growth rate of hydrogenotrophic SRB:

$$\mu_7 = \mu_{m,7} S_4 S_5 (1 - S_6^*/K_{1,7}) / ((K_{S,7} + S_4)(K_3 + S_5)) \quad (\text{A42})$$

Sludge loading rate:

$$\text{SLR} = \text{COD}_o / \text{HRT} / X_t \quad (\text{A43})$$

Organic removal rate:

$$\text{ORR} = (\text{COD}_o - \text{COD}_e) / \text{HRT} / X_t \quad (\text{A44})$$

Acetate removal rate:

$$\text{AcRR} = (2/3)(S_1' - S_1) + (4/7)(S_2' - S_2) + (S_3' - S_3) / \text{HRT} / X_t \quad (\text{A45})$$