

# The role of pH in the fermentative H<sub>2</sub> production from an acidogenic granule-based reactor

Yang Mu, Han-Qing Yu \*, Yi Wang

*School of Chemistry, Lab of Environmental Engineering, The University of Science and Technology of China, Hefei, Anhui 230026, China*

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## Abstract

The role of pH in the fermentative H<sub>2</sub> production from an upflow acidogenic granule-based reactor was investigated in this study. Experimental results show that all H<sub>2</sub> partial pressure, H<sub>2</sub> production rate and H<sub>2</sub> yield were pH-dependent, in the range of  $2.8 \times 10^4$ – $5.2 \times 10^4$  Pa, 61–145 ml-H<sub>2</sub> l<sup>-1</sup> h<sup>-1</sup> and 0.68–1.61 mol-H<sub>2</sub> mol-glucose<sup>-1</sup>, respectively. The maximum H<sub>2</sub> partial pressure was observed at pH 3.4, while both maximum H<sub>2</sub> production rate and H<sub>2</sub> yield were found at pH 4.2. Acetate, propionate, butyrate, *i*-butyrate, valerate, caproate and ethanol were present in the effluent of this UASB reactor, and their distribution was also pH-dependent. As pH was decreased from 4.2 to a lower level of 3.4 or increased to a higher level of 6.3, the fermentative type of this H<sub>2</sub>-producing reactor would shift from butyrate-type to caproate- or ethanol-type. Thermodynamic analysis was performed to explore the possible metabolic pathways of caproate and valerate formation. The metabolic pathway of caproate formation was pH-dependent, while that of valerate formation was pH-independent. A neural network model was designed, trained and validated. It was able to successfully describe the daily variations of H<sub>2</sub> partial pressure and H<sub>2</sub> yield of the reactor, and to predict its steady state performance at various pHs.

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## 1. Introduction

Hydrogen is an efficient energy carrier with high energy content per unit mass (122 kJ g<sup>-1</sup>). It does not produce any greenhouse gases and neither does it contribute to global warming. Hydrogen is also an important raw material in various chemical industries (Levin et al., 2003). Despite the green nature of H<sub>2</sub> as a fuel, it is still primarily produced from nonrenewable sources such as natural gas or petroleum hydrocarbons through steam reforming. In order for H<sub>2</sub> to become a more sustainable source of energy, H<sub>2</sub> should be produced through biological routes from wastes. Anaerobic fermentative production of H<sub>2</sub> decomposes organic substrates, and thus has an additional merit of converting organic wastes in the environment into

more valuable energy sources (Yu et al., 2004). Thus, it has recently attracted considerable attention as an effective way of recovering H<sub>2</sub> from organic wastes (Levin et al., 2003; Oh et al., 2004; Lin and Lay, 2005).

Many previous studies have demonstrated that the fermentative H<sub>2</sub>-producing process is greatly influenced by many factors, such as wastewater specificity, reactor configuration, hydraulic retention time (HRT), influent organic concentration, organic loading rate, pH, temperature, oxidation–reduction potential and nutritional requirements (Noike and Mizuno, 2000; Logan et al., 2002; Oh et al., 2004). Among these factors, pH has been found to be crucial to the distribution of acidogenic products (Gottschalk, 1986; Ren et al., 1997; Chen et al., 2002).

A substantial number of studies have been carried out to investigate the effect of pH on H<sub>2</sub> production in batch or continuous mode (Chen et al., 2002; Levin et al., 2003; Oh et al., 2004). However, the reported optimal pH for H<sub>2</sub> production is conflicting, varying from pH 9.0 to 4.0

\* Corresponding author. Tel.: +86 551 3607592; fax: +86 551 3601592.  
E-mail address: hqyu@ustc.edu.cn (H.-Q. Yu).

(Lay et al., 1999; Lay, 2000; Cai et al., 2004). Furthermore, little information is available in literatures concerning the role of pH in the fermentative H<sub>2</sub> production in a granule-based acidogenic reactor. Therefore, the main objective of this study was to explore the performance of H<sub>2</sub> production in an upflow anaerobic sludge blanket (UASB) reactor at various pHs, and to evaluate the influence of pH on the distribution of acidogenic products.

## 2. Material and methods

### 2.1. Substrate

A sucrose-rich synthetic wastewater was used as the substrate, supplemented with buffering chemicals and balanced nutrients as follows (unit in mg l<sup>-1</sup>): NH<sub>4</sub>HCO<sub>3</sub> 405; K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O 155; CaCl<sub>2</sub> 50; MgCl<sub>2</sub>·6H<sub>2</sub>O 100; FeCl<sub>2</sub> 25; NaCl 10; CoCl<sub>2</sub>·6H<sub>2</sub>O 5; MnCl<sub>2</sub>·4H<sub>2</sub>O 5; AlCl<sub>3</sub> 2.5; (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> 15; H<sub>3</sub>BO<sub>4</sub> 5; NiCl<sub>2</sub>·6H<sub>2</sub>O 5; CuCl<sub>2</sub>·5H<sub>2</sub>O 5; ZnCl<sub>2</sub> 5 (Oh et al., 2004). The pH value of synthetic wastewater was 7.0.

### 2.2. Reactor and inoculum

The bench-scale plexiglass-made UASB reactor was consisted of a reaction portion of 2.0 l and a gas–solids separator portion of 2.6 l. The UASB reactor was seeded with the sludge 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 6.33 g l<sup>-1</sup>, respectively. Prior to use, the seed sludge was first washed with tap water five times, and was then sieved to remove stone, sand and other coarse matters.

### 2.3. Operation of UASB

After the start-up of UASB, experiments were conducted to investigate the effect of pH on H<sub>2</sub> production of the UASB reactor at an HRT of 13 h and sucrose concentration of 10 g-COD l<sup>-1</sup> (COD: chemical oxygen demand). The pH of the mixed liquor of UASB was increased from 3.4 to 6.3 by adjusting the influent bicarbonate alkalinity (BA) through dosing NaHCO<sub>3</sub>. Each run lasted over four weeks to ensure reactor reaching pseudo-steady state before changing to the next condition. Effluent and biogas compositions were monitored. Only results obtained under pseudo-steady-state conditions were reported. The UASB reactor was kept at 39 ± 1 °C with heating bands throughout the entire operation.

### 2.4. Analyses

The amount of biogas produced was recorded daily using a gas meter. The composition of biogas, including H<sub>2</sub> and CH<sub>4</sub>, was analyzed with a gas chromatograph (Lunan, Model SP-6800A) equipped with a thermal con-

ductivity 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 °C, 105 °C and 60 °C, respectively. Argon was used as the carrier gas at a flow rate of 30 ml min<sup>-1</sup>. The concentrations of ethanol and volatile fatty acids (VFA), including acetate, propionate, butyrate, *i*-butyrate, valerate, and caporate, 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. The liquor samples were first centrifuged at 12000 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 °C and 300 °C, respectively. The initial temperature of the oven was 70 °C for 3 min followed with a ramp of 20 °C min<sup>-1</sup> 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<sup>-1</sup>. Sucrose concentration was determined by using anthrone-sulfuric acid method (Dubois et al., 1956), while the VSS and the BA were measured using the standard methods (APHA, 1995). The morphologies of the granules were observed using an image analysis system (Image-pro Express 4.0, Media Cybernetics) with an Olympus CX41 microscope and a digital camera (Olympus C5050 Zoom).

The actual change of Gibbs free energy ( $\Delta G$ ) of the reaction can be calculated with the following equation (Thauer et al., 1977):

$$\Delta G = \Delta G^0 + 2.303RT \log \frac{\{\text{products}\}}{\{\text{reactants}\}} \quad (1)$$

where  $\Delta G^0$  (kJ mol<sup>-1</sup>) is the value of  $\Delta G$  at pH 7.0 under standard conditions (i.e., all solutes are at the concentration of 1 M, and gases have partial pressure of 1.01 × 10<sup>5</sup> Pa).  $R$  is the universal gas constant, 8.314 J K<sup>-1</sup> mol<sup>-1</sup>,  $T$  is the absolute temperature in K,  $\{\}$  represents the chemical activity, which approximates molarity at low concentrations for aqueous matter, while partial pressure in atm for gaseous one.

## 3. Results and discussion

### 3.1. Start-up of UASB reactor

The start-up of the UASB reactor was conducted for 300 d in order to enrich the H<sub>2</sub>-producing microbes and establish a stable operation. In the start-up, the UASB reactor was initially fed with the synthetic wastewater of 5.61 g-COD l<sup>-1</sup> at an HRT of 30 h for 2 months, and the HRT was then shortened to 24 h for 3-month operation. Thereafter, the HRT was further shortened to 18 h for the subsequent operation, corresponding to a loading rate of 7.49 g-COD l<sup>-1</sup> d<sup>-1</sup>. After a further 3-month operation, acidogenic H<sub>2</sub>-producing granules were formed. After that,

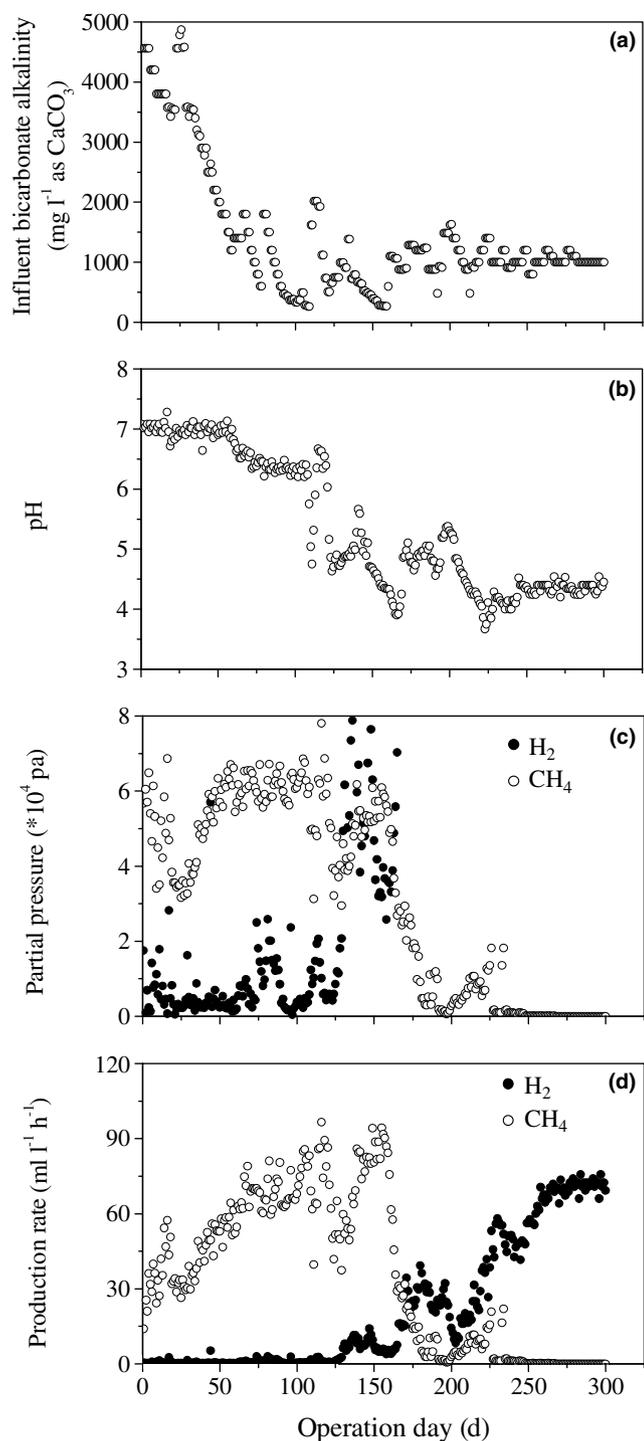


Fig. 1. Daily variations of: (a) influent bicarbonate alkalinity; (b) mixed liquor pH; (c) partial pressure of  $H_2$  and  $CH_4$  in biogas; and (d) production rate of  $H_2$  and  $CH_4$  during the start-up.

the UASB reactor was stably operated at a loading rate of  $7.49 \text{ g-COD l}^{-1} \text{ d}^{-1}$  for 2 months. Figs. 1 and 2 show the variation of the operating parameters during the start-up. As shown in Fig. 1(a) and (b), the pH of the UASB mixed liquor decreased from 7.0 to 4.0 by reducing the BA from 4000 to  $1000 \text{ mg l}^{-1}$  (as  $\text{CaCO}_3$ ). As a result, the  $CH_4$  par-

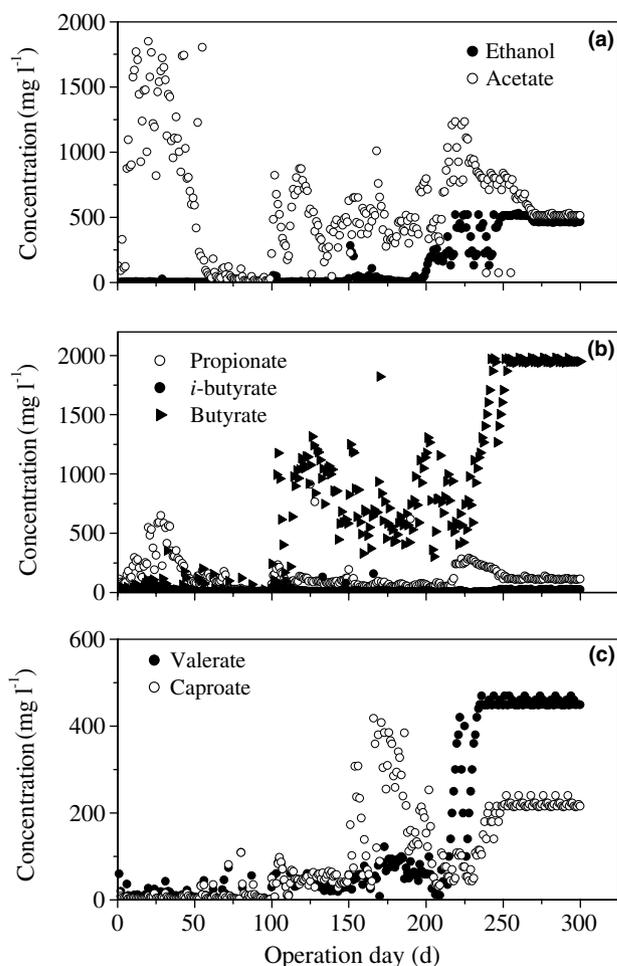


Fig. 2. Daily variations of (a) ethanol and acetate; (b) propionate, *i*-butyrate and butyrate; and (c) valerate and caproate during the start-up.

tial pressure decreased from  $6.0 \times 10^4 \text{ Pa}$  to nil. Correspondingly, the  $CH_4$  production rate increased from 20 to  $90 \text{ ml l}^{-1} \text{ h}^{-1}$ , but decreased to zero. On the contrary, the  $H_2$  partial pressure increased from nil to  $4.0 \times 10^4 \text{ Pa}$ , and the  $H_2$  production rate increased for zero to  $70 \text{ ml l}^{-1} \text{ h}^{-1}$ , attributed to the complete inhibition of the activity of methanogens and enrichment of the  $H_2$ -producing bacteria (Fig. 1(c) and (d)).

Fig. 2 shows the production profiles of VFA and ethanol during the start-up of UASB. Acetate was dominant during the start-up of 0–100 d. In 100–200 d, butyrate and acetate became the major products, and a significant production of caproate was observed. After 200 d, butyrate became dominant. In this period, acetate, propionate, valerate, and caproate were in the range of  $100\text{--}700 \text{ mg l}^{-1}$ , while the concentration of *i*-butyrate was below  $100 \text{ mg l}^{-1}$ . In the start-up period, fluctuations were observed for biogas, VFA and ethanol. These fluctuations might result from the decrease in pH and increase in the organic loading rate. However, after operation of 300 d, a successful start-up was achieved with the formation of the  $H_2$ -producing granules (Fig. 3).



Fig. 3. Images of the H<sub>2</sub>-producing granules.

### 3.2. Reactor performance at various pHs

#### 3.2.1. Sucrose degradation and BA dosage

As shown in Fig. 4a, sucrose was readily degraded at various pHs, and its degradation efficiency increased from 94% to 99% with an increase in pH from 3.4 to 6.3. This indicates that sucrose degradation was not affected considerably by the pH variation. A similar trend was observed for the degradation of proteins present in primary sludge at pH of 4.5–7.0 (Eastman and Ferguson, 1981). Fig. 4(b) shows that the BA also increased with increasing pH, from 500 mg l<sup>-1</sup> (as CaCO<sub>3</sub>) at pH 3.4 to 3500 mg l<sup>-1</sup> (as CaCO<sub>3</sub>) at pH 6.3.

#### 3.2.2. H<sub>2</sub> production

The variations of H<sub>2</sub> partial pressure in biogas, H<sub>2</sub> production rate, and H<sub>2</sub> yield as a function of pH are shown in Fig. 5. The H<sub>2</sub> partial pressure decreased with increasing pH, from 5.2 × 10<sup>4</sup> Pa at pH 3.4 to 2.8 × 10<sup>4</sup> Pa at pH 6.3 (Fig. 5(a)). The biogas was free of methane, due to the suppression of methanogenic activity. As illustrated in Fig. 5(b), the H<sub>2</sub> production rate increased from 61 to 145 ml l<sup>-1</sup> h<sup>-1</sup> with an increase in pH from 3.4–4.2, then decreased to 89 ml l<sup>-1</sup> h<sup>-1</sup> as pH was further increased to

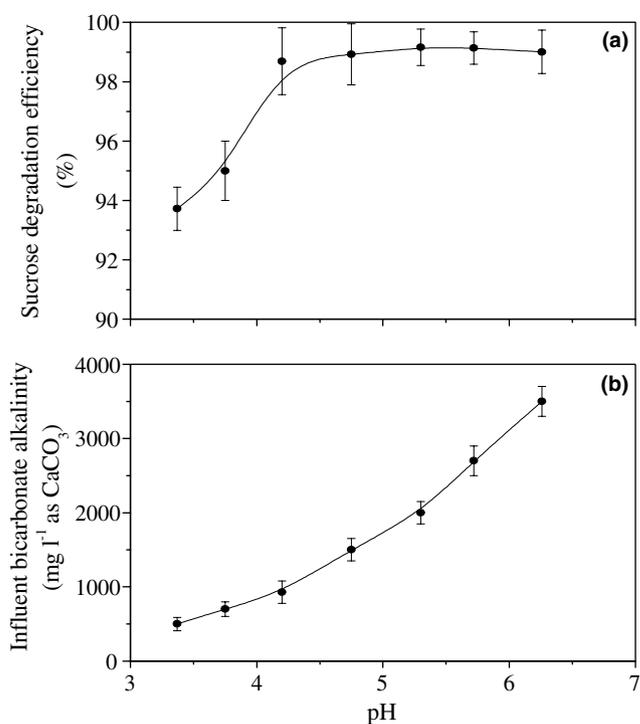


Fig. 4. Variations of (a) substrate degradation; and (b) influent bicarbonate alkalinity at various pHs.

6.3. The H<sub>2</sub> yield had a similar changing trend to that of H<sub>2</sub> production rate (Fig. 5(c)). It increased from 0.68 mol-H<sub>2</sub> mol-glucose<sup>-1</sup> at pH 3.4 to 1.61 mol-H<sub>2</sub> mol-glucose<sup>-1</sup> at pH 4.2, and then decreased to 1.00 mol-H<sub>2</sub> mol-glucose<sup>-1</sup> at pH 6.3.

#### 3.2.3. Formation of aqueous products

Table 1 summarizes the total VFA and ethanol concentrations in the effluent as well as the percentages of individual VFA and ethanol at various pHs. The VFA and ethanol concentration did not change significantly as pH increased from 3.4–5.3, in the range of 3474–3880 mg l<sup>-1</sup>, except for a higher concentration of 4145 mg l<sup>-1</sup> at pH 5.7 and a lower level of 2498 mg l<sup>-1</sup> at pH 6.3. Table 1 also shows that the distribution of VFA and ethanol was pH-dependent. The percentage of acetate decreased from 22.7% at pH 3.4 to 13.7% at pH 4.2, and then increased to 17.6 at pH 6.3. Ethanol had a similar trend to acetate. On the contrary, the percentage of butyrate increased from 21.6% at pH 3.4 to 52.1% at pH 4.2, then decreased to 23.0% at pH 6.3.

Table 1 also shows that the percentage of caproate had a high level of 24.0–28.9% at pH 4.8–6.3. Valerate was also an important product at pH > 3.8, accounting for 8.4–12.2%. Propionate had a level less than 6%, whereas *i*-butyrate was below 2%, regardless of the pH value. These results indicate that a mixed-type fermentation occurred in this H<sub>2</sub>-producing UASB reactor, i.e., butyrate-, ethanol- and caproate-type were all present.

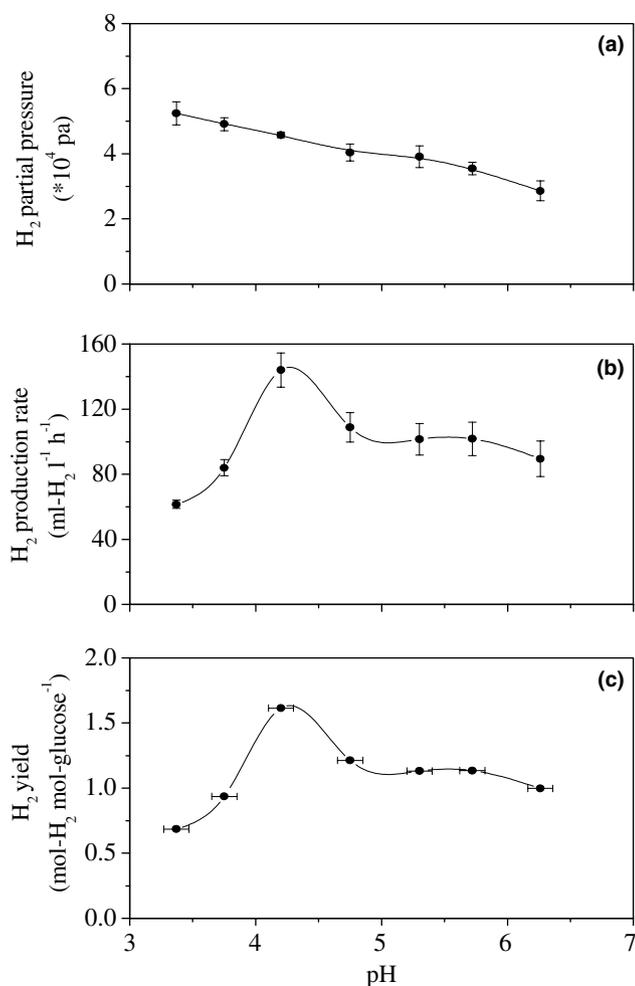


Fig. 5. Variations of: (a) H<sub>2</sub> partial pressure in biogas; (b) H<sub>2</sub> production rate; and (c) H<sub>2</sub> yield at various pHs.

The distribution of aqueous products in H<sub>2</sub> fermentation is often a crucial signal in assessing the efficiency of the H<sub>2</sub>-producing cultures (Cha and Noike, 1997). The ratio of butyrate/acetate (B/A ratio) has been used as an indicator for evaluating the effectiveness of H<sub>2</sub> production (White, 1995; Annous et al., 1996). As shown in Fig. 6, the H<sub>2</sub> yield decreased as the B/A ratio increased, suggesting that formation of butyrate seems to favor the H<sub>2</sub> production. This result was accord with those of other studies (White, 1995; Annous et al., 1996). However, the optimal

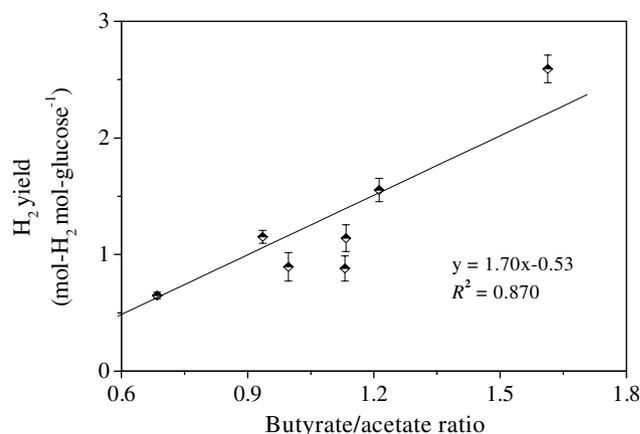


Fig. 6. Relationship between the H<sub>2</sub> yield and the ratio of butyrate/acetate.

B/A ratio for H<sub>2</sub> production heavily depends on the type of anaerobic cultures and substrates used (Nandi and Sengupta, 1998). For instance, when glucose was used as the substrate, the optimal B/A ratio for *Clostridium butyricum* (White, 1995) and *Bacillus methylotrophicum* (Anous et al., 1996) was 2.0 and 0.75, respectively. In this work, the optimal B/A ratio was 2.6 for this H<sub>2</sub>-producing granules with sucrose as a substrate. This further confirms that the optimal B/A ratio was substrate-dependent.

### 3.3. Thermodynamic analysis

In previous studies about fermentative H<sub>2</sub> production, butyrate was found as the dominant aqueous product. In an anaerobic H<sub>2</sub>-producing sequencing batch reactor, butyrate accounted for 65.0–71.5% of aqueous products (Lin and Jo, 2003). Butyrate was in a range of 31.2–45.6% of total VFA and alcohols in acidogenic H<sub>2</sub> production from sucrose using a mixed culture (Fang and Liu, 2002). In the experiment with pure batch cultures of *Rhodospseudomonas palustris* or *citrobacter* sp., ethanol and acetate were reported to be major compounds, which comprised more than 50% of total carbon consumed (Oh et al., 2003). An ethanol-type fermentation was observed in an acidogenic reactor with a mixed culture at pH 4.5 (Ren et al., 1997). However, in this study, caproate- and

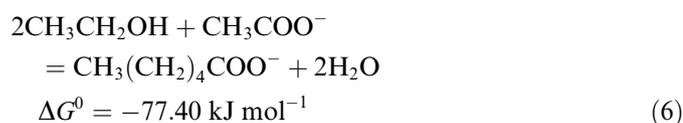
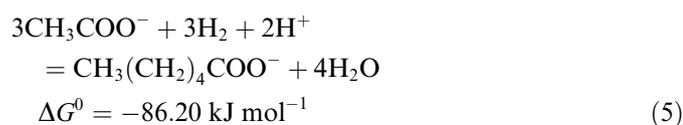
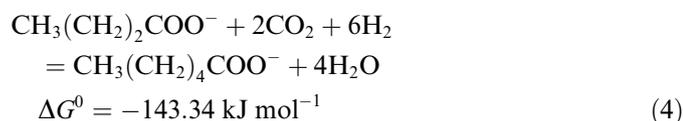
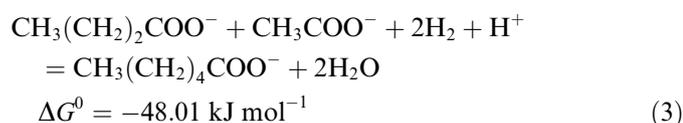
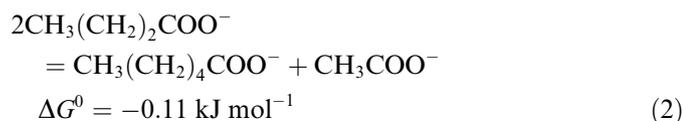
Table 1  
Distribution of VFA and ethanol in the UASB effluent at various pHs

pH	VFA + Eol (mg l <sup>-1</sup> )	HAc (%)	HPr (%)	<i>i</i> -HBu (%)	HBu (%)	HVa (%)	HCa (%)	Eol (%)
3.4	3635 ± 174	22.7 ± 1.4	5.3 ± 0.4	0.8 ± 0.2	21.6 ± 0.7	3.4 ± 0.4	13.2 ± 2.4	33.0 ± 3.1
3.8	3778 ± 200	18.7 ± 1.4	4.3 ± 0.4	0.7 ± 0.2	31.6 ± 0.7	8.4 ± 0.4	8.2 ± 2.4	28.1 ± 3.1
4.2	3738 ± 216	13.7 ± 1.0	3.1 ± 0.5	0.7 ± 0.1	52.1 ± 2.3	12.0 ± 0.9	5.8 ± 0.5	12.5 ± 0.4
4.8	3880 ± 373	14.0 ± 1.4	3.5 ± 0.6	0.6 ± 0.1	31.9 ± 3.0	12.2 ± 1.2	24.0 ± 3.9	13.8 ± 1.4
5.3	3474 ± 244	18.8 ± 0.6	2.7 ± 0.4	0.8 ± 0.1	24.3 ± 0.8	10.4 ± 1.4	28.9 ± 1.4	14.1 ± 0.9
5.7	4145 ± 196	16.2 ± 1.1	3.8 ± 1.1	0.9 ± 0.1	27.1 ± 1.0	10.7 ± 0.9	25.4 ± 1.8	15.9 ± 2.5
6.3	2498 ± 496	17.6 ± 2.2	4.4 ± 0.1	0.7 ± 0.2	23.1 ± 0.7	9.4 ± 1.3	24.8 ± 3.7	20.1 ± 0.3

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

valerate-type fermentations were found to be important in this H<sub>2</sub>-producing UASB reactor at various pHs. Fang and Liu (2002) also observed the formation of caproate and valerate in an acidogenic reactor, but their levels were less than 6%.

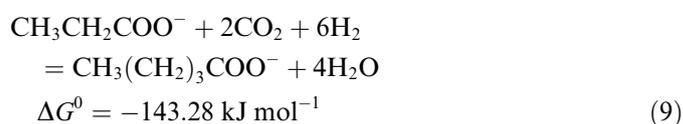
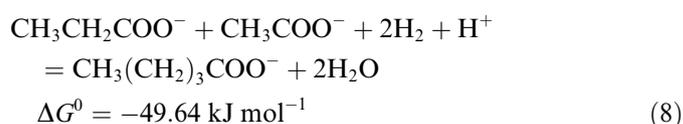
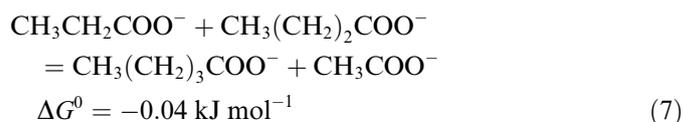
Five possible caproate [CH<sub>3</sub>(CH<sub>2</sub>)<sub>4</sub>COO<sup>-</sup>] forming pathways have been suggested as following (Thauer et al., 1977; Smith and McCarty, 1989):



According to the second thermodynamic law, a reaction can take place only if  $\Delta G$  is negative. Moreover, the absolute value of  $\Delta G$  must be larger than 2 kJ mol<sup>-1</sup>, which was the minimum amount of Gibbs free energy needed to sustain growth and/or conversion of a substrate (Smith and McCarty, 1989; Hickey and Switzenbaum, 1991). Since all terms needed for the calculation of  $\Delta G$  have been measured, the actual  $\Delta G$  values for the five reactions during the experiment can be calculated with Eq. (1). Table 2 lists the Gibbs free energy profiles of the five caproate-forming reactions (2)–(6) at various pHs. At pH < 4.2, reactions (2)–(6) were all responsible for caproate formation, while

only reaction 6 was possible at pH 5.3–6.3. At pH 4.2, reactions (2), (3), (5) and (6) could take place, but reactions (4) and (5) were not involved in the formation of caproate at pH 4.8. These results show that the metabolic pathway of caproate formation was pH-dependent.

On the other hand, three possible valerate [CH<sub>3</sub>(CH<sub>2</sub>)<sub>3</sub>COO<sup>-</sup>] forming reactions (reactions (7)–(9)), suggested by Smith and McCarty (1989), are listed as follows:



The Gibbs free energy profiles of the three valerate-forming reactions (7)–(9) at various pHs are also listed in Table 2. In a similar manner, it is judged that only reaction 9 should be responsible for the formation of valerate at various pHs. This indicates that the metabolic pathway of valerate formation was pH-independent.

It is noticed that H<sub>2</sub>, as an electron donor, could be consumed in the reactions (3)–(5), (8) and (9). As a result, the H<sub>2</sub> yield could be reduced. Furthermore, as the concentration of caproate and/or valerate becomes sufficiently high, they might result in a reduction of the pH gradient across the membrane and the total inhibition of all metabolic functions in the cell (Gottschalk, 1986). Hence, from a H<sub>2</sub> production point of view, appropriate strategies should be adopted to reduce the formation of caproate and valerate in a UASB reactor. One strategy is extracting H<sub>2</sub> from reactor continuously (Nielsen et al., 2001) and keeping the H<sub>2</sub> partial pressure at a low level, which made reactions (3)–(5), (8) and (9) unfavorable thermodynamically, i.e.,  $\Delta G > 0$ .

### 3.4. Modeling

Since the fermentative process in this H<sub>2</sub>-producing UASB reactor was complex, a black-box model, i.e., a neural network model with back propagation (Strik et al., 2005), was developed to predict the steady state performance of the reactor. Neural network consisted of a layer of input neurons, a layer of out neurons, and two hidden layers. The number of neurons in the input (*i*th) layer was 4. The two hidden layers consisted of 6 (*j*th layer) and 7 (*k*th layer) neurons each. The output (*l*th layer) had 1 neuron. The input vector consisted of a set of four variables: pH, HRT, substrate concentration (*S*), and influent

Table 2  
Gibbs free energy profiles of the caproate-forming (2)–(6) and the valerate-forming (7)–(9) reactions at various pHs

pH	$\Delta G$ for reactions (kJ mol <sup>-1</sup> )								
	2	3	4	5	6	7	8	9	
3.4	-2.61	-7.41	-21.73	-6.19	-62.41	-0.19	-1.69	-130.35	
3.8	-4.32	-8.73	-10.75	-5.30	-62.48	1.17	-1.95	-128.18	
4.2	-8.60	-9.31	2.79	-2.32	-58.26	0.76	-1.62	-127.03	
4.8	-2.50	-2.72	26.86	4.41	-55.16	1.61	-0.31	-128.14	
5.3	0.04	0.31	47.57	7.86	-54.77	3.12	1.70	-128.09	
5.7	-1.29	2.27	61.64	12.84	-56.14	1.54	3.40	-129.31	
6.3	-0.34	7.86	80.72	22.51	-54.96	1.40	7.90	-130.46	
3.4	-2.61	-7.41	-21.73	-6.19	-62.41	-0.19	-1.69	-130.35	

BA, whereas the output was one variable:  $H_2$  partial pressure or  $H_2$  yield.

A total of 149 experimentally derived data sets were obtained from this study. Of these data sets, 129 sets were used for training the neural network model, whereas 20 data sets were kept aside for validating (Fig. 7).

Fig. 8 shows the comparison between the experimental values for various output and corresponding values obtained by training and validating the model. The training determination coefficients ( $R^2$ ) of the  $H_2$  partial pressure (0.931) and  $H_2$  yield (0.906) show that the neural networks learned the relationship between input and output well. The validating  $R^2$  values of the  $H_2$  partial pressure (0.950) and  $H_2$  yield (0.933) were even greater, suggesting that the generalization capacity of the model

was very good. The lower training  $R^2$  values than the validating ones were most likely attributed to noise in the training data (Strik et al., 2005). Thus, in the subsequent simulations the validated neural network model was used to predict the effect of pH variation on the  $H_2$  partial pressure and  $H_2$  yield under steady state conditions. The results in Fig. 9 show that the predicted results were consistent with the measured ones for both  $H_2$  partial pressure and  $H_2$  yield at various pHs, suggesting that this neural network model was appropriate. Moreover, as shown in Fig. 9(b), the  $H_2$  yield would decline dramatically as pH was decreased from 4.0 to a lower level or increased from 6.0 to a higher level. This further confirms that a precise pH control should be adopted for a  $H_2$ -producing UASB reactor.

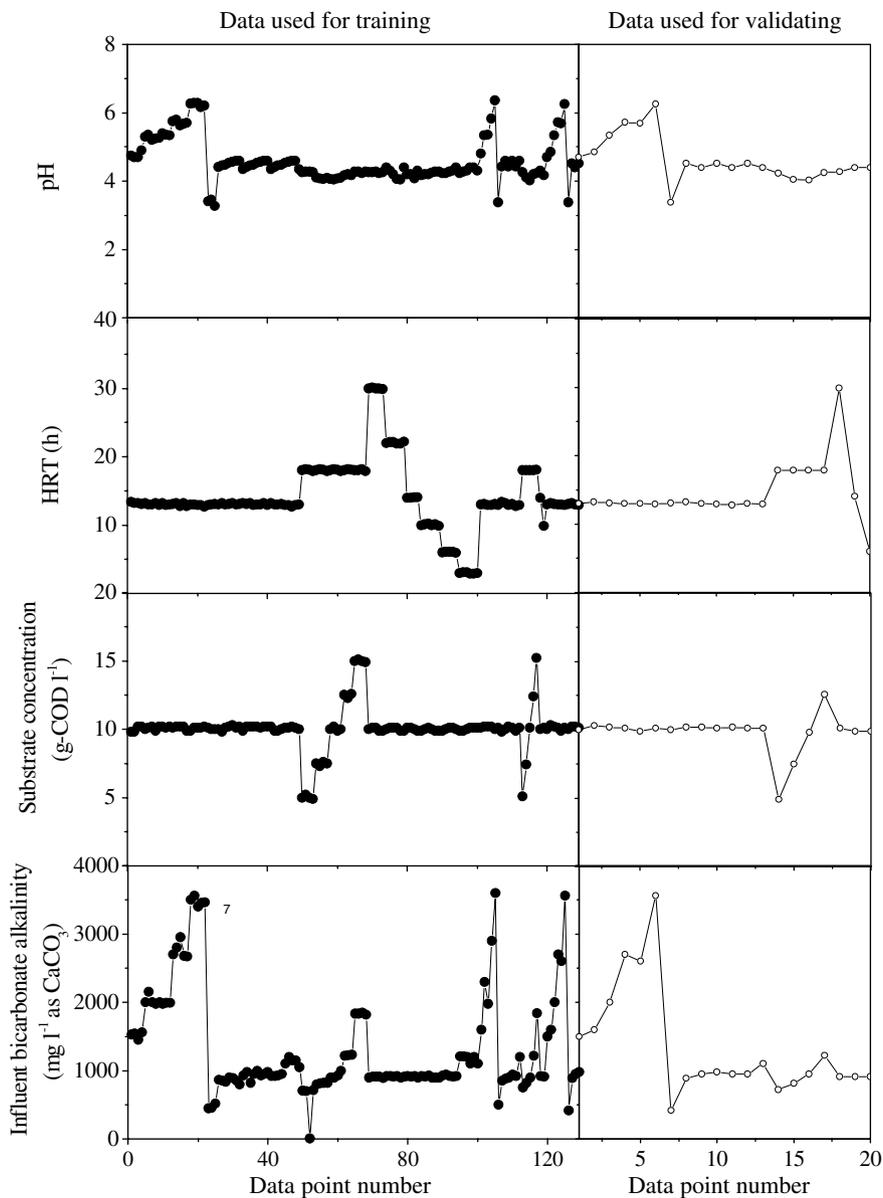


Fig. 7. Sets of input variables used for training and validating neural network model.

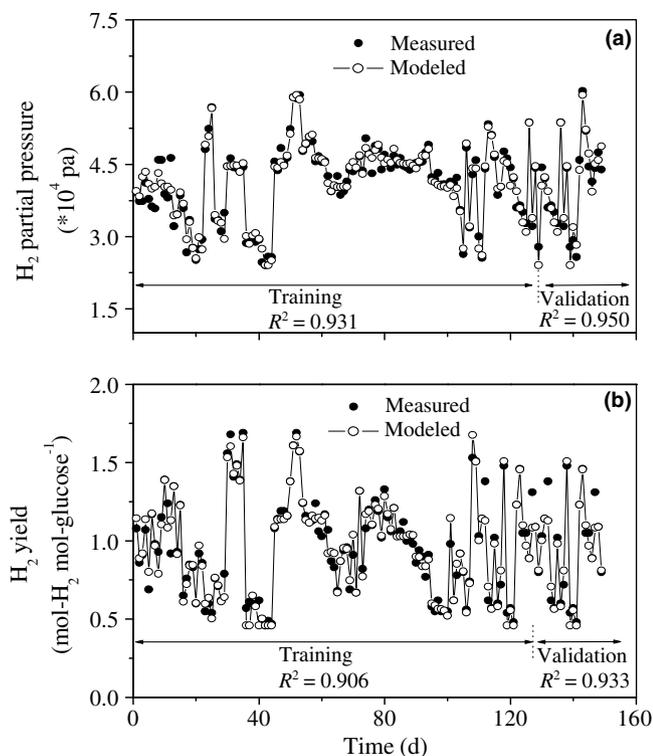


Fig. 8. Training and validating the neural network model with: (a) H<sub>2</sub> partial pressure; and (b) H<sub>2</sub> yield.

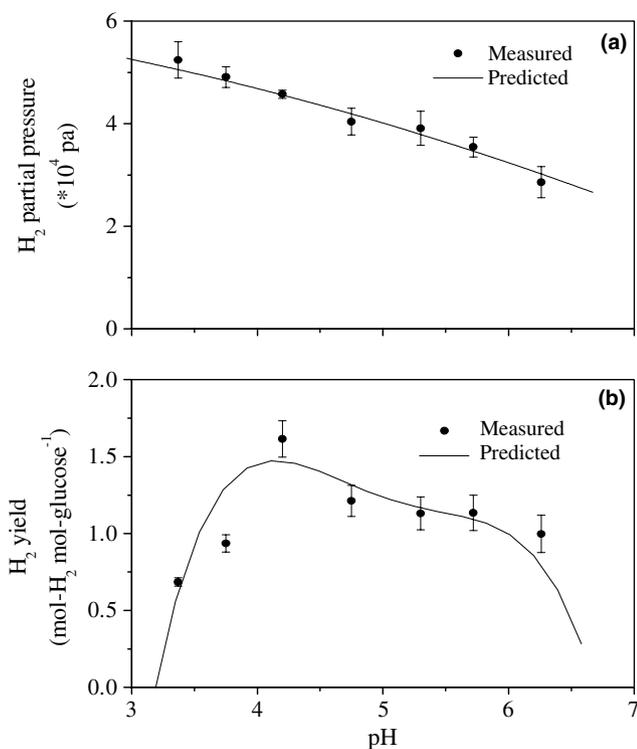


Fig. 9. Prediction of: (a) H<sub>2</sub> partial pressure; and (b) H<sub>2</sub> yield at various pHs with the neural network model.

#### 4. Conclusions

The experimental results show that the pH was crucial to the H<sub>2</sub> production in a granule-based acidogenic reactor. The H<sub>2</sub> partial pressure in biogas, H<sub>2</sub> production rate and H<sub>2</sub> yield were all pH-dependent. The maximum H<sub>2</sub> partial pressure,  $5.3 \times 10^4$  Pa, was observed at pH 3.4. While the maximum value of both H<sub>2</sub> production rate and H<sub>2</sub> yield,  $145 \text{ ml-H}_2 \text{ l}^{-1} \text{ h}^{-1}$  and  $1.61 \text{ mol-H}_2 \text{ mol-glucose}^{-1}$ , were found at pH 4.2. The distribution of aqueous products was also pH-dependent. Butyrate, acetate and ethanol were dominant at various pHs and their distribution was pH-dependent. When pH was decreased from 4.2 to a lower level or increased to a higher level, the fermentative type would shift from butyrate-type to caproate- or ethanol-type. Thermodynamic analysis indicates that the caproate formation was pH-dependent, while valerate formation was pH-independent. An established neural network model was able to successfully describe the daily variations of H<sub>2</sub> partial pressure and H<sub>2</sub> yield, and to predict the steady state performance of the UASB reactor at various pHs.

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