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Comparative performance of two upflow anaerobic biohydrogen-producing reactors seeded with different sludges

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Abstract

Comparisons were made between the performance of two upflow anaerobic biohydrogen-producing reactors. One reactor (R1) was seeded with methanogenic sludge, but was operated at low pH levels, whereas another (R2) was seeded with heat-pretreated sludge. Experiment results showed that H_2 partial pressure in biogas, H_2 production rate and H_2 yield were pH-dependent in the two reactors. The optimum pH for H_2 partial pressure was observed at pH 3.10 for R1 and 4.00 for R2, respectively. The optimum pH for maximum H_2 production rate and H_2 yield was 4.00 for both R1 and R2. Acetate, propionate, butyrate, *i*-butyrate, valerate, caproate and ethanol were main aqueous products and their distribution was also significantly influenced by pH. For R1, caproate was dominant at pH < 4.00 and the butyrate-type fermentation predominated at pH 4.5–6.0. For R2, butyrate was dominant at pH > 3.60, while the ethanol-type fermentation was important at pH < 3.60. With a thermodynamic analysis, possible metabolic pathways for the formation of caproate and valerate in both reactors were suggested.

Keywords: Acidogenesis; H_2 production; pH; Upflow anaerobic sludge blanket (UASB)

1. Introduction

Recently, H_2 has emerged as one of the most promising carriers of new energy because it is clean, recyclable and efficient. In addition, H_2 can be used as an important raw material in various chemical industries [1]. However, H_2 is still primarily produced from fossil fuels, such as natural gas, petroleum and coal, through steam reforming or from water through electrolysis and thermochemical decomposition. These processes are costly and not environment friendly. Therefore, biological H_2 production process, which is favorable for low costs, has been explored by many researchers in the past few years.

Biological H_2 production can be classified as photo- and dark-fermentation processes [2]. Compared with H_2 production by photosynthetic bacteria or algae, dark fermentative H_2 production process with anaerobic acidogenic culture has advantages due to its higher production rate [1]. Furthermore, dark fermentative bacteria utilize organic matters as a substrate, and thus have an advantage of converting organic wastes into more valuable energy sources. Therefore, it has recently attracted considerable attention as an effective way of harvesting H_2 from organic wastes [1,3].

In order to achieve a high H_2 yield, preventing the growth of H_2-consuming methanogens is one key consideration. Many pervious studies have focused on manipulating culture conditions to block methanogenesis, e.g., operating at low pHs and low temperatures [4,5]. Another method for enriching the H_2-producing cultures is a simple heat–shock treatment to remove non-spore-forming bacteria, such as methanogens, from anaerobic inoculum [6–8]. These two methods have been extensively investigated and their relative effectiveness has been compared in batch cultures [9]. However, there is little information about the comparative performance between them when the two methods are applied to continuous H_2-producing bioreactors.

On the other hand, the upflow anaerobic sludge blanket (UASB) reactor is an extensively used anaerobic treatment system because of its high treatment efficiency and excellent process stability. In the last two decades, UASB process

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has prevailed successfully for anaerobic treatment of various types of wastewater to produce methane [10]. Recently, it has been proven that UASB system is also a promising system for hydrogen production [11–14].

Besides, pH plays an important role in the H_2-producing process, as it has a significant influence on the hydrogenase activity and on the metabolism pathways, e.g., utilization of carbon and energy sources, efficiency of substrate degradation, synthesis of proteins and various types of storage material and release of metabolic products from cells [15]. Moreover, pH variation can affect cell morphology and structure and, therefore, flocculation and adhesion phenomena [16]. If pH is not maintained in the desired range, it could inhibit H_2 production or cause a microbial population shift, resulting in cessation of H_2 production [6]. A considerable number of studies have been carried out to evaluate the effects of pH on H_2 production in various types of reactors, such as continuously stirred tank reactor (CSTR) and trickling biofilter [17,18]. However, to our best knowledge, little information is available in literature concerning the role of pH in fermentative H_2 production in a UASB reactor.

Therefore, the main goal of this study was to evaluate the significance of pH in H_2 production from UASB reactors, and to compare the performance of reactors with different inoculums. One reactor was seeded with a non-heat-treated inoculum and was shifted to acidogensis for H_2 production by lowering pH, while another was seeded with heat-pretreated sludge. In addition, a thermodynamic analysis was performed to elucidate the possible formation pathways of caproate and valerate. It is expected that the results obtained from this study could provide some valuable information for the operation of H_2-producing bioreactors.

2. Materials and methods

2.1. Reactors, seed sludge and substrate

The experiment was conducted in parallel in two UASB reactors. Each plexiglas-made reactor had a working volume of 4.0 l with an internal diameter of 100 mm (Fig. 1). The two reactors were operated at 35 ± 1 °C with heating bands.

Seed sludge was taken from a full-scale anaerobic 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 with tap water twice, and was then sieved to remove stone, sand and other coarse matters. Thereafter, some sludge without heat treatment was seeded into a UASB reactor (R1) with a sludge concentration of 20.0 g-VSS/l. Other sludge was heated at 105 °C for 1.5 h to inactivate methanogens and to enhance the H_2-producing bacteria, and was then seeded into another UASB reactor (R2) with a sludge concentration of 20.0 g-VSS/l.

A sucrose-rich synthetic wastewater was used as the substrate, 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; $ZnCl_2$ 5.

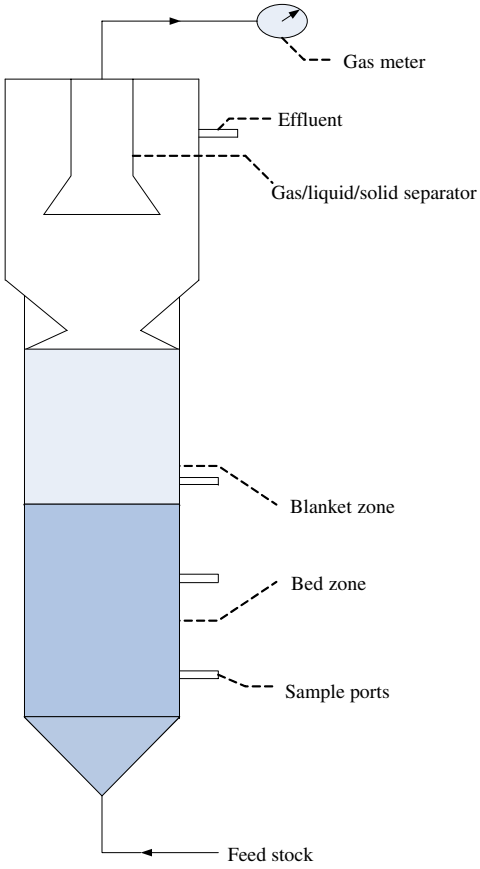

Fig. 1. Setup of the UASB reactors.

2.2. Experimental

The start-up of the two UASB reactors was conducted for 5 months to enrich H_2-producing microbes and to establish a stable operation. In the start-up, the UASB reactors were initially fed with the synthetic wastewater of 5.0 g/l at a hydraulic retention time (HRT) of 46.0 h for 1 month. In the following 2 months, the substrate concentration was gradually increased to 10.0 g/l and the HRT was shortened to 25.0 h. Thereafter, the HRT was further shortened to 17.0 h for the subsequent operation, corresponding to a loading rate of 14.1 g/l/d. In the start-up, owing to the production of various volatile Fatty acids (VFA), $NaHCO_3$ was added into the influent to adjust the pH of the mixed liquor. For R1, the pH of the mixed liquor was decreased from 7.00 to approximate 4.00 by reducing the influent alkalinity. As a result, the CH_4 partial pressure decreased from 0.60 atm to nil, but the H_2 partial pressure increased from nil to 0.45 atm. A hydrogen production rate of 105 ml-H_2/l/h and a corresponding hydrogen yield of 1.25 mol-H_2/mol-glucose were obtained. This attributed to the complete inhibition of the activity of methanogens and the enrichment of the H_2-producing cultures. For R2, H_2 was produced without detectable methane from the very beginning of start-up

due to the heat pretreatment, which could remove non-spore-forming methanogens. After 5-month operation with a decrease in pH from 7.00 to 4.00 by reducing the influent alkalinity, H_2 could be steadily produced with a partial pressure of 0.45 atm, a production rate of 100 ml-H_2/l/h and a corresponding yield of 1.13 mol-H_2/mol-glucose.

After the start-up of two UASB reactors, experiments were conducted to evaluate the influence of pH on H_2 production in R1 and R2 at a HRT of 17.0 h and a sucrose concentration of 10 g/l. The pH of the mixed liquor in both reactors was increased from 3.10 to 6.00 by adjusting the influent alkalinity through dosing $NaHCO_3$. At each run, the two reactors were operated for more than 10 times of HRT to establish a pseudo-steady state. Aqueous effluent, biogas amount and composition were continuously monitored and the reactors were assumed to reach the pseudo-steady state when all the items were within 5% deviation. In this paper, only the results obtained under the pseudo-steady state were reported.

2.3. Analyses

The volume of biogas produced was recorded daily using a gas meter with water displacement method. The contents of H_2 and CH_4 in biogas was 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 VFA, 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 by using anthrone–sulfuric acid method [19], while VSS was measured according to the Standard Methods [20].

3. Results

3.1. Substrate degradation

At pH < 3.10, the sucrose degradation efficiency in R2 was much lower than that in R1 (Fig. 2), indicating that the microbial system in R2 was more sensitive to low pH and had a lower activity than that of R1. Sucrose was readily degraded in both reactors, and its degradation efficiency increased with an increasing pH. With a pH increase from 3.10 to 6.00, the sucrose degradation efficiency increased from 93.1% to 99.4% for R1 and from 85.0% to 99.4% for R2.

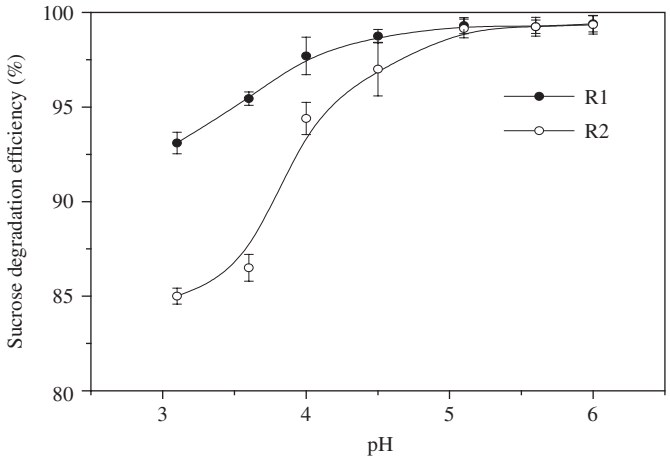

Fig. 2. Variation of substrate degradation at various pHs for R1 and R2.

3.2. Gaseous products

In the two H_2-producing reactors, both H_2 and CO_2 were produced as the main gaseous products. Methane was only detected at a high pH value in R1 and R2. For R1, the H_2 partial pressure decreased from 0.52 to 0.11 atm with a pH increase from 3.10 to 6.00 (Fig. 3a). Methane was detected with a partial pressure of 0.02 atm in R1 at pH 6.00. For R2, the H_2 partial pressure increased from 0.42 atm at pH 3.10 to a peak value of 0.48 atm at pH 4.00, but then declined to approximately 0.003 atm at pH 6.00 (Fig. 4a). Methane was also detected in R2 at pH > 5.10, and increased from 0.01 atm at pH 5.10 to 0.13 atm at pH 6.00.

As shown in Fig. 3b, the H_2 production rate increased from 64 to 127 ml/l/h with a pH increase from 3.10 to 4.00 for R1, and then it decreased to 55 ml/l/h as pH was further increased to 6.00. Similarly, for R2, the H_2 production rate increased from 43 to 105 ml/l/h as pH increased from 3.10 to 4.00, but declined to about 0.4 ml/l/h as pH was increased to 6.00 (Fig. 4b).

Figs. 3c and 4c illustrate the H_2 yield as a function of pH in the two reactors. The H_2 yield of R1 increased from 0.74 mol-H_2/mol-glucose at pH 3.10 to 1.51 mol-H_2/mol-glucose at pH 4.00, and then decreased to 0.62 mol-H_2/mol-glucose at pH 6.00 (Fig. 3c). The H_2 yield in R2 has a similar trend as that in R1. It increased from 0.53 mol-H_2/mol-glucose at pH 3.10 to 1.19 mol-H_2/mol-glucose at pH 4.00, and thereafter decreased to about 0.005 mol-H_2/mol-glucose at pH 6.00 (Fig. 4c).

3.3. Aqueous products

VFA and alcohols are the main aqueous products for H_2 production in the two reactors. Tables 1 and 2, respectively, summarize the total VFA and ethanol concentration in the UASB effluent as well as the percentages of individual VFA and ethanol at various pHs for R1 and R2.

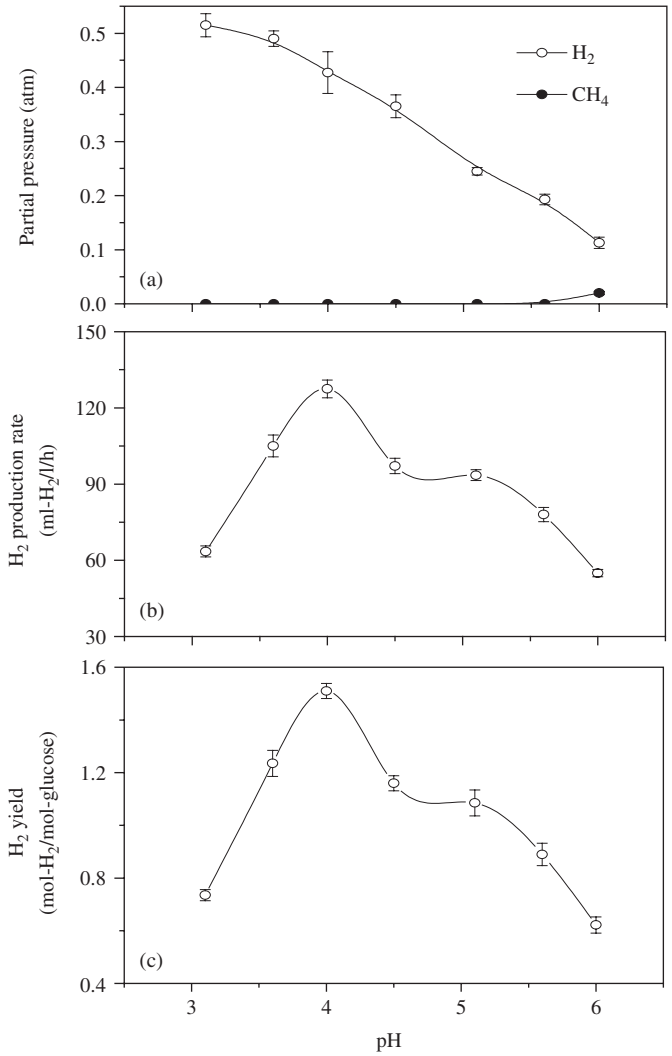

Fig. 3. Variation of: (a) H_2 partial pressure in biogas, (b) H_2 production rate and (c) H_2 yield at various pHs in R1.

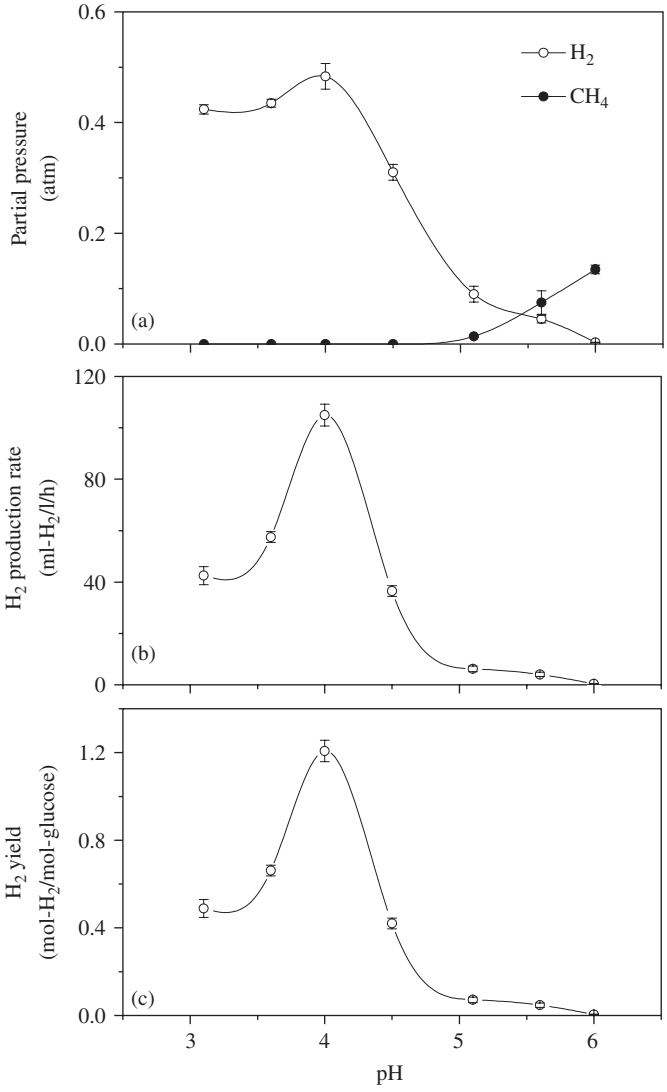

Fig. 4. Variation of: (a) H_2 partial pressure in biogas, (b) H_2 production rate and (c) H_2 yield at various pHs in R2.

As listed in Table 1, the total VFA and ethanol concentration in R1 did not significantly change as pH was increased from 3.10 to 5.10, in the range of 3021–3336 mg/l, expect for a higher concentration of 4047 mg/l at pH 5.60 and a lower level of 2836 mg/l at pH 6.00. However, the distribution of VFA and ethanol was highly pH-dependent. The acetate percentage slightly changed in the range of 18.9–23.0%, as pH increased from 3.10 to 5.10; but it jumped to over 30% at pH > 5.60. Ethanol accounted for a large proportion when pH was low, with a percentage of 27.3% at pH 3.10 and 13.5% at pH 3.60. However, when pH was further increased, its percentage significantly declined to less than 8%. On the other hand, the butyrate percentage increased from 8.0% to a peak of 44.9% at pH 4.50, and then declined to 27.3% at pH 6.00. Caproate was astonishingly high at pH < 4.00, but decreased with an increase in pH. Propionate and valerate were also important products at pH < 4.50, accounting for 10.0–14.0% and 9.8–11.8% of total VFA and ethanol, respectively. *Iso*-butyrate was consistently below 1% regardless of the change of pH.

As seen from Table 2, the total VFA and ethanol concentration in R2 effluent substantially increased from 2091 to 4041 mg/l as pH was increased from 3.10 to 4.00, but slightly changed with a further pH increase to 5.60, in the range of 3915–4230 mg/l. However, a much higher concentration of 5680 mg/l was observed at pH 6.00. The distribution of VFA and ethanol in R2 effluent was also pH-dependent. Butyrate was the main product in the range of 31.8–52.3%. However, at pH 3.10, butyrate accounted for only 12.0%. The percentage of acetate did not change significantly as pH increased from 3.10 to 4.50, in the range of 22.1–25.0%, but it sharply increased to 41.7% at pH 6.00. Ethanol was in a high level at a low pH, accounting for 45.0% at pH 3.10 and 30.0% at pH 3.60, but it significantly declined to less than 7% at pH > 3.60. Propionate and caproate were also important products in R2, in the range of 7.0–9.5% and 3.3–8.0%, respectively. The distribution of valerate and *i*-butyrate in R2 effluent was similar to that of R1.

Table 1
Distribution of VFA and ethanol in the effluent of R1 at various pHs

pH	VFA + Eol (mg/l)	HAc (%)	HPr (%)	*i*-HBu (%)	HBu (%)	HVa (%)	HCa (%)	Eol (%)
3.10 ± 0.10	3215 ± 165	23.0 ± 1.3	1.0 ± 0.1	0.2 ± 0.0	8.0 ± 0.3	0.5 ± 0.1	40.0 ± 2.6	27.3 ± 3.3
3.60 ± 0.10	3336 ± 173	19.1 ± 1.0	1.4 ± 0.2	0.1 ± 0.0	10.4 ± 0.5	0.7 ± 0.1	54.8 ± 3.2	13.5 ± 2.1
4.00 ± 0.10	3021 ± 144	18.9 ± 1.1	2.4 ± 0.3	0.4 ± 0.1	12.4 ± 2.1	2.7 ± 0.3	55.8 ± 2.9	7.4 ± 0.3
4.50 ± 0.10	3147 ± 184	20.2 ± 1.2	10.0 ± 0.8	0.5 ± 0.1	44.9 ± 3.1	9.8 ± 1.0	9.6 ± 1.1	5.0 ± 0.8
5.10 ± 0.10	3239 ± 201	22.7 ± 1.4	10.2 ± 0.7	0.1 ± 0.0	40.0 ± 2.7	10.7 ± 1.3	10.0 ± 0.3	6.3 ± 0.2
5.60 ± 0.10	4047 ± 195	32.2 ± 1.7	14.0 ± 1.1	0.6 ± 0.1	27.4 ± 1.2	11.2 ± 0.8	11.3 ± 0.5	3.3 ± 0.2
6.00 ± 0.10	2836 ± 216	33.5 ± 2.1	12.3 ± 0.5	0.6 ± 0.1	27.3 ± 0.6	11.8 ± 1.2	12.9 ± 1.1	1.6 ± 0.1

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

Table 2
Distribution of VFA and ethanol in the effluent of R2 at various pHs

pH	VFA + Eol (mg/l)	HAc (%)	HPr (%)	*i*-HBu (%)	HBu (%)	HVa (%)	HCa (%)	Eol (%)
3.10 ± 0.10	2091 ± 156	25.0 ± 1.2	7.0 ± 0.3	0.5 ± 0.1	12.0 ± 0.3	2.5 ± 0.2	8.0 ± 1.5	45.0 ± 2.8
3.60 ± 0.10	3014 ± 163	23.0 ± 1.1	7.0 ± 0.5	0.1 ± 0.0	32.0 ± 0.7	1.0 ± 0.1	6.9 ± 0.2	30.0 ± 2.1
4.00 ± 0.10	4041 ± 146	22.1 ± 1.2	7.3 ± 0.2	1.0 ± 0.1	52.3 ± 2.0	4.8 ± 0.2	5.7 ± 0.3	6.8 ± 0.2
4.50 ± 0.10	3915 ± 135	25.0 ± 1.1	7.2 ± 0.3	0.1 ± 0.0	51.1 ± 3.2	7.1 ± 0.3	3.3 ± 0.2	6.2 ± 0.2
5.10 ± 0.10	4018 ± 210	29.4 ± 1.1	8.0 ± 0.3	0.1 ± 0.0	48.5 ± 2.5	7.4 ± 0.4	4.0 ± 0.1	2.6 ± 0.1
5.60 ± 0.10	4230 ± 197	30.7 ± 2.0	9.5 ± 0.6	0.6 ± 0.1	45.4 ± 1.7	9.0 ± 0.8	3.3 ± 0.2	1.5 ± 0.1
6.00 ± 0.10	5680 ± 225	41.7 ± 2.5	8.9 ± 0.4	0.9 ± 0.2	31.8 ± 0.7	10.2 ± 0.4	4.1 ± 0.3	2.4 ± 0.1

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

3.4. Thermodynamic analysis on the formation of caproate and valerate

Caproate and valerate were of a high level, and even became dominant at certain pH values in this study. This result is different from those of most previous studies concerning H_2-production. Five possible caproate-forming reactions that might occur in the two reactors were as follows (ΔG^0 : kJ/mol) [21–23]:

$$2CH_3(CH_2)_2COO^- = CH_3(CH_2)_4COO^- + CH_3COO^-, \quad \Delta G^0 = -0.11, \tag{1}$$

$$CH_3(CH_2)_2COO^- + CH_3COO^- + 2H_2 + H^+ = CH_3(CH_2)_4COO^- + 2H_2O, \quad \Delta G^0 = -48.01, \tag{2}$$

$$CH_3(CH_2)_2COO^- + 2CO_2 + 6H_2 = CH_3(CH_2)_4COO^- + 4H_2O, \quad \Delta G^0 = -143.34, \tag{3}$$

$$3CH_3COO^- + 3H_2 + 2H^+ = CH_3(CH_2)_4COO^- + 4H_2O, \quad \Delta G^0 = -96.70, \tag{4}$$

$$2CH_3CH_2OH + CH_3COO^- = CH_3(CH_2)_4COO^- + 2H_2O, \quad \Delta G^0 = -77.40, \tag{5}$$

where ΔG^0 is the change of Gibbs free energy [21] at pH 7.0 under standard conditions (i.e., all solutes are at the concentration of 1 M, and gases have partial pressure of 1 atm). The actual change of Gibbs free energy (ΔG) of the reactions can be calculated with the following equation [21]:

$$\Delta G = \Delta G^0 + 2.303RT \log \frac{\{\text{products}\}}{\{\text{reactants}\}},$$

where R is the universal gas constant, 8.314 J/mol/K, T is the absolute temperature in K, and { } represents the chemical activity, which approximates molarity at low concentration for aqueous matter, while partial pressure in atm for gaseous ones.

Fig. 5 illustrates the variation of Gibbs free energy for caproate formation at various pHs in the two reactors. For R1, reactions (2)–(5) were all thermodynamically possible at pH < 4.00. However, reaction (2) was unlikely responsible for the formation of caproate under this condition because the ΔG values of reaction (2) were less than 2 kJ/mol at these pH values, the minimum amount of Gibbs free energy needed to sustain growth and/or conversion of a substrate [22]. Furthermore, in reactions (3) and (4), H_2 would be consumed as an electron donor, which might reduce the H_2 yield. Nevertheless, as shown in Fig. 3, H_2 yield was higher when pH was less than 4.00. Therefore, reactions (3) and (4) were also unlikely responsible for the high levels of caproate at pH < 4. This leaves reaction (5) as the only reaction potentially responsible for caproate formation at pH < 4.00 in R1. However, reaction (3) might be associated with the high percentage of caproate at pH > 5.10 in R1, since this reaction was both thermodynamically spontaneous and H_2-consuming. Similarly, as shown in Fig. 5b, reaction (5) should also be the reason for the high values of caproate at pH < 4.0 in R2.

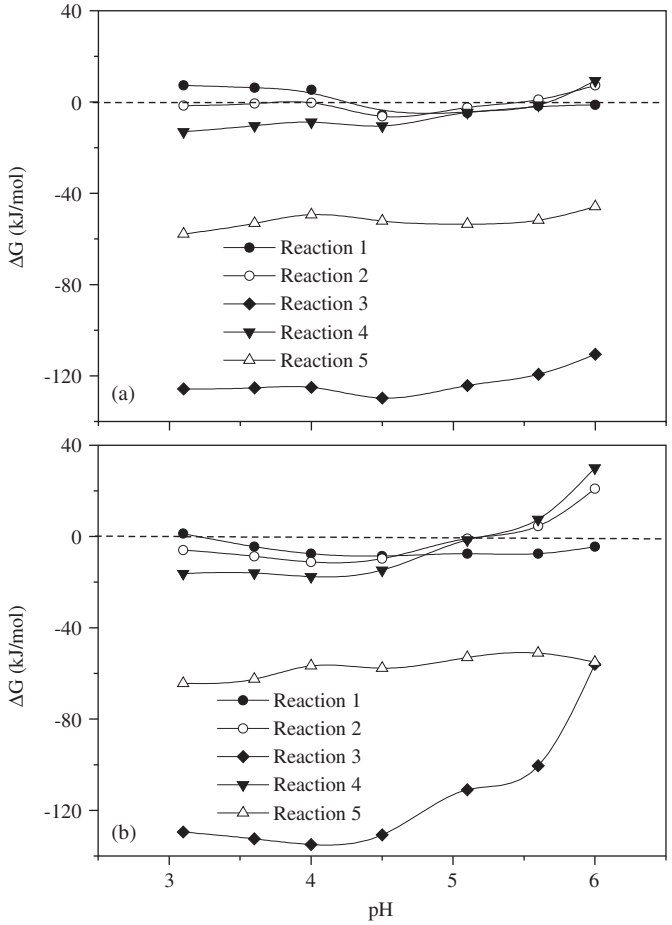

Fig. 5. Variation of Gibbs free energy for caproate formation at various pHs in: (a) R1; and (b) R2.

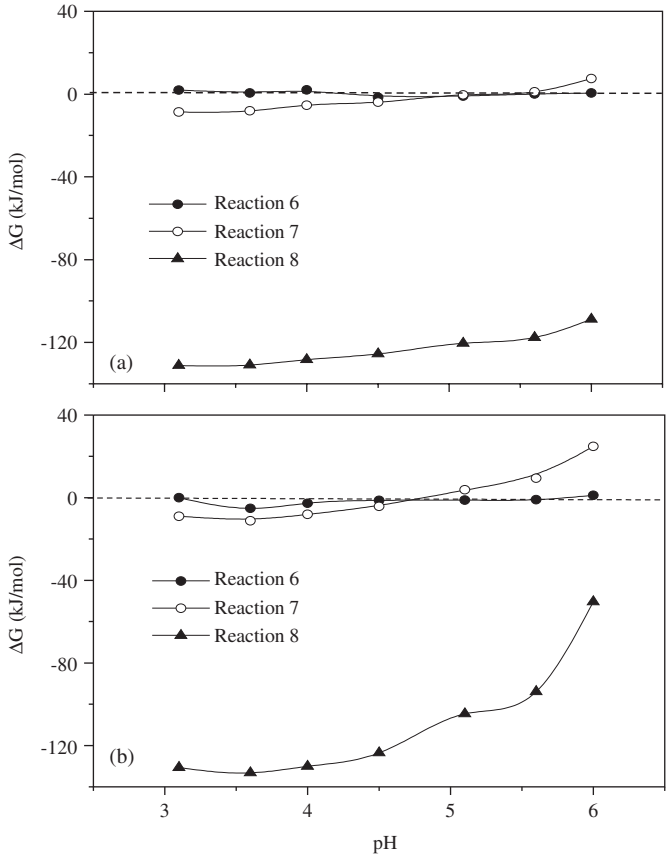

Fig. 6. Variation of Gibbs free energy for valerate formation at various pHs in: (a) R1; and (b) R2.

Three possible valerate-forming reactions (reactions (6)–(8)), suggested by Smith and McCarty [22], were listed as follows (ΔG^0 : kJ/mol):

$$CH_3CH_2COO^- + CH_3(CH_2)_2COO^- = CH_3(CH_2)_3COO^- + CH_3COO^-, \quad \Delta G^0 = -0.04, \tag{6}$$

$$CH_3CH_2COO^- + CH_3COO^- + 2H_2 + H^+ = CH_3(CH_2)_3COO^- + 2H_2O, \quad \Delta G^0 = -49.64, \tag{7}$$

$$CH_3CH_2COO^- + 2CO_2 + 6H_2 = CH_3(CH_2)_3COO^- + 4H_2O, \quad \Delta G^0 = -143.28. \tag{8}$$

The Gibbs free energy profiles of these three valerate-forming reactions at various pHs in the two reactors are shown in Fig. 6. In a similar way, reaction (8) was judged to be responsible for valerate formation at pH > 4.50 in both reactors, since it was not only thermodynamically possible but also H_2 consuming, which is in accordance with the low yield of H_2 at pH > 4.50 in the two reactors.

4. Discussion

Experimental results showed that the H_2-producing USAB system was sensitive to pH changes and the reactor performance differentiated from each other in various aspects. Performance of the two reactors is compared in Table 3. The maximum values of H_2 partial pressure in biogas, H_2 production rate and H_2 yield for R1 were 0.52 atm, 127 ml-H_2/l/h and 1.51 mol-H_2/mol-glucose, respectively, while for R2, they were 0.48 atm, 105 ml-H_2/l/h and 1.19 mol-H_2/mol-glucose, respectively. The optimum pH for maximum H_2 partial pressure was 3.10 for R1 and 4.00 for R2. The maximum H_2 production rate and the H_2 yield in the two reactors were achieved at pH 4.00. Moreover, methane was detected at a partial pressure of 0.02 atm only at pH 6.00 for R1, while for R2, methane could be detected at pH > 5.10 and its partial pressure increased to 0.13 atm at pH 6.00. Some previous studies have reported that heat pre-treatment could consistently eliminate the production of measurable concentration of methane [6,8,9]. In this work, however, methane could be detected in R2 at high pH levels, suggesting that the heat pre-treatment could not completely remove the methanogens and that methanogenic activity could be partially resumed as the growth conditions became favorable. These results indicate that the cultures with heat pre-treatment in R2 were less stable and were able to

Table 3
Maximum values of H_2 partial pressure, H_2 production rate and H_2 yield and corresponding optimum pHs: comparative performance of the two reactors

Reactor	H_2 partial pressure		H_2 production rate		H_2 yield	
	Maximum value (atm)	Optimum pH	Maximum value (ml-H_2/l/h)	Optimum pH	Maximum value (mol-H_2/mol-glucose)	Optimum pH
R1	0.52 ± 0.02	3.10 ± 0.10	127 ± 9	4.00 ± 0.10	1.51 ± 0.06	4.00 ± 0.10
R2	0.48 ± 0.02	4.00 ± 0.10	105 ± 4	4.00 ± 0.10	1.19 ± 0.05	4.00 ± 0.10

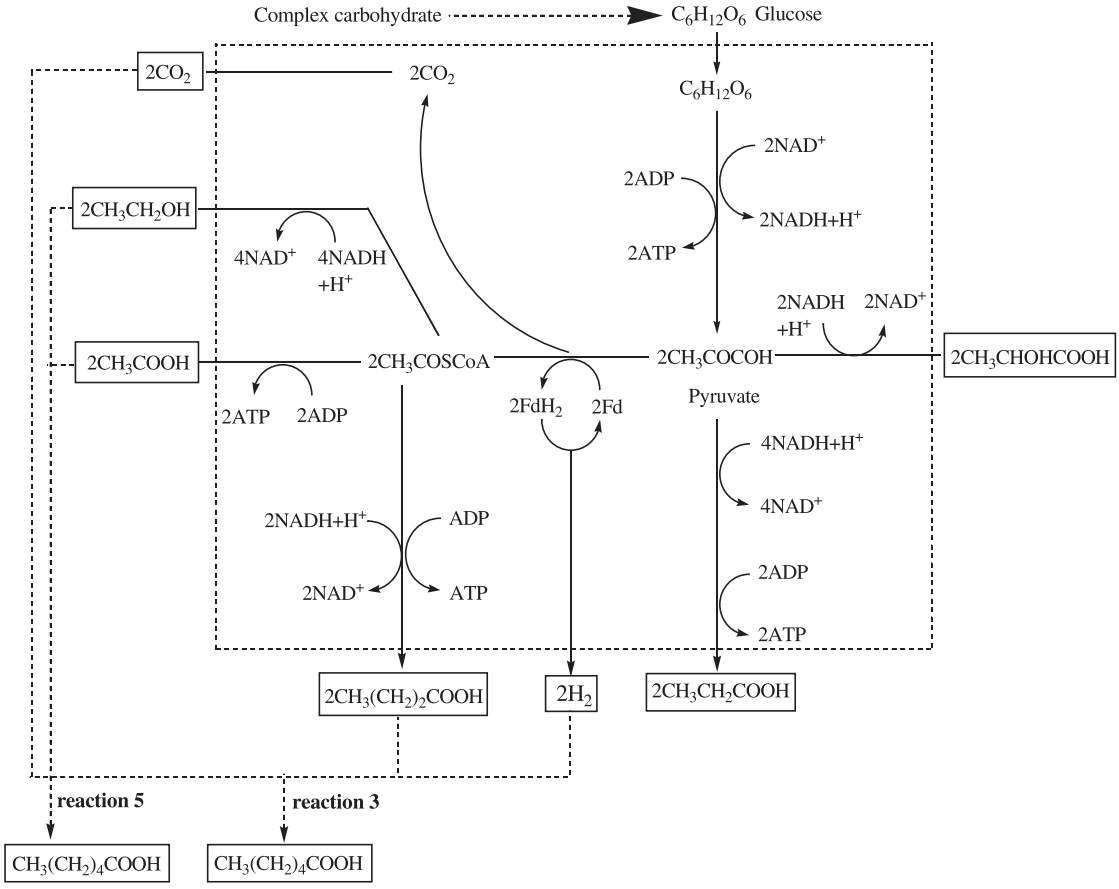

Fig. 7. Possible pathways of caproate formation.

produce H_2 continuously in a much narrower pH range than that in R1.

The distribution of VFA and ethanol in the two reactors was also different. Tables 1 and 2 show that the mixed-type fermentation occurred in both reactors and their significance was pH-dependent. For R1, caproate-type fermentation was dominant at pH < 4.00, but butyrate type was dominant at pH > 4.50, although caproate was still in a high level. For R2, butyrate type predominated at pH > 4.00, and caproate was also in a significant level at pH < 3.60. Moreover, as shown in Tables 1 and 2, valerate was an important product at pH > 4.50 in both reactors. All of these indicate that the influence of pH on the metabolic pathways was significant and that microbial population and their biological activities were also affected to a large degree by the variation of pH. This sensitivity issues a real challenge to a precise control of pH in the scale-up of the H_2-producing UASB reactors.

In previous studies regarding fermentative H_2 production, butyrate was usually found as the dominant aqueous product. In a high-rate anaerobic sequencing batch reactor, butyrate was detected as the main product and accounted for 65.0–71.5% of the total aqueous products [24]. Acetate, propionate and butyrate were the major VFAs produced and butyrate was dominant in a batch experiment carried out in a CSTR seeded with anaerobic sewage sludge acclimated with sucrose [25]. In the experiments with pure cultivation of *Clostridium thermolacticum* in a continuous bioreactor, ethanol has been reported to be obtained in a similar proportion as acetate for all dilution rates [26]. Ethanol was one of the major aqueous products with an average concentration of 864–2715 mg/l in an UASB

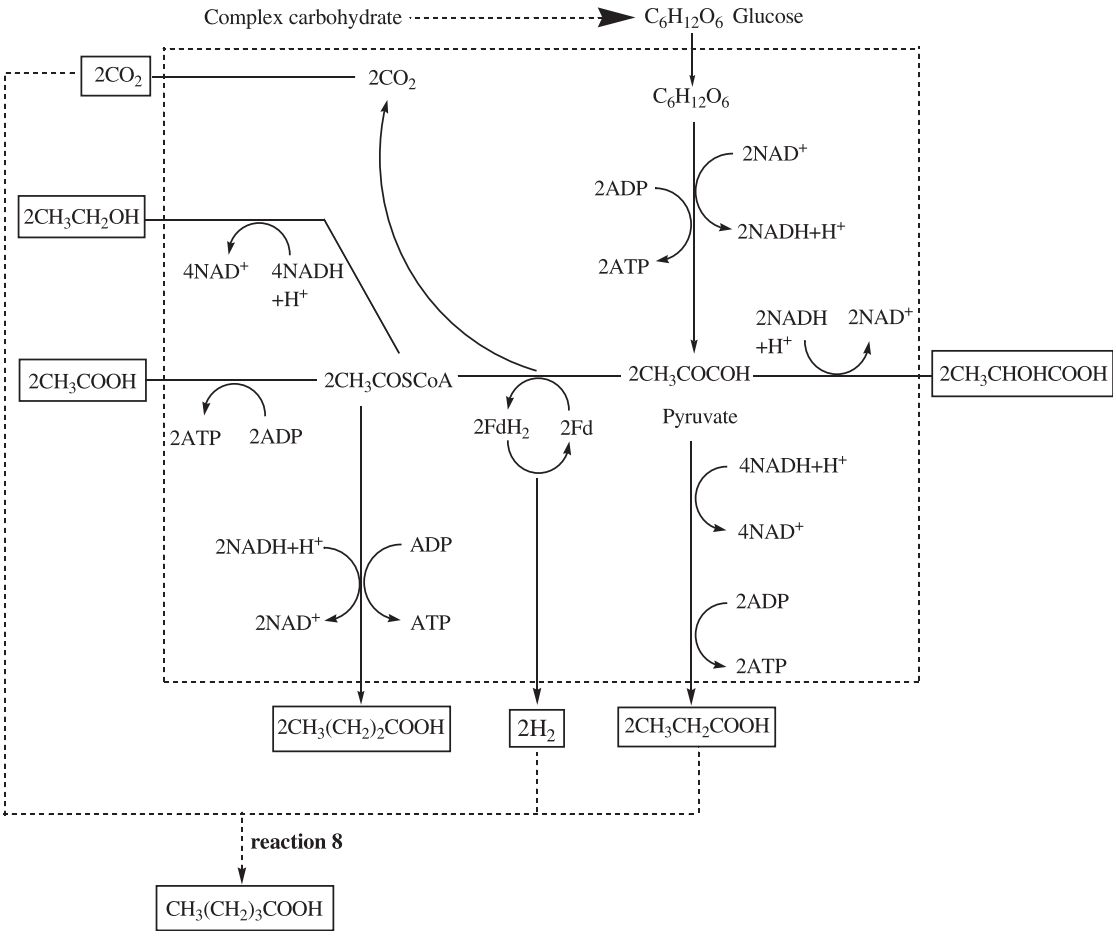

Fig. 8. A possible pathway of valerate formation.

reactor converting sucrose into H_2 [11]. However, in this study, caproate-type fermentation was found to be dominant in R1 at pH < 4.00. Although other researchers have also found caproate formation in fermentative H_2 production, it was usually in small quantity [27]. In this study, caproate was even dominant under certain conditions. The thermodynamic analysis suggests that caproate could be formed in two possible ways. One needed H_2 as the electron donor (reaction (3)), while another could form caproate independent of H_2 according to reaction (5). The possible metabolic pathways for caproate formation are shown in Fig. 7.

On the other hand, the thermodynamic analysis on the formation patterns of valerate indicates that reaction (8) was potentially responsible for its formation. This reaction required H_2 as the electron donor and consumed propionate and carbon dioxide. Fig. 8 illustrates the possible metabolic pathway for valerate formation. However, it should be noticed that Figs. 7 and 8 only indicate the possible pathways of the formation of caproate and valerate through reactions (3), (5) and (8), but do not reflect the stoichiometric ratio of the reactions. This warrants further investigations.

The different performance of the two reactors might be associated with the diversity of microbial population in the inoculums. A mixed-type fermentation with mixed cultures, closely related to species in the genera *Citrobacter*, *Clostridium* or *Klebsiella* was found in a complete-mixed fermentor producing H_2 by manipulating the mixed liquor at pH 5.5 [27]. While in the heat-treated sludge, *Clostridium acetobutylicum* was identified to be the major bacteria [7]. Furthermore, some specific bacteria might be responsible for the formation of caproate with such a high level in R1 in this study. For instance, reaction (5) was found in the ethanol–acetate fermentation of *Clostridium kluyveri*, in which ethanol and acetate were converted to butyrate and caproate [21]. When the operation was favorable for reaction (5), caproate was formed.

In previous studies [6,12,28], the favorable pH for dark fermentation is primarily located in the range of 5.00–6.50. However, in this study, the optimal pH for biohydrogen production was found to be around 4.00 in the light of maximum hydrogen production rate and hydrogen yield. This optimum pH value was remarkably lower than those previously reported. Such a difference may also be attributed to the specific bacterial community structure of the reactors in this study. Therefore, the species and population of microbes should be identified since they are very important not only for elucidating the metabolic pathways of carbohydrate but also for explaining the performance of the H_2-producing UASB systems. This should be one of the focuses in the future study.

5. Conclusions

In the two biohydrogen-producing UASB reactors, the H_2 production performance was highly pH-dependent. The optimum pH for H_2 partial pressure was 3.10 for R1 and 4.00 for R2, respectively, whereas the maximum H_2 production rate and H_2 yield were achieved at pH 4.00 for both reactors. Acetate, propionate, butyrate, *i*-butyrate, valerate, caproate and ethanol were main aqueous products in both reactors and their distribution was significantly influenced by pH as well. For R1, caproate was dominant at pH < 4.00 and butyrate-type fermentation predominated at pH 4.5–6.0. For R2, butyrate was dominant at pH > 3.60, while ethanol-type fermentation was important at pH < 3.60. A thermodynamic analysis indicates that caproate might be formed in two possible ways: one needed H_2 as the electron donor while another was independent of H_2. On the other hand, the formation of valerate required H_2 as the electron donor and consumed propionate and carbon dioxide.

Acknowledgments

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