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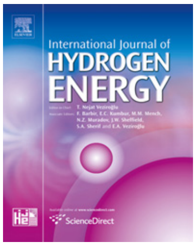

Biological hydrogen production in continuous stirred tank reactor systems with suspended and attached microbial growth

Nan-Qi Ren*, Jing Tang, Bing-Feng Liu, Wan-Qian Guo

State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology,
No.202 Haihe Road, Harbin 150090, China

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ABSTRACT

Fermentative H_2 production in continuous stirred tank reactor (CSTR) system with bacteria attached onto granular activated carbon (GAC) was designed to produce H_2 continuously. The H_2 production performances of CSTR with suspended and attached-sludge from molasses were examined and compared at various organic loading rates (8–40 g COD/L/d) at hydraulic retention time of 6 h under mesophilic conditions (35 °C). Both reactor systems achieved ethanol-type fermentation in the pH ranges 4.5–4.8 and 3.8–4.4, respectively, while ORP ranges from −450 to −470 mV and from −330 to −350 mV, respectively. The hydrogen production rate in the attached system was higher compared to that of the suspended system (9.72 and 6.65 L/d/L, respectively) while specific hydrogen production rate of 5.13 L/g VSS/d was higher in the suspended system. The attached-sludge CSTR is more stable than the suspended-sludge CSTR with regard to hydrogen production, pH, substrate utilization efficiency and metabolic products (e.g., volatile fatty acids and ethanol) during the whole test.

1. Introduction

Human beings are eager for sustainable and alternative energy since global environmental problems and energy crisis are becoming more and more severe nowadays. Hydrogen is an ideal choice because it is clean, recyclable, possessing a high-energy yield (122 kJ/g) and most important; hydrogen production via biological means has the potential to eliminate environmental deterioration that is derived from the utilization of conventional fossil fuels [1]. Biological hydrogen production falls into two main categories: photosynthetic and fermentative ones. In contrast to photosynthetic hydrogen production process, fermentative process has the advantages of high efficiency of hydrogen evolution, light-independency, control simplicity, low production cost, and high feasibility of industrialization [2,3]. Moreover, the fermentative hydrogen production can simultaneously utilize organic wastes and produce other useful by-products such as organic acids and alcohols [4].

Continuous stirred tank reactor (CSTR) has been the most frequently used configuration in continuous hydrogen production researches [5–9]. In such a reactor, hydrogen-producing bacteria are suspended in the well-mixed liquor and suffer less from the mass-transfer resistance, but cells are washed-out at a low hydraulic retention time.

Thus, a variety of high-rate bioreactor systems, including fixed-bed reactor [10], packed bed reactor [11,12], granular-sludge

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* *Corresponding author*. Tel.: +86 451 86282008.
E-mail address: rnq@hit.edu.cn (N.-Q. Ren).

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bed reactor (e.g., CIGSB) [13–16], up-flow anaerobic sludge blanket reactor (UASB) [17,18] and fluidized bed reactor (e.g., DTFBR) [19–22], were developed for hydrogen production to enhance biomass retention by using immobilized-cell systems via surface attachment, self-flocculation, and gel entrapment approaches, among which the surface attachment approach was the one most frequently used. In general, attached growth systems, although not suitable for high solids concentration in the influent, allow for better retention of active microbial mass, lower hydraulic retention times and in many cases suffer from lower product inhibition than the suspended growth systems [23].

In foregoing bioreactors, marked decrease of mass-transfer efficiency was present after a term operation due to the agglomeration of massive number of cells and lack of efficient mixing. So there is no enough shear force to assist separation of the biogas attached to the sludge particles. These bubbles would make the particles float and cover the liquid surface on top of the bioreactor, resulting in inefficient and unstable hydrogen fermentation and even a reactor shut-down [21]. The approaches to improve mass-transfer efficiency via recycle of liquid and biogas were universally adopted [15]. Also, the height to diameter ratio of the column-shaped configurations was adjusted to elevate the liquid up-flow velocity [16].

However, systematic studies regarding process comparison between attached growth systems and conventional suspended growth systems under identical operating conditions have been scarcely reported. An efficient and stable performance of hydrogen fermentation in the CSTR system with bacteria attached onto granular activated carbon (GAC) was developed in this study. Direct comparison of the attached-sludge CSTR with conventional suspended-sludge CSTR for H_2 production was assessed by varying the organic loading rate (OLR) range from 8 to 40 g COD/L/d and varying pH at consistent OLRs of 32 and 40 g COD/L/d, which would probably provide basic knowledge for the choice of bioreactor in practical fermentative hydrogen production.

2. Materials and methods

2.1. Sludge inoculation and setup

Experiments were performed in two identical CSTR systems as shown in Fig. 1. Each reactor had a working volume of 5.2 L and was operated at a consistent temperature of 35 ± 1 °C. A gas–liquid–solid three-phase separator was installed inside the CSTR to promote retention of sludge. The reactor was sealed to assure anaerobic condition.

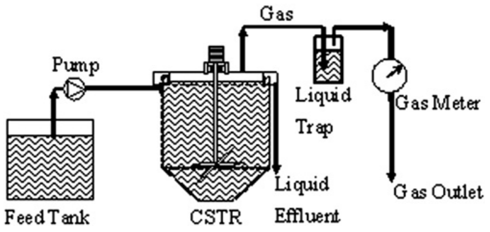

Fig. 1 – Schematic of continuous stirred tank reactor system.

Normal molasses, the substrate used for hydrogen fermentation containing about 53% sugars, were diluted with water to a certain concentration (2000–10 000 mg COD/L). The molasses used throughout the study were collected from a local sugar refining industry, whose characteristics had been described in a previous study [5]. The COD:N:P of the influent was maintained at a ratio of 1000:5:1 to ensure the growth demand of microorganisms.

The seed sludge was collected from sewage sludge and screened to eliminate large particulate materials. After 2 weeks of aerobic cultivation using molasses as the substrate in a batch reactor, acclimated seed sludge was inoculated in CSTRs, one of which was also added granular activated carbon (GAC) with an average diameter of 1 mm at a volume (mL) to weight (g) ratio of 10:1 as the support medium for biofilm attachment. After 24 h of aerobic cultivation, biofilm formed outside and inside the GAC. Then continuous flow operation of two bio-hydrogen production reactors commenced. The initial biomass and OLR were 7.3 g/L and 12 g COD/L/d, respectively.

2.2. Analytical methods

Volatile fatty acids (VFAs) and ethanol in the effluent were quantified with a gas chromatography (GC-122, Shanghai Analytical Apparatus Corporation, China) equipped with a flame ionization detector (FID) and a 2.0-m stainless (5.0 mm inside diameter) column, packed with Porapak GDX-103 on 60/80 mesh, which was operated isothermally at 190 °C. The injector and detector were both operated at 220 °C. Nitrogen was used as a carrier gas at a flow rate of 30 mL/min. Biogas composition was analyzed on an SP-2305 gas chromatograph (GC) column with a thermal conductivity detector and a 2.0 m stainless column filled with Porapak Q (50/80 mesh). Nitrogen was used as a carrier gas at a flow rate of 40 mL/min.

The procedures described in Standard Methods of Ref. [24] were used to determine chemical oxygen demand (COD), total suspended solid (TSS) and volatile suspended solid (VSS). Oxidation reduction potential (ORP) and pH were measured by a pHS-25 acidity voltmeter. Biogas production was measured using a wet-gas meter calibrated to a temperature of 25 °C and pressure of 1 atm condition.

For comparison purposes, CSTRs with suspended and attached microbial growth were operated in parallel. Steady-state conditions were considered to be attained when the biogas production and the proportion of soluble fermentative products were consistent within 5% for three consecutive days. Data were collected for 24 HRTs after the steady-state conditions were reached.

3. Results and discussion

3.1. H_2 production in start-up process

A high–low OLR alternation was taken as the start-up strategy of two microbial growth systems for quick and effective establishment of highly efficient hydrogen producing population and ethanol-type fermentation. In the present study, both CSTRs were fed continuously with diluted molasses at a strength of approximately 4000 mg COD/L to reach an OLR of 16 g COD/L/d with an HRT of 6 h in the first 24 HRT cycle, then the OLR was adjusted to 8 g COD/L/d by reducing the substrate concentration to 2000 mg COD/L in the second 24 HRT cycle, finally the OLR was restored to 24 g COD/L/d in the third 24 HRT cycle. In order to compare start-up conditions of attached and suspended-sludge CSTRs, bio-hydrogen production rate, composition of biogas and soluble metabolites, ORP and substrate utilization efficiency were monitored during the course of continuous hydrogen fermentation. The data for attached and suspended-sludge CSTRs are presented in Figs. 2 and 3, respectively.

The removal rate of COD in attached-sludge CSTR reached as high as 66% at the first day, because most of the organic materials were absorbed by activated carbon. After the carrier was saturated, the removal rate of COD was about 15–17%, which was similar to that of the suspended. Figs. 2e and 3e show that ORP in attached and suspended-sludge reactors ascended to −330 and −390 mV, respectively, in the first 24 HRT cycle of 16 g COD/L/d, and sharply declined to −410 and −460 mV when OLR was shifted down to 8 g COD/L/d, then as the OLR increased, ORP in attached-sludge reactor went back to −340 mV while that of suspended was retained at around −460 mV. It is implied that the activated carbon is a protector of bacteria, building a niche safer than that of the suspended.

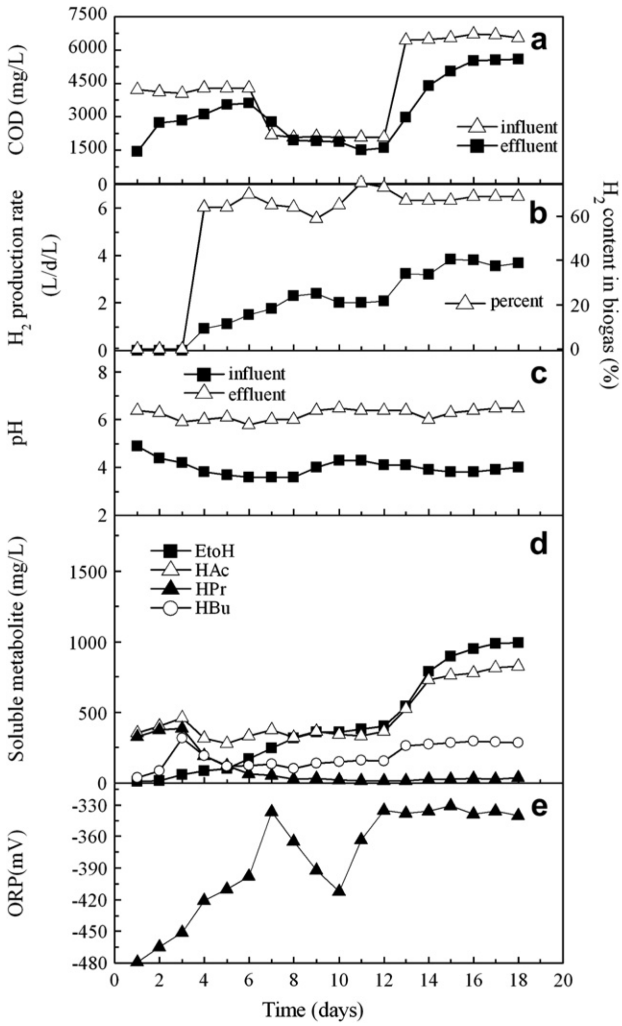

Fig. 2 – Performance of CSTR with attached microbial growth during start-up stage.

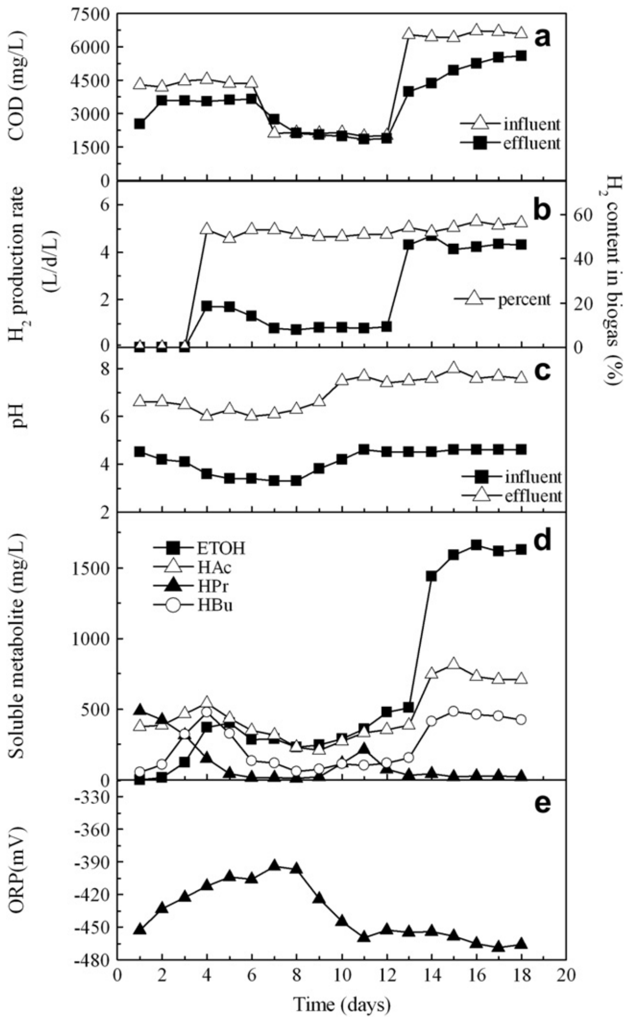

Fig. 3 – Performance of CSTR with suspended microbial growth during start-up stage.

In both the cases, the biogas came into generation after 3 days of anaerobic cultivation and primarily consisted of CO_2 and H_2, while CH_4 was undetectable. During the first 24 HRT cycle, the pH in two reactors dropped dramatically below 4.0, then in the second 24 HRT cycle, sodium bicarbonate was added to maintain a pH range of 4.2–4.6 in the suspended-sludge reactor, while the attached-sludge bioreactor was stable at pH 3.8–4.4 spontaneously, which maybe the reason due to which hydrogen content in biogas was higher in attached reactor (63–70%) than that of suspended reactor (50–57%).

Typical soluble metabolites included ethanol (EtOH), acetate (HAc), propionate (HPr), and butyrate (HBu). The changes in soluble metabolites in two bioreactors during the start-up period were almost similar in trend (Figs. 2d and 3d). In the first 3 days, propionate and acetate were the dominant soluble metabolites while no hydrogen was produced. From day 4, propionate decreased gradually, and ethanol and butyrate increased slowly while remarkable hydrogen was produced. Along with the increase of OLR from 8 to 24 g COD/L/d, the concentrations of ethanol and acetate dramatically increased (accounting for 80–85% of the total soluble microbial products) and ethanol-type fermentation products formed one day later. It is deduced that fluctuations between certain high and low OLRs induced the metabolic shift of biochemical reactions in H_2-producing bacteria, which resulted in a substantial increase of hydrogen production rate [6]. Ethanol concentrations of attached and suspended-sludge bioreactors increased from 404.2 to 1095.7 mg/L and from 476.9 to 1723.8 mg/L, accounting for 63.1% and 72.3%, respectively. Meanwhile, the hydrogen production rates in the two bioreactors increased from 2.08 to 3.85 L/d/L and from 1.69 to 4.32 L/d/L (Figs. 2b and 3b), respectively. The above results show that ethanol-type fermentation [8,25–27] was established in both H_2 production systems.

Steady hydrogen productions of both reactors were achieved within 18 days by using high–low OLR alternation strategy. For CSTR system with suspended H_2-producing sludge in other literature, nearly 43 days were required for the start-up to reach a constant effluent quality and biomass concentration [28]. The results seem to indicate that the start-up strategy employed was effective in eliminating the competition of non-H_2 producer for the substrate and allowed rapid establishment of efficient H_2-producing population, in consequence, conducive to quick and effective start-up and formation of ethanol-type fermentation of both bio-hydrogen production systems.

3.2. *Effect of OLR and pH on hydrogen production*

The differences in hydrogen production rate were attributed to the difference in the microbial population and the operating conditions, such as OLR and pH control. Figs. 4 and 5 illustrate the impacts of OLR and pH on the hydrogen production in attached and suspended-sludge reactors, respectively.

One of the factors that affects the metabolic pathways and consequently the H_2-producing efficiency in dark fermentative H_2 production is pH [29]. Overall assessment of the data in Figs. 4 and 5 shows that pH 4.0 was optimal for the attached-sludge reactor while pH 4.5 was favorable for the suspended-sludge reactor. Substantial suppression in hydrogen production rate was noted as the pH fell beyond the optimum range. The highest hydrogen production rate obtained for CSTR with attached-sludge was 9.72 L/d/L, which occurred at pH 4.0 when the reactor was operated at an OLR of 40 g COD/L/d, and 6.65 L/d/L at pH 4.5 under OLR of 32 g COD/L/d for the CSTR with

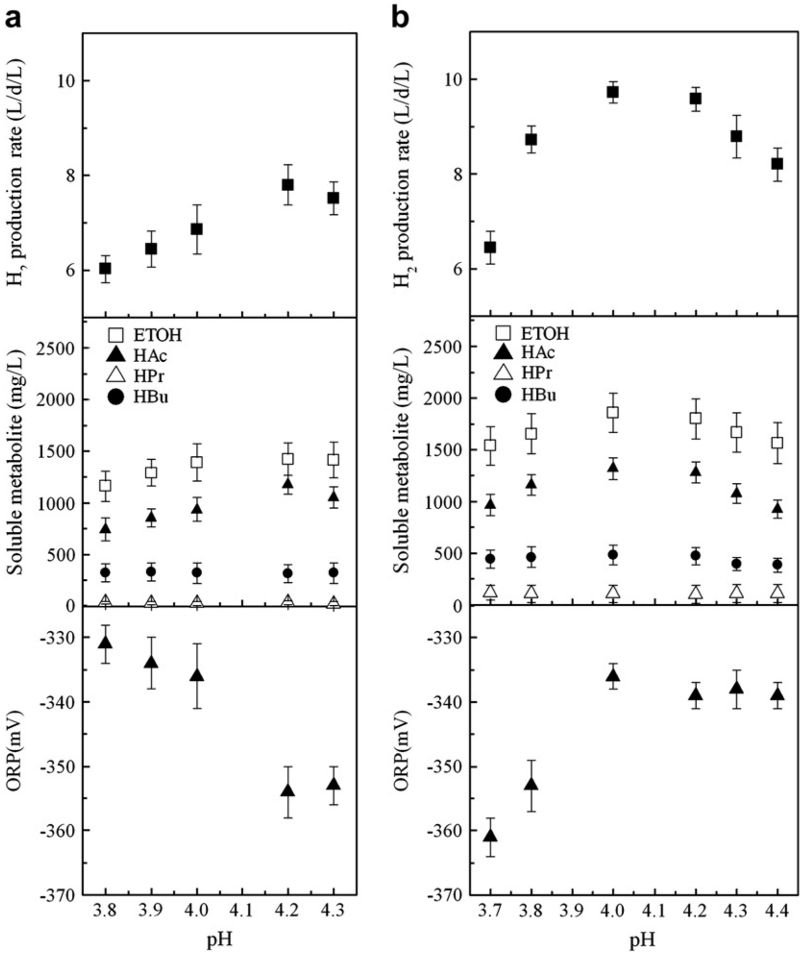

Fig. 4 – Effect of pH on hydrogen fermentation in CSTR with attached microbial growth. (a) OLR = 32 g COD/L/d, (b) OLR = 40 g COD/L/d. Five data obtained at steady-state were used for the determination of the mean values and standard deviations.

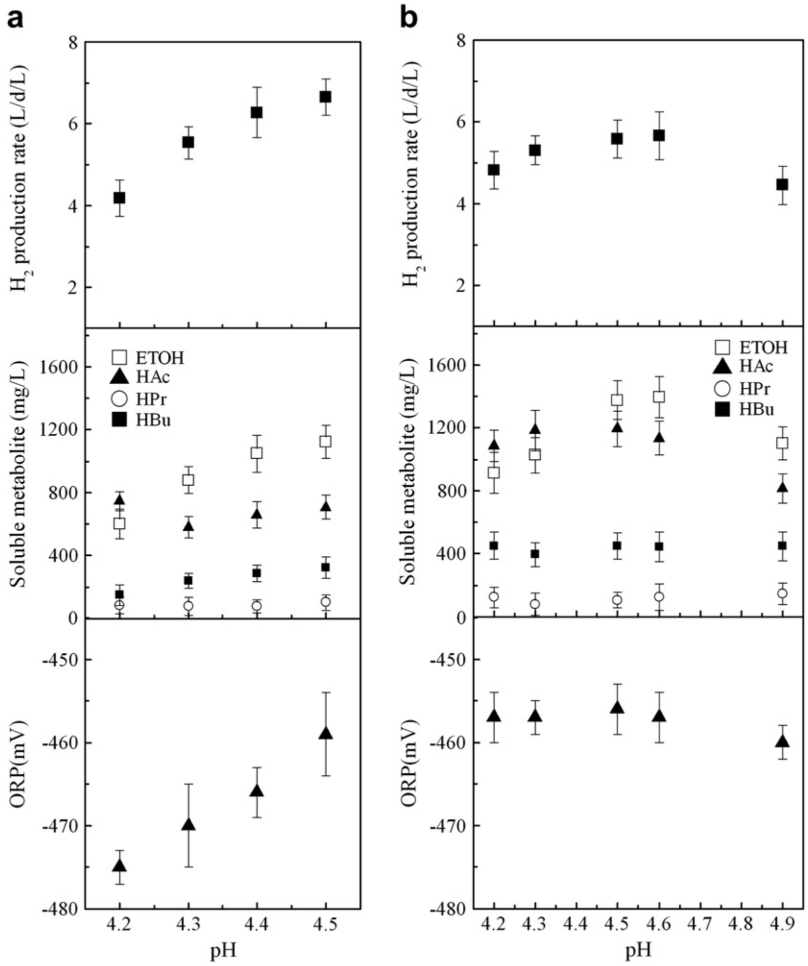

Fig. 5 – Effect of pH on hydrogen fermentation in CSTR with suspended microbial growth. (a) OLR = 32 g COD/L/d, (b) OLR = 40 g COD/L/d. Five data obtained at steady-state were used for the determination of the mean values and standard deviations.

suspended-sludge. Thus, the optimal pH for H_2 production in attached-sludge CSTR seems to be more acidic than the optimal pH for H_2 production in suspended-sludge CSTR.

The composition of soluble metabolites in suspended-sludge reactor was found to be more sensitive to the changes in pH than that of attached-sludge reactor. For attached growth system, the metabolite composition was quite similar for all pHs and OLRs tested, except that relatively more soluble metabolites were produced in high OLR (40 g COD/L/d). As shown in Fig. 4, the predominant soluble metabolite was ethanol (accounting for 52% of the total soluble metabolites), followed by acetate (accounting for 31% of the total soluble metabolites). For suspended growth system, ethanol and acetate were also predominant soluble metabolites. The concentration of ethanol was lower than acetate at $\text{pH} < 4.3$, while in the conditions of pH above 4.3, ethanol was produced more than acetate, as shown in Fig. 5.

H_2 may be directly utilized for the formation of HPr, so HPr is considered an unfavorable metabolite for H_2 production [1]. It was found that relatively more HPr was produced in activated carbon-supported fixed-bed reactor compared to that of the suspended-sludge system while the bacteria carried out butyrate-type fermentation [10]. In the present study, HPr was relatively insignificant (less than 10% of the total soluble metabolites) all through the operation after start-up while ethanol-type fermentation was carried out in both attached and suspended CSTRs, suggesting that the metabolic pathway was fit for bio-hydrogen production, especially attached microbial growth system.

pH is also an important parameter in the control of methanogenic activity [30]. The biogas produced consisted of H_2 and CO_2 and was free of CH_4 during the experimental period. It is important to note that the naturally lower pH contributes to the inhibition of methanogenic activity.

OLR is an important parameter dictating the system performance and operational cost of fermentative H_2 production [6]. In the suspended CSTR, H_2 production rate increased slightly from 4.18 to 6.65 L/d/L at an OLR = 32 g COD/L/d as the pH increased from 4.2 to 4.5. The total quantity of soluble metabolites and ORP increased considerably as the pH increased. When operated at an OLR of 40 g COD/L/d, H_2 production rate improved from 4.82 to 5.67 L/d/L as pH changed from 4.2 to 4.6, then decreased to 4.45 L/d/L as pH further altered to 4.9. ORP was maintained stable in all pHs tested. However, ORP of attached CSTR was fluctuant with the pH and exhibited opposite trends at an OLR of 32 and 40 g COD/L/d.

Operating at an OLR of 40 g COD/L/d (Figs. 4b and 5b), the attached system produced a significantly higher amount of soluble metabolites and gas products than the suspended, which is opposite to the situation occurring at a lower OLR of 24 g COD/L/d (Figs. 2 and 3). It suggested that the attached system suffered from mass-transfer resistance at a low substrate concentration, as the increase of OLR, substrate concentration grads around the carriers increased, breaking through the block in mass transfer ultimately. On the other hand, effect of products inhibition on the suspended system emerged as the OLR increased. According to self-adjusting mechanism of H_2-producing bacteria, excessive fermentative products will terminate the process of H_2 production. For the attached system, H_2-producing bacteria are protected by GAC carriers, enduring much higher concentration of end products. The biomass concentration reached 15.5 g VSS/L in attached-sludge CSTR, which is much higher than that obtained in suspended-sludge CSTR (6.48 g VSS/L). The ability to maintain high biomass concentration highlights the key factor for the remarkable hydrogen production efficiency of the attached system, though the maximum specific hydrogen production rate of 5.13 L/g VSS/d was obtained in suspended-sludge CSTR.

4. Conclusions

The study demonstrated a novel and feasible attached-sludge CSTR configuration for the fermentative H_2 production from molasses. The start-up strategy of high–low OLR alternation was shown to be effective in accelerating the process of start-up and establishment of ethanol-type fermentation of both attached and suspended CSTRs. Also H_2 production performance of the attached and suspended-sludge CSTRs at various OLRs and pHs was studied and compared. Optimal operational parameters of attached and suspended-sludge CSTRs were as follow: pH around 4.0 and 4.5, ORP −330 to −350 mV and −450 to −470 mV, OLR 40 and 32 g COD/L/d, respectively. The highest hydrogen production rate of attached-sludge CSTR was 9.72 L/d/L obtained at an OLR of 40 g COD/L/d and pH 4.0, while the highest hydrogen production rate of suspended-sludge CSTR was 6.65 L/d/L obtained at an OLR of 32 g COD/L/d and pH 4.5. The attached system is noticeable for the stabilization regarding hydrogen production, pH, substrate utilization efficiency and metabolic products (e.g., volatile fatty acids and ethanol), and endurance of high concentration metabolites at high OLR.

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