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International Journal of Hydrogen Energy 32 (2007) 185–191
International Journal of HYDROGEN ENERGY
www.elsevier.com/locate/ijhydene

Biohydrogen production in a granular activated carbon anaerobic fluidized bed reactor

Zhen-Peng Zhang[a,b], Joo-Hwa Tay[a,b], Kuan-Yeow Show[a,*], Rong Yan[b], David Tee Liang[b], Duu-Jong Lee[c], Wen-Ju Jiang[d]

[a]*School of Civil and Environmental Engineering, Nanyang Technological University, 639798 Singapore, Singapore*
[b]*Institute of Environmental Science and Engineering, Nanyang Technological University, 637723 Singapore, Singapore*
[c]*Department of Chemical Engineering, National Taiwan University, Taipei 10617, PR China*
[d]*Department of Environmental Science and Engineering, Sichuan University, Chengdu 610065, PR China*

Available online 18 September 2006

Abstract

A new approach to immobilize mixed culture of hydrogen-producing bacteria was examined by growing on granular activated carbon in an anaerobic fluidized bed reactor. Production of hydrogen by the immobilized culture was assessed at a consistent pH of 4.0 and at a temperature of 37 °C. The system was operated by shortening the hydraulic retention time (HRT) from 4 to 0.5 h at 10 g/L influent strength, or by increasing the influent concentration of glucose from 10 to 30 g/L at 1 h HRT. The biogas produced was composed of H_2, CO_2 and free of CH_4. Hydrogen composition decreased from 61% to 57% with the reduction in HRT, while it stabilized at 59% as feed strength varied. The dissolved metabolite products were acetate and butyrate, with smaller quantities of other volatile fatty acids and alcohols. The hydrogen production rate and specific hydrogen production rate were linearly correlated to the effective organic loading rate, which was calculated on the basis of organic loading and glucose conversion rate, giving the respective maximum rates of 2.36 L/L h and 4.34 mmol-H_2/g-VSS h. The attached biofilm concentration was retained as high as 21.5 g/L. It is concluded that a substantial quantity of retained biomass would enable the reactor to run at the high organic loading rates and thus enhance the production rates of hydrogen gas.

Keywords: Biohydrogen production; Granular activated carbon; Anaerobic fluidized bed reactor; pH; Organic loading rate

1. Introduction

Biohydrogen production by anaerobic fermentation has attracted worldwide attention owing to the fact that hydrogen can be produced substantially at a high rate form renewable organic matters [1]. The studies on continuous fermentative hydrogen production in the laboratory-scale had been conducted using suspended-cell systems and immobilized-cell systems since 1980s [2–5]. The hydrogen production rate (HPR) has been considered as an important index to evaluate the performance of continuous hydrogen-producing processes [6]. However, continuous stirred tank reactor (CSTR) process, a typical representative of suspended-cell systems, usually exhibits poor performance in HPR since it is unable to maintain high levels of hydrogen-producing biomass at a short hydraulic retention time (HRT) due to its intrinsic structure [3,5].

To achieve satisfactory HPR, immobilized-cell systems have become popular alternatives to suspended-cell systems for continuous biohydrogen production since they are more capable of maintaining higher biomass concentration even at shorter HRTs [6,7]. The previous studies lead us to believe that the cell immobilization techniques including cell-entrapping and cell-attaching methods improve cell retention and HPR in the granular reactors [8], fixed-bed reactors [6] and fluidized bed reactors [9]. However, in compassion with attaching methods, the immobilized cells created by gel entrapping techniques are easily suffered from mass transfer resistance [10]. Moreover, the biogas produced inside the gel could lead to damages to the structure of the immobilized bioparticles [7].

* Corresponding author. Tel.: +65 6790 5282; fax: +65 6791 0676.
E-mail address: ckyshow@ntu.edu.sg (K.-Y. Show).

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doi:10.1016/j.ijhydene.2006.08.017

Although anaerobic fluidized bed reactor (AFBR) processes with attached biofilm have been extensively studied in the field of wastewater treatment for many years due to their potential to offer the advantages, e.g. accumulation of a large amount of biomass on the support medium, high organic loading rate (OLR), short HRT operation, and good mixing characteristics [11–13], they are not yet reported in anaerobic hydrogen production. Therefore, the present study focused on continuous biohydrogen production by mixed culture growing on the support medium in an AFBR. Granular activated carbon (GAC) was used as the support medium since it served excellently in microbial colonization [14,15]. The effects of HRT and feed concentration on hydrogen fermentation were investigated, and the performance of granular activated carbon anaerobic fluidized bed reactor (GAC-AFBR) system was also evaluated in the present study.

2. Materials and methods

2.1. Seed sludge

Activated sludge and digested sludge obtained from Ulu Pandan Water Reclamation Plant (Singapore) were used as inocula. The aim of two sludge mixture used was to widen the microbial diversity for those hydrogen-producing bacteria from natural resources. The collected sludge was mixed together a volume ratio of 1:1 and final concentrations of suspended solid (SS) and volatile suspended solid (VSS) concentration were 9.55 and 8.35 g/L, respectively.

2.2. Feed composition

The synthetic wastewater mainly consisting of glucose was used as feed, which also contained the following nutrients at a constant nutrient: carbon ratio per 10 g glucose: 200NH_4Cl, 200peptone, 200KH_2PO_4, 30$FeCl_36H_2O$, 20$MgCl_2 \cdot 6H_2O$, 10$MnCl_2 \cdot 4H_2O$, 5$CoCl_2 \cdot 6H_2O$, 6$CaCl_2 \cdot 2H_2O$, 5$Cu(NO_3)_2 \cdot 3H_2O$, 5$ZnSO_4 \cdot 7H_2O$, and 5$NiSO_4 \cdot 6H_2O$.

2.3. Experimental set-up and support medium

The schematic diagram of the experimental system is shown in Fig. 1. The AFBR consisted of a glass tubular section of 40 mm internal diameter and 500 mm height with a conic bottom. Over this, an upper section of 80 mm internal diameter and 150 mm length was mounted to prevent carry over of suspended particles into the effluent and also to serve as a gas holder. Forty mm height glass bead with 5 mm in diameter was located in the reactor bottom as influent distributor. The column has four sampling ports located at 50, 120, 250 and 400 mm above the reactor bottom. These ports were used to extract liquid and bioparticle samples along the reactor. The effluent was recycled through a recycle pump connecting effluent outlet and feed inlet at a fixed recycling rate of 250 mL/min throughout. The pH of mixed liquid in the reactor was controlled at 4.0 constantly by automatic titration using respective peristaltic pumps connecting to an integrated controller with 2M NaOH and 2M HCl. The reactor was operated at a consistent temperature of 37 °C by a heating blanket. The readings of peristaltic pumps and pH meter were calibrated weekly. The production of biogas was measured daily using water displacement method, and biogas volume was calibrated to a temperature of 25 °C and pressure of 1 atm condition. GAC was used as the support medium, and its physical characteristics were offered by the supplier as follows: size range, 500–800 μm; surface area, 1100–1250 m^2/g; bulk density, 440–480 g/L; and real density, 1450 g/L (Kimic Chemitech, Singapore). Ninety gram of GAC was loaded into the reactor, occupying an initial bed height of 165 mm.

2.4. Startup and operation of AFBR

Six hundred milliliter mixed seed sludge with a concentration of 8.35 g-VSS/L, initially heated at 105 °C for 45 min to kill non-spore formers, was used to inoculate the reactors. After loading support medium and seed sludge and approximately 0.5 L feed solution into reactors, the reactor was flushed with nitrogen gas for 10 min to create an anaerobic environment. The startup of GAC-AFBR was described elsewhere [16]. In short, after one week batch cultivation, the OLR was enhanced stepwise by shortening HRT. The reactor reached the steady-state after three months of operation at a HRT of 1.08 h, and then the HRT was lengthened to 4 h. The HRT was shortened stepwise from 4 to 0.5 h at an influent concentration of 10 g/L to investigate the effect of HRT on hydrogen fermentation. Similarly, the influence of feed strength was studied by increasing its concentration from 10 to 30 g-glucose/L at a HRT of 1 h. The reactor was run 20–80 cycles of the corresponding HRT to ensure steady-state conditions were reached. Each steady-state condition was lasted over one week to collect the gaseous and aqueous metabolites daily and bioparticle samples two or thrice. Steady-state conditions defined in the present study were considered to be reached when the variations of products and biomass concentration were less than 10%. Only those data obtained under steady-state conditions are reported.

2.5. Analytical methods

Biogas contents: The biogas was analyzed by a micro-gas chromatograph (Varian 4900, USA) equipped with a thermal conductivity detector. Hydrogen was analyzed using a Molsieve 5A Plot column with argon as carrier gas at 60 °C; methane and carbon dioxide were analyzed using a Propac Q column with helium as carrier gas at 60 °C.

Aqueous samples: Aqueous samples were filtered through a 0.45 μm membrane before analysis. Volatile fatty acids (VFAs) and alcohol were determined by a gas chromatograph (Agilent 6890, USA) equipped with a mass selective detector (220 °C), injector (200 °C) and a 30 m × 0.25 μm HP-FFAP fused-silica capillary column. The temperature of the oven was initially maintained at 60 °C for 4 min, increased to 168 °C at a ramp of 6 °C/ min and lastly heated to 200 °C at 10 °C/ min and kept for 2 min. Helium was used as the carrier gas. Glucose

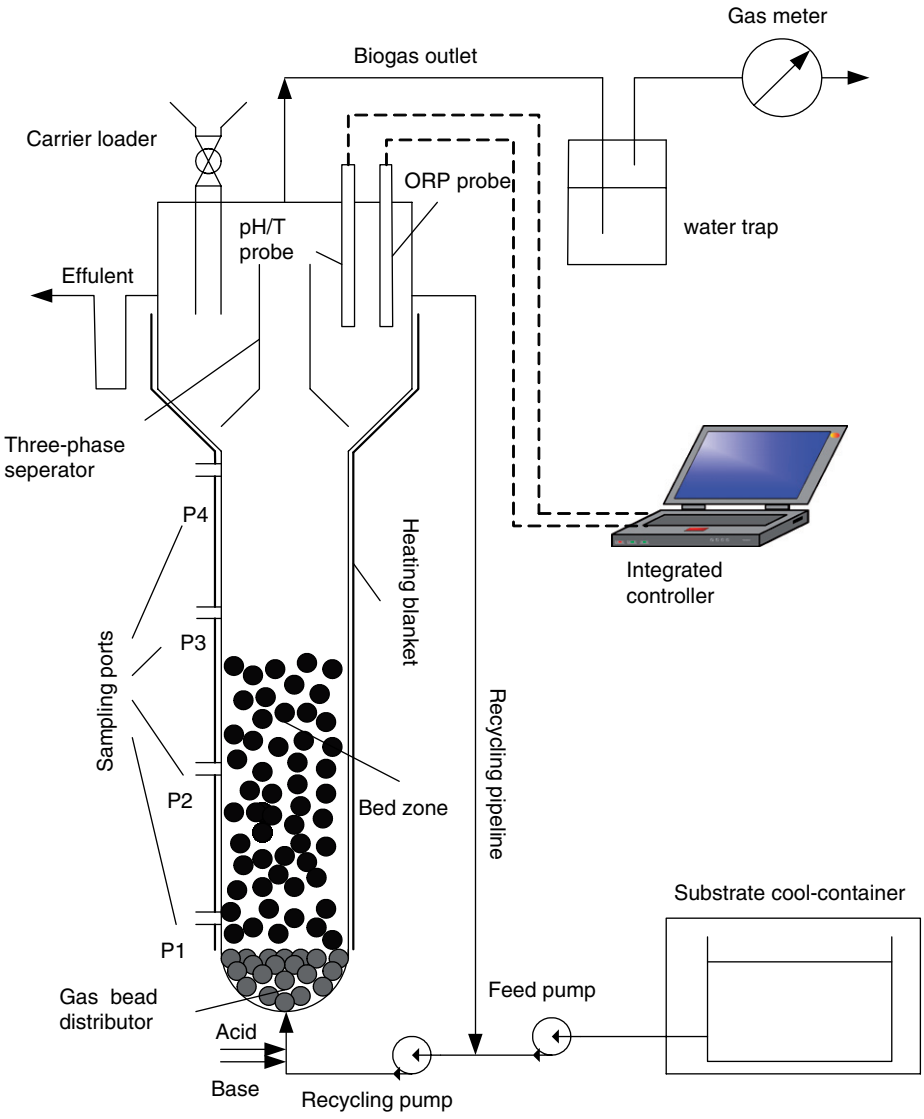

Fig. 1. Schematic diagram of the granular activated carbon anaerobic fluidized bed reactor system.

concentration was determined by the phenol-sulfuric acid method [17].

Attached biomass: All the bioparticle samples were taken from the Sampling port 2 (Fig. 1). A slurry sample of known volume was gently washed to remove the SSs and sonicated, and the detached biomass and clean support media were then collected separately. The weight ratio of attached volatile solids (AVS) and support medium was determined by measuring the mass of AVS and corresponding clean support medium. The AVS concentration was calculated as the total AVS over the reactor working volume. Measurements of biomass and support medium mass were performed according to the standard methods [18].

Microbial observation: Microbial observation was conducted by using a scanning electron microscope (SEM) (Stereosan 420; Leica Cambridge Instruments). The bioparticle samples were gently washed with phosphate buffer solution and allowed to settle naturally. The bioparticles were then fixed with 4% paraformaldehyde and left for 4 h. The fixed bioparticles were dehydrated by successive passages through 40%, 60%, 80% and 100% ethanol and then dried Critical Points Dryer (E3000) (VG Microtech, East Grinstead, West Sussex, UK) and finally observed by SEM. The same amount of clean GAC ($\sim$ 0.3 g) was supplemented after the bioparticle sampling.

3. Results and discussion

3.1. Effect of HRT

The effect of HRT on hydrogen production was evaluated as the reactor was operated at a constant pH of 4.0 and influent concentration of 10 g-glucose/L. Fig. 2 depicts steady-state results obtained at a varying HRT from 0.5 to 4 h. The biogas produced was composed of hydrogen, carbon dioxide, and free of methane, indicating the absence of methanogens in the system might be as a consequence of the lower pH

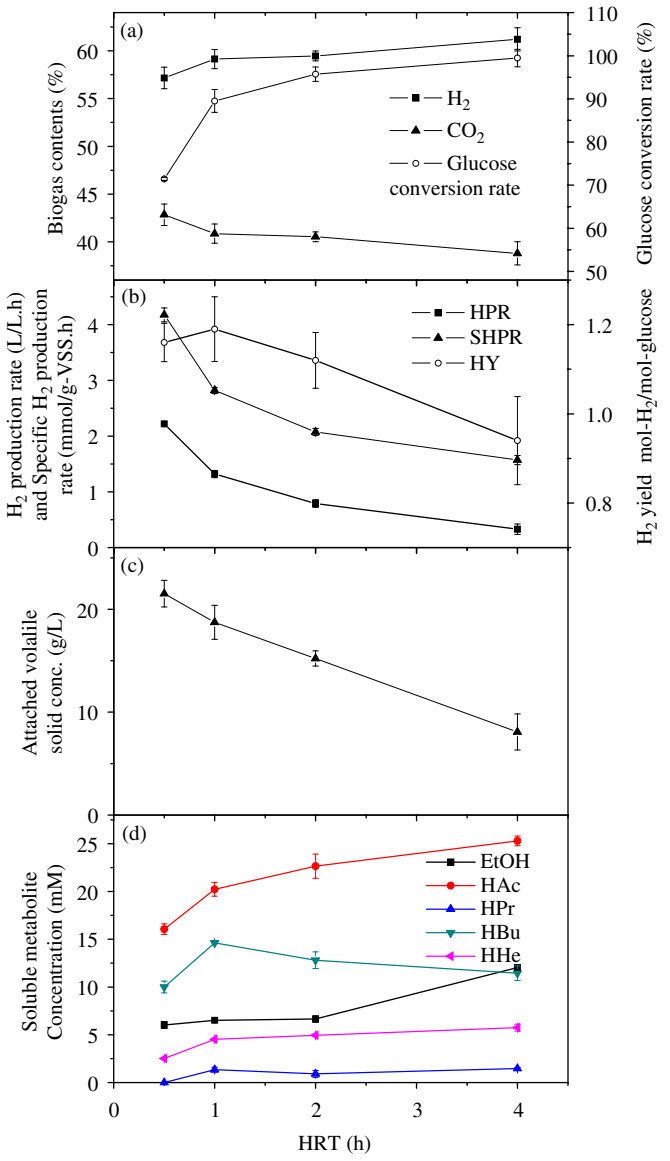

Fig. 2. Effect of HRT on the performance of AFBR with a feed strength of 10 g/L: (a) biogas contents and glucose conversion rate; (b) hydrogen production rate, specific hydrogen production and hydrogen yield; (c) attached volatile solid concentration; (d) soluble metabolites (EtOH = ethanol; HAc = acetate; HPr = propionate; HBu = butyrate; HHe = hexanoate).

operation. Hydrogen composition decreased slightly from 61.2% to 57.2% as the HRT was shortened. While a hydrogen yield of 0.94 mol-H_2/mol-glucose was found at 4 h HRT it stabilized at 1.12–1.19 (average of 1.16 ± 0.03) at the HRT of 0.5–2 h. This indicates the shift of metabolic flux might occur after transition from 4 to 2 h HRT where more substrate is shunted to reaction end products instead of bacterial growth or maintenance, leading to an increased hydrogen yield. Both HPR and SHPR increased significantly with the shortened HRT, giving the maximum at the shortest HRT of 0.5 h of 2.22 L/L h and 4.18 mmol-H_2/g-VSS h, respectively. However, the glucose conversion rate decreased apparently, from 99.47% at 4 h to 71.44% at 0.5 h HRT. The attached biomass was highly correlated to HRT. It increased from 8.1 g-VSS/L at 4 h to 21.5 g-VSS/L at 0.5 h.

Fig. 2(d) reveals the composition of dissolved metabolites associated with the HRT. In general, the products in descending order were acetate (43–46%), butyrate (20–31%), ethanol (14–21%), and hexanoate (7–10%), followed by an insignificant amount of propionate (0–3%). The metabolites decreased slightly as the HRT was shortened with the exception of butyrate which initially increased, but decreased with further decrease in HRT from 1 to 0.5 h. Notably, hexanoate was detected in a range of 2.51–5.74 mM, which was seldom observed in fermentative hydrogen production using acclimated anaerobic sludge, indicating that part of the glucose might be utilized in a metabolic pathway which is unclear at this stage of study. Also, an insignificant amount of propionate production, even absence at 0.5 h HRT suggests that the activity of propionate formers might be inhibited by the low pH and sensitive to a short HRT, which has been reported by other researchers [19–21]. This seems to provide evidence that the higher hydrogen composition achieved in the present study which is much higher than other studies [6,22] might result from less consumption of hydrogen in the formation of propionic acid [Eq. (1)]

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O. \quad (1)$$

It has been reported that besides HRT, the dissolved product compositions are influenced by the operation pH. Generally, contrary to the pH of 5.5–6.5, lower pH (4.0–5.0) favored the production of alcohols, predominating over VFAs. However, the finding of metabolite distribution is not in substantial agreement with the results of several studies conducted in close pH conditions [23–25]. Ren and his team [23] reported that acetate and ethanol were produced as the dominant metabolites equally, but less butyrate and propionate were produced when the reactor was operated at a pH of around 4.5; Kim and his co-worker [24] found that butyrate was dominated initially, but butanol became the major product, associated with the remarkable decrease in butyrate after a long term operation at a pH of 4.3. Yu and his group [25] found that propionate and ethanol accounted for 40% and 26% of acidification products in the effluent at pH 4.5. This lack of consistency indicates that other operation parameters, such as dominant microbial population and substrate influence the distribution in the metabolite compositions.

3.2. Effect of feed concentration

To evaluate the effect of feed concentration on hydrogen production, a test was conducted at 1 h HRT and varying influent glucose concentration, from 10 to 30 g/L. Constant hydrogen content which accounted for approximately 59.2% in the evolved biogas was observed throughout the test, indicating that biogas composition is independent on the feed strength (Fig. 3a). The hydrogen yield decreased slightly from 1.19 to 1.10 mol-H_2/mol-glucose as influent glucose concentration increased, with an average of 1.16 ± 0.05 mol-H_2/mol-glucose (Fig. 3b). This indicates that feed concentration has somewhat negative impact on the hydrogen yield, which is in

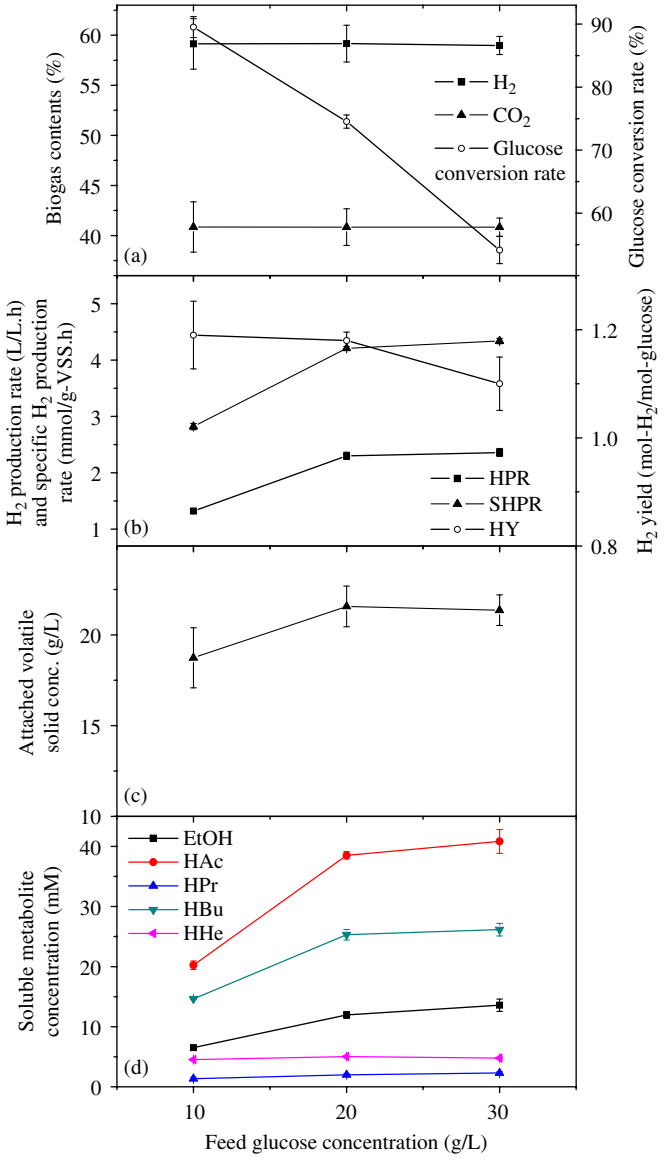

Fig. 3. Effect of feed concentration on the performance of AFBR at a HRT of 1 h: (a) biogas contents and glucose conversion rate; (b) hydrogen production rate, specific hydrogen production and hydrogen yield; (c) attached volatile solid concentration; (d) soluble metabolites (the symbols are the same as in Fig. 2).

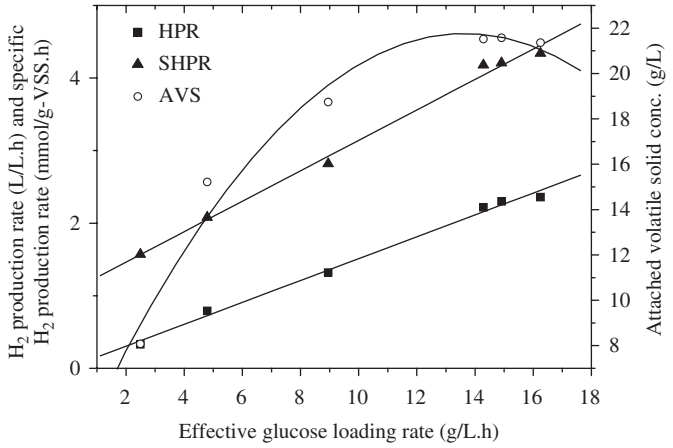

Fig. 4. Hydrogen production rate, specific hydrogen production rate and attached volatile solid concentration as a function of the effective organic loading rate.

accordance with the other studies [25,26]. While the HPR and SHPR increased with feed strength and reached the maxima of 2.36 L/L h and 4.34 mmol-H_2/g-VSS h, respectively, at the highest feed strength of 30 g/L they both had tapered off as the feed strength varied from 20 to 30 g/L. The glucose conversion rate decreased significantly with the feed concentration, and only 54.15% of glucose was utilized at 30 g/L as compared to 89.52% at 10 g/L, indicating that unutilized glucose was retained in the system as high as 13.8 g/L here. However, there was no substrate inhibition effect on hydrogen production observed throughout the test. It is likely that the compact biofilm structure enables microbial consortia to be free of the inhibition of high substrate concentration. Some researchers found that the inhibiting concentration of substrate on acidogenic bacteria was estimated to be about 5–6 g-glucose/L in the suspended cultures [27,28]. These results imply that the microorganisms in the biofilm matrix could react more actively to a stressful environment than those in the form of suspension. The attached biomass concentration increased slightly, from 18.7 g-VSS/L at 10 g-glucose/L to 21.6 g-VSS/L at 20 g/L, but had leveled off as the glucose concentration increased further. As for dissolved products, they consisted of acetate (43–47%), butyrate (30–31%), ethanol (14–15%), hexanoate (5–10%) and propionate (2–3%). Acetate, butyrate and ethanol initially increased but leveled off as the feed strength increased, while hexanoate and propionate did not vary much during the whole period.

The results shown in Figs. 2 and 3 indicate that the AVS concentration, HPR and SHPR seems to be influenced strongly by the OLR. To illustrate the relationship clearly, the HPR, SHPR, and AVS are plotted VS the effective OLR which is calculated on the basis of glucose conversion rate and OLR employed in Fig. 4. The statistical results imply that the HPR ($R=0.997$, $p<0.001$) and SHPR ($R=0.997$, $p<0.001$) were linearly correlated to the effective OLR. This finding is consistent with the previous studies investigating the influence of OLR on hydrogen production [26,29]. Also, the attached biomass ($R^2=0.973$, $p<0.001$) increased significantly, but not linearly with the effective OLR, indicating that the enhanced OLR could not always stimulate the increase in attached biomass concentration. In fact, besides OLR other factors such as hydraulic conditions, bioparticle collision as suggested by the previous studies [30,31] might regulate and affect the attached biomass quantity, although they are not discussed in the present study.

3.3. Evaluation of reactor performance

It has been generally believed that the hydrogen yield is highly related to the dominant microorganisms and environmental conditions in anaerobic hydrogen fermentation processes, but seems to be independent on the reactor configuration. For example, a comparable hydrogen yield of 1.6–2.1 mol-H_2/mol-glucose was achieved in CSTR [32],

up-flow anaerobic sludge blanket reactor [25] and fixed-bed reactor [6] using *Clostridium*-rich mixed cultures. Although Fig. 5 illustrates that rod-shaped bacteria are dominated on the biofilm which look like *Clostridium* species, the hydrogen yield of 1.19 mol-H_2/mol-glucose in the present study is much lower than the maximum reported (2.45 mol-H_2/mol-glucose) using *Clostridium*-rich mixed cultures [33]. It is likely that the low pH influences hydrogen conversion efficiency of hydrogen-producing bacteria. The previous studies indicates that hydrogen yield peaked at an optimum pH range of 5.2–5.7, but decreased significantly as the pH decreased to 4.7 [34,35]. These results may be explained by considering the activity of hydrogenase, an iron-containing enzyme of *Clostridium* sp. is inhibited by a low pH [36,37], although the activity of *Clostridium butyicum* could be noticed even at pH 4.0 [24]. Moreover, more substrate might be required to maintain bacterial growth in a stressful environment, which also results in a low hydrogen yield. An attempt had been made to operate AFBRs under the same conditions except higher pHs (data not shown). The results showed that significant increase in methane production was observed when the pH was higher than 4.0, indicating that the existing methanogens presumably survived after heat pretreatment or were contaminated by the external environments. The role-play of pH in hydrogen production by the GAC-AFBR system is needed to clarify in the further study.

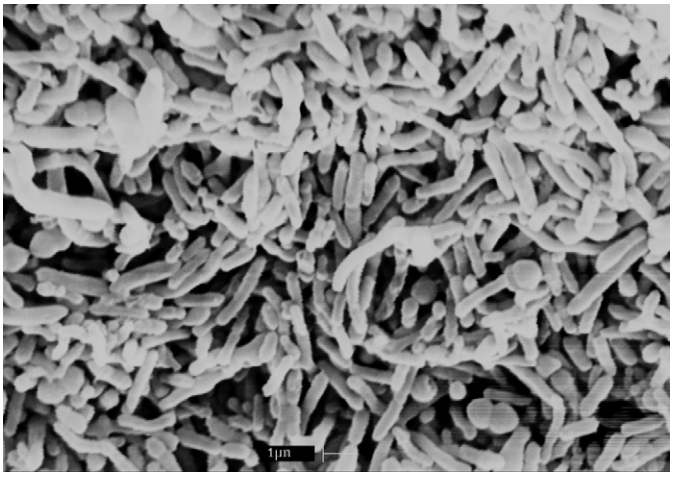

Fig. 5. Scanning electron microscopy of the attached bacteria in the GAC-AFBR (magnification: 5000×).

The production rate of hydrogen gas has been used to evaluate the process performance [38]. The results of the previous studies summarized in Table 1 clearly point out that the efficiency of hydrogen fermentation is influenced by the biomass level retained in the systems. Comparing with suspended-cell systems, the immobilized-cell systems achieved a biomass concentration of 15.8–44 g/L even at a short HRT (0.5–2 h), which essentially lead to an elevated production rate of hydrogen. In the present study, the maximal thickness of well-developed biofilm was estimated to be 40 μm. In general, less limitation of mass transfer encounters when the biofilm thickness is less than 80 μm [39]. This, along with the good fluidization characteristics indicates the GAC-AFBR system could function free of mass transfer resistance which is the fatal drawback of some other cell immobilization processes and techniques for biohydrogen production, e.g. fixed-bed reactor and agar gel entrapping technique. In fact, Kumar and Das [10] obtained a slight increase in the HPR in the rhomboid reactor using immobilized cell on lingocellulosic materials as compared to the tubular and tapered reactors due to the enhanced turbulence and reduction in gas hold-up. They also compared different techniques of cell immobilization and concluded that attaching methods give better performance in the HPR as compared to entrapping methods in the packed-bed reactors [10]. Therefore, up to 21.5 g-VSS/L attached biomass concentration and good mass transfer efficiency enable the present GAC-AFBR system to be superior in HPR to suspended-cell system [3,5,27], and even to other immobilized-cell systems including fixed-bed/packed-bed reactors [6,10,40], biofilter reactor [26], and fluidized bed reactor with entrapped-cell [9], with the exception of the carrier-induced granular sludge bed (CIGSB) process whose HPR reached 7.3 L//L h [8]. Another potential advantage of the present system is that low pH operation would contribute significantly to the cost reduction in the alkali amount required for pH control during the process of hydrogen fermentation.

4. Conclusions

Based on the experimental results obtained, it can be concluded that the formation of biofilm significantly increases

Table 1
Comparative study on the efficiency of hydrogen fermentation processes

Process	Bacterial growth mode/support medium	Optimal HRT (h)	HPR (L/L h)	Highest biomass conc. (g-VSS/L)	References
CSTR	Suspension/none	6	0.15	0.8	[34]
CSTR	Suspension/none	6	0.58	1.7	[22]
AFBR	Entrapment/alginate gel	2	0.93	—	[9]
Packed-bed	Flocculation/none	1.5	1.42	17	[40]
Packed-bed	Attachment/ligocelluosic agroresidues	1.08	1.85	44	[10]
Fixed bed	Attachment/activated carbon	1	1.32	15.8	[6]
CIGSB	Flocculation/none	0.5	7.33	26.1	[8]
Tricking biofilter	Attachment/fibrous polymeric material	4	1.07	24	[26]
GAC-AFBR	Attachment/GAC	1	2.36	21.5	This study

biomass level up to 21.5 g-VSS/L, leading to elevation of the production rate of hydrogen gas. The gaseous metabolites contained 57–61% H_2 and the rest was CO_2. Hydrogen composition increased slightly with HRT, but was independent on the influent concentration of glucose. The dissolved products were predominated by acetate and butyrate, with smaller quantities of ethanol, hexanoate and propionate. The HPR and SHPR were linearly correlated to the effective OLR, giving the respective maximum rates of 2.36 L/L h and 4.34 mmol-H_2/g-VSS h, whereas hydrogen yield stabilized at 1.16 mol-H_2/mol-glucose with an exception of 0.94 mol-H_2/mol-glucose at 4 h HRT. These results indicate that the GAC-AFBR system is superior in the production rate of hydrogen gas to suspended and most immobilized processes reported so far.

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