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Biohydrogen production with mixed anaerobic cultures in the presence of high-concentration acetate

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ABSTRACT

Inhibition of acetate addition on hydrogen production from sucrose-rich synthetic wastewater by mixed anaerobic culture was investigated in this study. Experimental results showed that the added acetate had a significant influence on both substrate degradation and hydrogen production during the fermentation process. The distribution of aqueous products was also influenced by the acetate addition. Modified logistic equations were able to simulate the acidogenesis process well, while a noncompetitive product inhibition model was successfully used to describe the inhibitory effects of acetate addition on both substrate degradation and hydrogen production. From kinetic analysis, the maximum specific rate $r_{max} = 584.9\,mg/g\,VSS/h$, inhibition constant $K_C = 8.27\,g/l$ and the exponent of inhibition $n = 1.53$ were estimated for sucrose degradation, whereas $r_{max} = 221.7\,ml/g\,VSS/h$, $K_C = 9.44\,g/l$ and $n = 1.52$ were calculated for hydrogen production. In addition, the $C_{I,50}$ values of added acetate on the specific hydrogen production rate and hydrogen yield were 11.05 and 31.90 g/l, respectively. The fermentation patterns in the reactor were substantially changed because of the acetate addition, especially at higher dosages.

1. Introduction

Hydrogen has emerged as one of the most promising carriers of new energy because it is clean, recyclable and efficient. In addition, it can be used as an important industrial raw material in hydrogenation processes [1]. For instance, hydrogen is used to produce lower molecular weight compounds, saturate compounds, crack hydrocarbons or remove sulfur and nitrogen compounds. Biological hydrogen production through dark-fermentation processes has recently attracted considerable attention as an effective way of harvesting hydrogen from organic wastes because of its high production rate [1,2].

In the acidogenesis of organic wastes, hydrogen, carbon dioxide, volatile fatty acids (VFAs) and alcohols are simultaneously produced. During biological reaction processes, the VFAs can accumulate to a high level and thus may be stimulatory, inhibitory or even toxic to fermentative bacteria, depending on their concentration [3–7]. The inhibition mechanism of VFAs on fermentative bacteria has been analyzed previously. It has been assumed that both undissociated and dissociated forms of these acids may act as uncouplers for the growth of microorganisms [8,9]. On the other hand, inhibition to methane production by propionate and acetate was influenced by pH, suggesting that the inhibition was due to the free acids rather than the acid anions [3]. Besides, the ionic strength in solution would increase if a high level of dissociated VFAs is present in the culture, which will result in cell lysis and thus cause inhibitory effects on microorganisms [10].

When harvesting hydrogen from organic wastes, acetate, propionate and butyrate are usually the main aqueous

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products [11–13]. Endproduct inhibition on hydrogen production by the extreme thermophile, *Caldicellulosiruptor saccharolyticus*, has been reported by van Niel et al. [10]. However, little information is available in literature regarding the inhibitory effects of VFAs on hydrogen production with mesophilic mixed hydrogen-producing microorganisms. A previous study of our group has demonstrated the inhibition of butyrate on hydrogen production with mixed anaerobic cultures [14]. Both acetate and butyrate are main aqueous products in hydrogen-producing process and acidogenic hydrogen production is usually characterized as butyrate-type fermentation [15–17]. Acetate may have different inhibitory mechanisms and kinetics from butyrate. Therefore, this study was conducted to investigate the influence of added acetate on hydrogen production as a supplement to our previous work. It is expected that the results obtained from this study could provide useful information for the operation of acidogenic hydrogen production processes.

2. Development of the model describing batch hydrogen production processes

In general, the reaction for hydrogen production with a limiting substrate can be formulated as follows:

$$aS \xrightarrow{X} X + b_i P_i, \quad i = 1 \ldots n, \tag{1}$$

where the bacterium, X, consumes substrate, S, to produce more bacteria and products, P_i. In batch tests with growth-associated products, the rates of bacterial growth, substrate consumption and product formation are generally expressed as follows:

$$r_X = -Y_X \times r_S, \tag{2}$$

$$r_{P_i} = -Y_{P_i} \times r_S, \tag{3}$$

$$r_{P_i} = \frac{Y_{P_i}}{Y_X} \times r_X, \tag{4}$$

where r_S, r_X and r_{P_i} are the rates of substrate consumption, bacterial growth and product formation, respectively, and Y_X is the cell yield and Y_{P_i} is the product yield.

A number of mathematical models might be used to describe the bacterial growth in a batch culture. Among them, the logistic equation was expressed as

$$X = X_0 + \int_0^t r_X \, dt = X_0 + \frac{A}{1 + \exp^{-[(\mu_m \times e/A)(\lambda - t) + 1]}}. \tag{5}$$

The consumed substrate could be calculated using the following equation:

$$\begin{aligned} S &= \int_0^t r_S \, dt = -\int_0^t \frac{r_X}{Y_X} \, dt = -\frac{1}{Y_X} \int_0^t r_X \, dt \\ &= -\frac{1}{Y_X} \times \frac{A}{1 + \exp^{-[(\mu_m \times e/A)(\lambda - t) + 1]}} \\ &= \frac{-A/Y_X}{1 + \exp^{-[((-\mu_m/Y_X) \times e/-A/Y_X)(\lambda - t) + 1]}}. \end{aligned} \tag{6}$$

The term $-A/Y_X$ can be replaced by S_{max}, defined as potential maximal substrate consumed, while $-\mu_m/Y_X$ can be defined as the maximum rate of substrate degraded, R_{max}. Therefore, Eq. (6) was rewritten as

$$S = \frac{S_{max}}{1 + \exp^{-[(R_{max} \times e/S_{max})(\lambda - t) + 1]}}. \tag{7}$$

In a similar way, the following equation was obtained:

$$\begin{aligned} P_i &= \int_0^t r_{P_i} \, dt = \int_0^t \frac{Y_{P_i}}{Y_X} \times r_X \, dt = \frac{Y_{P_i}}{Y_X} \int_0^t r_X \, dt \\ &= \frac{Y_{P_i}}{Y_X} \times \frac{A}{1 + \exp^{-[(\mu_m \times e/A)(\lambda - t) + 1]}} \\ &= \frac{A \times Y_{P_i}/Y_X}{1 + \exp^{-[((\mu_m \times Y_{P_i}/Y_X) \times e/A \times Y_{P_i}/Y_X)(\lambda - t) + 1]}}. \end{aligned} \tag{8}$$

The term $A * Y_{P_i}/Y_X$ can be replaced by $P_{max,i}$, defined as potential maximal product formed, whereas $\mu_m * Y_{P_i}/Y_X$ can be defined as the maximum rate of product formed, $R_{max,i}$. This results in the following equation:

$$P_i = \frac{P_{max,i}}{1 + \exp^{-[(R_{max,i} \times e/P_{max,i})(\lambda - t) + 1]}}. \tag{9}$$

In this work, Eqs. (7) and (9) were used to describe the acidogenic hydrogen production processes.

3. Experimental

3.1. Seed sludge

The anaerobic sludge used in this study was obtained from a full-scale upflow anaerobic sludge blanket reactor treating citrate-producing wastewater. The pH and volatile suspended solids (VSS) of the seed sludge were 7.1 and 6.3 g/l, respectively. Prior to use, the sludge was first washed twice with tap water, and then sieved to remove stone, sand and other coarse matters. Subsequently, the sludge was heated at 105 °C for 1.5 h to inactivate methanogens and to enhance the hydrogen-producing bacteria.

3.2. Batch experiments

The experimental setup is shown in Fig. 1. Experiments were conducted in a batch mode with a 5-l fermentor (Baoxin Biotech Ltd., Shanghai) in duplicate. Agitation of the fermentation broth was provided by a six-bladed impeller. In each run, the reactor was first seeded with the heat-pretreated sludge of 12.7 g VSS/l and dosed with acetic acid of 0, 5, 15, 25, 35 and 50 g/l (added as acetate sodium), respectively. A sucrose-rich synthetic wastewater was used as the substrate at a fixed concentration of 25.0 g COD/l (COD: chemical oxygen demand), supplemented with buffering chemicals and a sufficient amount of inorganic nutrients as follows (in mg/l): NH_4HCO_3 405; $K_2HPO_4 \cdot 3H_2O$ 155; $CaCl_2$ 50; $MgCl_2 \cdot 6H_2O$ 100; $FeCl_2$ 25; NaCl 10; $CoCl_2 \cdot 6H_2O$ 5; $MnCl_2 \cdot 4H_2O$ 5; $AlCl_3$ 2.5; $(NH_4)_6Mo_7O_{24}$ 15; H_3BO_3 5; $NiCl_2 \cdot 6H_2O$ 5; $CuCl_2 \cdot 5H_2O$ 5 and $ZnCl_2$ 5. The mixture was then filled to 3.0 l with deionized water and was sealed. After that, the fermentor was purged with nitrogen gas for 5 min prior to test to ensure anaerobic condition during the experiments.

The temperature and agitation rate were constantly controlled at 37 °C and 150 rpm, respectively. The pH of the mixed liquor was kept constant at 5.5 by feeding 4 M NaOH or 2 M HCl solutions via respective peristaltic pumps. The biogas

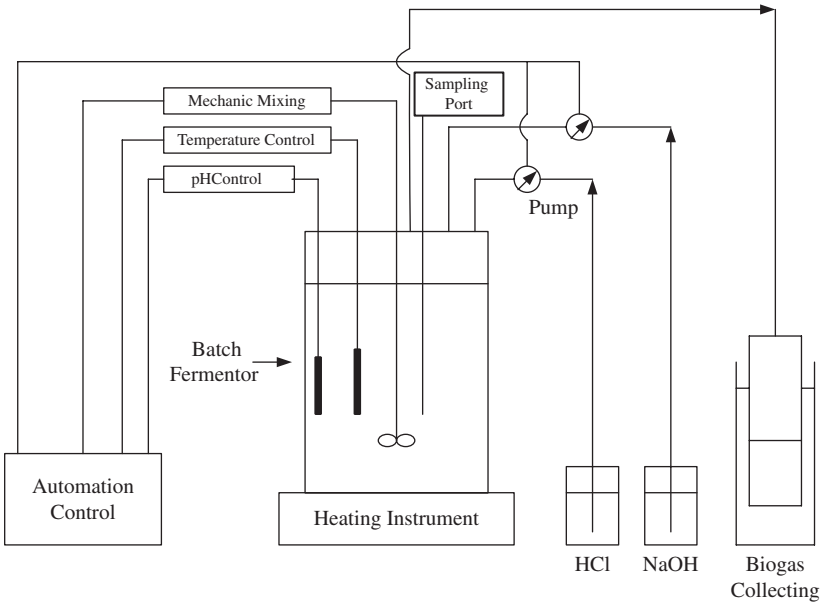

Fig. 1 – The schematic of the experimental setup.

was sampled using a syringe for biogas content analysis, and a liquor sample was taken from the reactor at the same time.

3.3. *Analyses*

The volume of biogas produced was recorded using water replacement equipment. The biogas contents were analyzed with a gas chromatograph (Lunan, Model SP-6800A) equipped with a thermal conductivity detector and a 1.5 m stainless-steel column packed with 5 Å molecular sieve. The temperatures of injector, detector and column were kept at 100, 105 and 60 °C, respectively. Argon was used as the carrier gas at a flow rate of 30 ml/min. The concentrations of ethanol and VFAs, including acetate, propionate, butyrate, i-butyrate, valerate and caproate, in the effluent were determined with another gas chromatograph (Agilent, Model 6890NT) equipped with a flame ionization detector and a 30 m × 0.25 mm × 0.25 μm fused-silica capillary column (DB-FFAP). The liquor samples were first centrifuged at 12 000 rpm for 5 min, and were then acidified by formic acid and filtrated through 0.2-μm membrane and finally measured for free acids. The temperatures of the injector and detector were 250 and 300 °C, respectively. The initial temperature of the oven was 70 °C for 3 min followed with a ramp of 20 °C/min for 5.5 min and to final temperature of 180 °C for 3 min. Nitrogen was used as carrier gas with a flow rate of 2.6 ml/min. Sucrose concentration was determined using anthrone-sulfuric acid method [18], while COD and VSS were measured according to the Standard Methods [19].

4. Results and discussion

4.1. *Sucrose degradation*

Although sucrose degradation was significantly influenced by the concentration of added acetate, the sucrose degradation process could be well simulated by Eq. (7) at all acetate dosages. For example, Fig. 2 illustrates the sucrose degradation pattern as a function of incubation time at acetate dosages of 0 and 35 g/l. The solid lines were drawn using a nonlinear regression with "Microsoft Origin 7.0". All the parameter values fitting using Eq. (7) at various added acetate concentrations are summarized in Table 1. With the increase in added acetate concentration, the sucrose was degraded increasingly slowly and the lag time (λ_1) became longer, indicating the significant negative effects of the added acetate on the substrate degradation. However, the final sucrose degradation efficiencies exceeded 96% regardless of the acetate dosages, provided that the incubation time was sufficiently long.

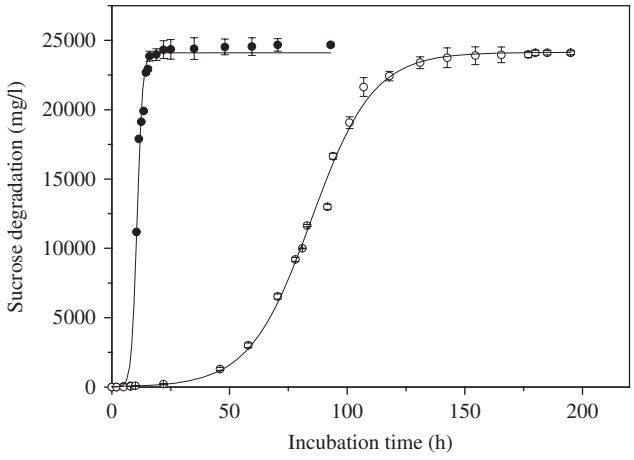

Fig. 2 – Sucrose degradation as a function of incubation time at acetate dosages of 0 (•) and 35 (○) g/l.

The profiles of specific sucrose degradation rate with incubation time at the acetate dosages of 0 and 35 g/l are shown in Fig. 3. The lag time at 35 g/l of added acetate was

Table 1 – Simulation results for sucrose degradation by Eq. (7) at various added acetate concentrations

Added acetate (g/l)	S_{max} (mg/l)	R_{max} (mg/l/h)	λ_1 (h)	R^2
0	24 102	7437	11.9	0.983
5	24 151	5051	20.7	0.995
15	24 119	2180	58.3	0.989
25	24 244	1200	76.0	0.974
35	24 138	661	97.9	0.994
50	24 121	395	198.2	0.998

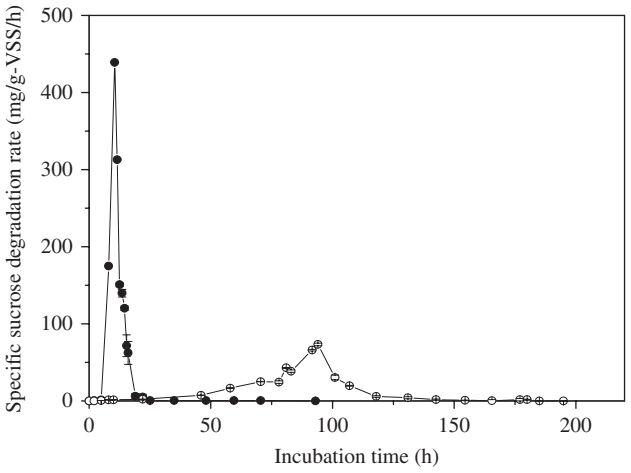

Fig. 3 – Specific sucrose degradation rate as a function of incubation time at acetate dosages of 0 (•) and 35 (○) g/l.

much longer than that of the control. In addition, the peak value was much lower and appeared about 85 h later than that of the control, suggesting the inhibitory effect of the added acetate on the reaction activity of the sludge.

4.2. *Hydrogen production*

Both hydrogen and carbon dioxide were produced as the gaseous products, while methane was undetectable due to the heat treatment of the seed sludge. During the fermentation process, the cumulative H_2 production at various acetate concentrations could be modeled by Eq. (9) with regression coefficients of 0.99. Fig. 4 illustrates the hydrogen production versus incubation time at the acetate dosages of 0 and 35 g/l as an example. The parameter values derived from data fitting using Eq. (9) are listed in Table 2. The potential maximal hydrogen produced (P_{max}) and maximum hydrogen production rate (R_{max}) both declined with increasing acetate dosage. This reveals that the addition of acetate imposed significant inhibitory effects on hydrogen production. This was also confirmed by the profiles of specific hydrogen production rate (SHPR) at acetate dosages of 0 and 35 g/l (Fig. 5).

As given in Tables 1 and 2, the lag time (λ_2) had a similar increasing trend to that for sucrose degradation process (λ_1). The values of λ_2 in the acetate dosage of 0–35 g/l approxi-

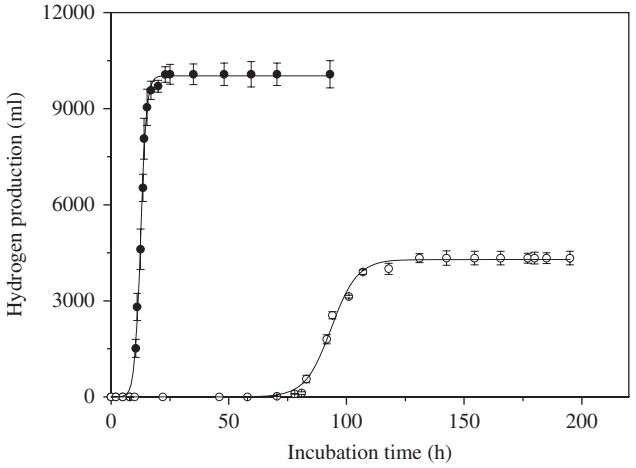

Fig. 4 – Cumulative hydrogen production as a function of incubation time at acetate dosages of 0 (•) and 35 (○) g/l.

Table 2 – Simulation results of hydrogen production by Eq. (9) at various added acetate concentrations

Added acetate (g/l)	P_{max} (ml)	R_{max} (ml/h)	λ_2 (h)	R^2
0	10 028	2827	13.8	0.995
5	9800	2004	22.2	0.994
15	7934	1009	61.5	0.990
25	6153	498	81.1	0.988
35	4289	280	99.0	0.993
50	4136	197	310.3	0.991

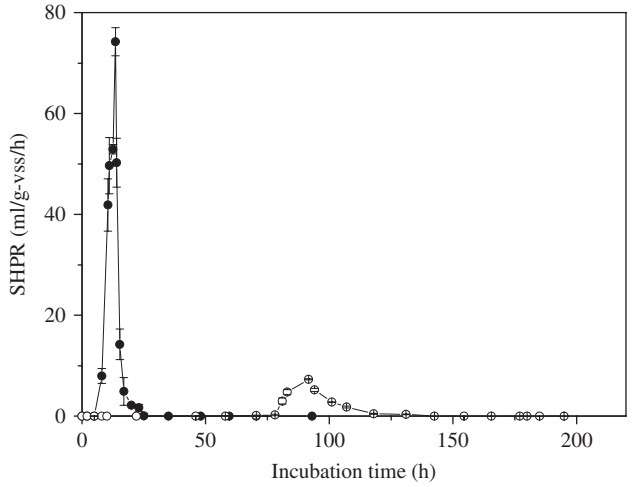

Fig. 5 – SHPR as a function of incubation time at acetate dosages of 0 (•) and 35 (○) g/l.

mated to those of λ_1 within the same acetate dosage range. However, when the added acetate concentration finally reached 50 g/l, λ_2 was longer than λ_1 by more than 100 h. In this case, when the substrate began to be degraded, the

hydrogen production might not start. Thus, at 50 g/l of added acetate, the sucrose might be first digested into other products, such as various long chain fatty acids, rather than hydrogen [20]. Hydrogen production did not begin until a long period after sucrose was initially degraded. This is in good agreement with the production of VFAs at 50 g/l of acetate dosage which is described later.

Table 3 summarizes the effects of added acetate on SHPR, hydrogen yield (HY) and the maximum hydrogen partial pressure (MHPP). SHPR could be calculated by dividing the maximum hydrogen production rate, which was obtained from the differential coefficient of the results of Eq. (9), by the sludge concentration, while HY could be estimated by dividing the total volume of produced hydrogen in each test by the amount of consumed sucrose.

SHPR decreased as the added acetate concentration increased, from 74.2 to only 5.2 ml/g VSS/h with an increase in acetate dosage from 0 to 50 g/l. The change of HY also had a similar decreasing trend, from 1.04 mol H_2/mol glucose at 5 g/l of added acetate to 0.44 mol H_2/mol glucose at 50 g/l of added acetate. As given in Table 3, MHPP initially declined with increasing added acetate, from 0.50 atm at 5 g/l of added acetate to 0.40 atm at 25 g/l of added acetate; however, when the acetate concentration was increased to 35 g/l and further to 50 g/l, MHPP did not change significantly and kept at around 0.40 atm.

The relative toxicity activity is usually used to quantify the inhibition caused by an inhibitor and the inhibition can be expressed by a simple index $C_{I,50}$, which represents the added inhibitor concentration at which the activity is reduced by 50% [21]. Fig. 6 plots the bioactivities of SHPR and HY relative to the control against the added acetate concentration. The $C_{I,50}$ values of added acetate for SHPR and HY were 11.05 and 31.90 g/l, respectively. The difference between the $C_{I,50}$ values for SHPR and HY might be attributed to the fact that these two parameters had different meanings. SHPR presented the hydrogenic activity of unit weight of the sludge in the reactor, while HY denoted the ability of the sludge converting sucrose into hydrogen. Therefore, the added acetate at an identical concentration might cause more drastic inhibitory effects on the hydrogenic activity of unit weight of the sludge than on its ability to convert sucrose into hydrogen.

Table 3 – Effects of added acetate concentration on hydrogen production

Added acetate (g/l)	SHPR (ml/g VSS/h)	HY (mol H_2/mol glucose)	MHPP (atm)
0	74.2 ± 1.2	1.06 ± 0.05	0.54 ± 0.05
5	52.6 ± 0.8	1.04 ± 0.03	0.50 ± 0.01
15	26.5 ± 0.6	0.84 ± 0.02	0.47 ± 0.02
25	13.1 ± 0.3	0.65 ± 0.01	0.40 ± 0.04
35	7.3 ± 0.5	0.46 ± 0.04	0.40 ± 0.03
50	5.2 ± 0.6	0.44 ± 0.01	0.39 ± 0.01

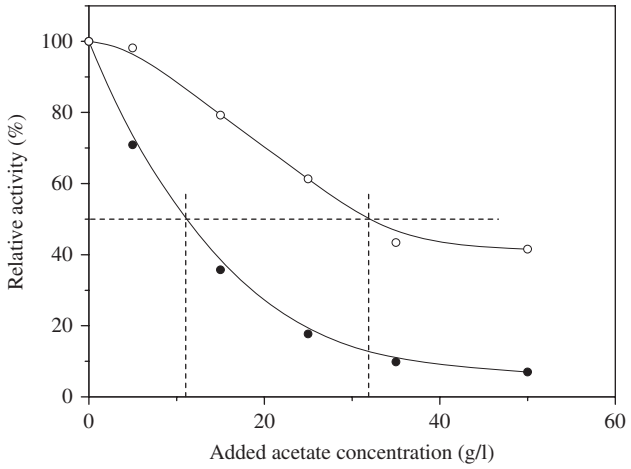

Fig. 6 – Effects of added acetate on relative bioactivity of SHPR (•) and HY (○).

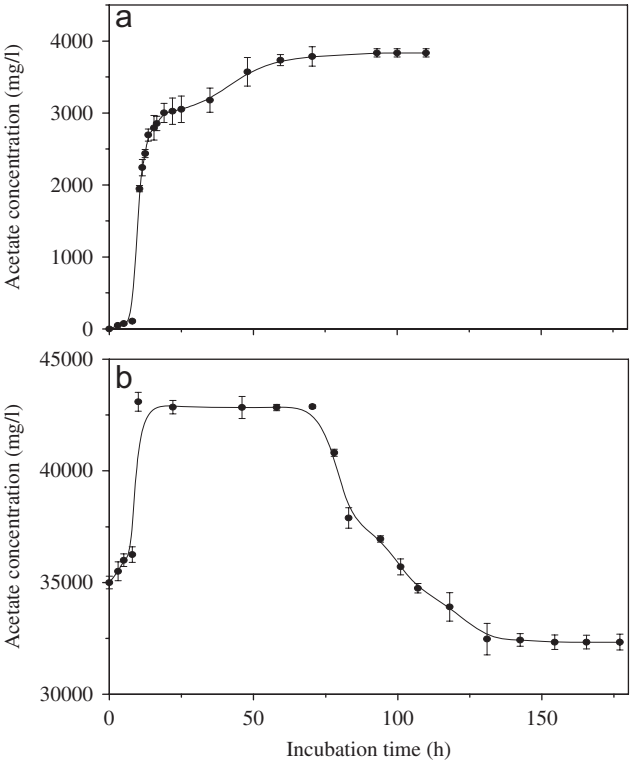

Fig. 7 – Total acetate concentration as a function of incubation time at acetate dosages of (a) 0 g/l and (b) 35 g/l.

4.3. *Formation of aqueous products*

VFAs and alcohols are the main aqueous products in the hydrogen-producing reactor, and acetate and butyrate were predominant [15–17,22]. Fig. 7 illustrates the changes of total acetate concentration at acetate dosages of 0 and 35 g/l. In the control, the cumulative acetate production had a similar increasing trend to that of the hydrogen production for the control (Fig. 7a). Nevertheless, at 35 g/l of acetate dosage, after a short increasing period the total acetate concentration declined sharply until hour 130 (Fig. 7b). An interesting

phenomenon was that the final total acetate concentration for the dosage of 35 g/l acetate was even lower than the acetate dosage at the very beginning. This was in good accordance with the increase in other VFAs. On the other hand, the changes of butyrate concentration in the control, as shown in Fig. 8, also had a similar increasing trend to that of the hydrogen production. However, at 35 g/l of acetate dosage, the butyrate concentration had a much lower increasing rate and needed much longer time to reach equilibrium. This reveals the significant negative effects of the added acetate on the sludge bioactivity.

The final distribution of the aqueous products at various acetate dosages was significantly influenced by the addition of acetate (Table 4). However, in accordance with many other studies [15,17], the butyrate-type fermentation dominated in this hydrogen-producing reactor. With the addition of acetate up to 25 g/l, the butyrate concentration increased from 7294 mg/l in the control to 15 058 mg/l, whereas when acetate dosage was increased, it declined to 14 281 mg/l at 35 g/l of acetate dosage and further to 11 336 mg/l at 50 g/l of acetate dosage.

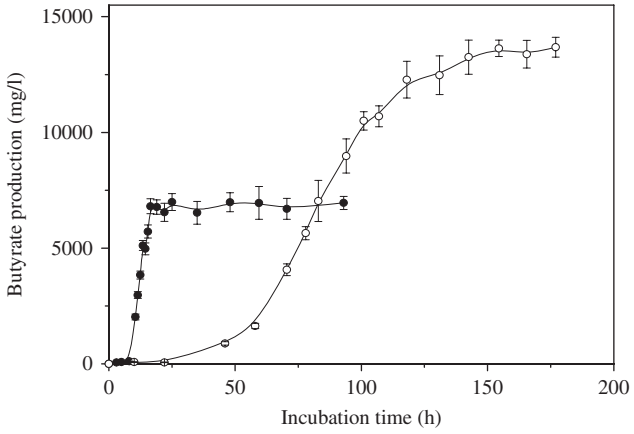

Fig. 8 – Total butyrate concentration as a function of incubation time at acetate dosages of 0 (•) and 35 (○) g/l.

The ethanol concentration initially increased significantly, compared with that of the control, and remained almost unchanged as the acetate dosage was increased to 25 g/l. However, it substantially increased again with a further increase in acetate dosage. The changes of the propionate concentration had a similar trend to those for ethanol. Valerate, caproate and i-butyrate were also present in the reactor effluent, but in very low levels and did not change significantly as the added acetate was less than 35 g/l. However, as the acetate dosage was increased to 50 g/l, the concentrations of these three VFAs were all increased to relatively higher levels.

As mentioned above, at acetate dosages of 35 and 50 g/l, the total acetate concentration at the conclusion of the tests was even lower than the dosage at the very beginning. This indicates that the added acetate was partially transformed to other types of VFAs and/or alcohols, resulting in the high levels of ethanol, propionate, i-butyrate, valerate and caproate at higher acetate dosages [20,22]. These results suggest that the fermentation patterns in the reactor were substantially altered because of the addition of acetate, especially at higher dosages.

4.4. *Inhibition modeling*

A noncompetitive inhibition equation was used to evaluate the degree of inhibition [3,23–26]:

$$r = \frac{r_{max}}{1 + (C/K_C)^n}, \quad (10)$$

where r is the specific rate of sucrose degradation or hydrogen production (mg/g VSS/h or ml/g VSS/h), calculated from Eqs. (7) aor (9) at an acetate dosage of C (g/l), r_{max} is the maximum value of r, K_C is the inhibition constant (g/l) and n is the exponent of inhibition.

The calculation results are shown in Figs. 9 and 10. The solid lines were derived with a nonlinear regression and the parameter values were estimated. For sucrose degradation, $r_{max} = 584.9$ mg/g VSS/h, $K_C = 8.27$ g/l and $n = 1.53$; for hydrogen production, $r_{max} = 221.7$ ml/g VSS/h, $K_C = 9.44$ g/l and

Table 4 – Effects of acetate dosage on the distribution of final aqueous products

Added acetate (g/l)	Concentration of final aqueous products (mg/l)							Acetate produced[a] (mg/l)
	HAc	HPr	i-HBut	HBu	HVa	HCa	Eol	
0	4091±153	548±32	165±28	7294±352	38±8	37±5	276±28	4091±153
5	8 245±128	658±46	145±21	9283±463	33±6	46±9	787±35	3245±128
15	16 586±136	780±28	160±19	13 042±510	53±10	53±11	651±42	1586±136
25	25 324±320	779±53	132±27	15 058±508	45±9	51±8	810±31	324±320
35	32 634±215	944±36	153±23	14 281±475	101±23	99±15	2154±128	−2366±215[b]
50	46 378±163	1041±57	660±44	11 336±421	155±18	363±32	1722±154	−3622±163[b]

Note: HAc, acetate; HPr, propionate; i-HBu, i-butyrate; HBu, butyrate; HVa, valerate; HCa, caproate; Eol, ethanol.

[a] The concentration of acetate produced was the difference between the total acetate detected in the solution and the added acetate at the very beginning.

[b] "–" means that the concentrations of acetate at the conclusion were lower than the dosages at the very beginning.

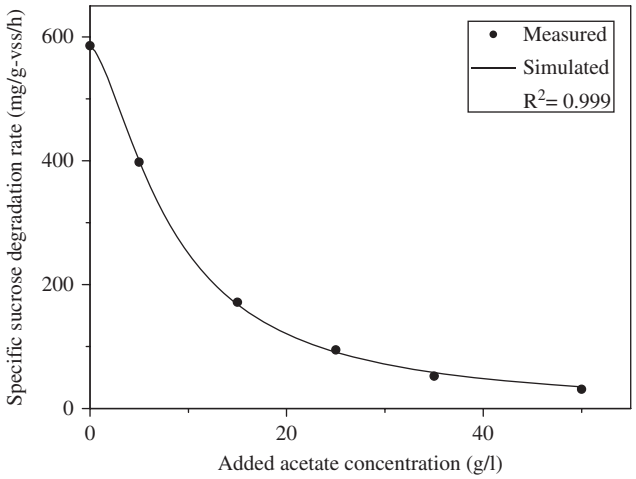

Fig. 9 – Relationship between the specific sucrose degradation rate and added acetate concentration.

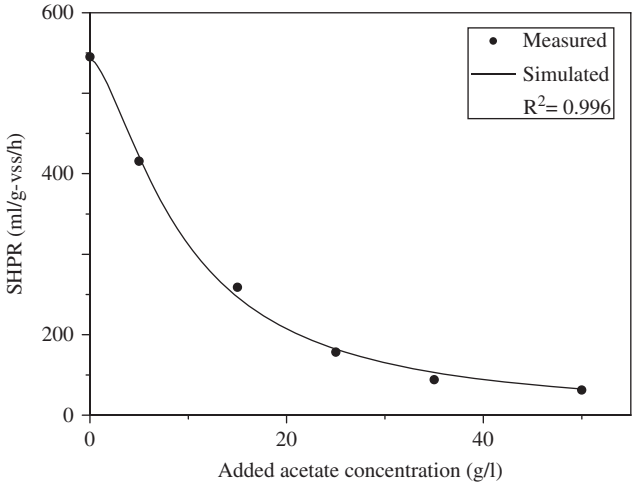

Fig. 10 – Relationship between SHPR and added acetate concentration.

$n = 1.52$. The values of K_C and n of sucrose degradation are approximate to those of hydrogen production, indicating that Eq. (10) was able to describe well the inhibitory effects of added acetate on both degradation of substrate and formation of products.

5. Conclusions

The added acetate had a significant negative influence on the hydrogen production in the acidogenesis of sucrose. The distribution of VFAs and alcohols produced was also influenced by the acetate addition. The fermentation patterns in the reactor were substantially changed because of the acetate addition, especially at higher dosages. Modified logistic equations were able to simulate the acidogenesis process well. A noncompetitive product inhibition model was successfully used to describe the inhibitory effects of acetate addition on both substrate degradation and hydrogen production. From kinetic analysis, the maximum specific rate $r_{max} = 584.9\,mg/g\,VSS/h$, inhibition constant $K_C = 8.27\,g/l$ and the exponent of inhibition $n = 1.53$ were estimated for sucrose degradation, whereas $r_{max} = 221.7\,ml/g\,VSS/h$, $K_C = 9.44\,g/l$ and $n = 1.52$ were calculated for hydrogen production. In addition, the $C_{I,50}$ values of added acetate on the SHPR and HY were 11.05 and 31.90 g/l, respectively.

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