Desalination and Water Treatment
www.deswater.com

doi:10.5004/dwt.2020.25203

180 (2020) 201–210
March

Comparison of fermentative hydrogen production from molasses in two continuous stirred tank reactors: CSTR versus ACR

Qing Fu[a], Sheng Chang[a,*], Yue-Ting Fan[a], Jian-Zheng Li[b]

[a]*State Environmental Protection Key Laboratory of Source Water Protection, Chinese Research Academy of Environmental Sciences, Beijing 100012, China, emails: changsheng83@163.com/changsheng@craes.org.cn (S. Chang)*
[b]*State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China*

Received 27 July 2018; Accepted 24 November 2019

ABSTRACT

The performance of fermentative hydrogen production bioreactors at different organic load rates (OLRs) in both a continuous stirred tank reactor with an inner gas–liquid–solid three-phase separator installed (ICSTR) and anaerobic contact reactor (ACR) was investigated. Five OLRs were examined, ranging from 12 to 36 g COD/L-d, with influent diluted molasses concentrations ranging from 4,000 to 8,000 mg COD/L and hydraulic retention time (HRT) ranging from 8 to 4 h. For the ICSTR, when the OLR was below 24 g COD/L-d, the hydrogen yield maintained at about 1.74 mol/mol glucose-converted, while the system failed to operate when the chemical oxygen demand (COD) was increased to 8,000 mg/L or the HRT was shorted to 4 h, this is because of *t* the low buffer ability and biomass washing out. However, for the ACR system, hydrogen production rate (HPR) and hydrogen yield increased linearly along with the increasing of OLR due to the elevating enhancement of biomass. The HPR and hydrogen yield peaked at 3.77 L/L-d and 1.83 mol/mol glucose-converted under the OLR of 36 g COD/L-d, respectively. The results showed that ethanol and acetate were predominated soluble products. The molar hydrogen yield was correlated linearly with the ethanol-acetate ratios, indicating hydrogen consumption by homoacetogenic bacteria, which significantly affected the hydrogen production.

Keywords: Fermentative hydrogen production; Continuous stirred tank reactor; Anaerobic contact reactor; Organic loading rate; Hydrogen yield

1. Introduction

The international community has become increasingly interested in the biological production of hydrogen gas over the last three decades. Among different biological processes for hydrogen production, dark fermentation is the most commercially feasible H_2 biological production method because of its potential for direct use of wastewater streams, and organic wastes and its high production rate [1–3]. Research on dark fermentative H_2 production has been conducted in a variety of reactor systems. The continuously stirred tank reactor (CSTR), the most common operational mode, has been widely studied to continuously produce H_2 from carbohydrates using mixed cultures under mesophilic conditions [4–9]. In general, mixing in the reactor can improve the mass transfer between the substrate and micro-organisms. However, a typical suspended-cell system of CSTR usually exhibits poor performance in hydrogen production rate (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 [7,9]. To enhance the HPR, immobilization processes of hydrogen-producing culture are most popular and have been developed extensively, due to the elevated biomass retention as compared to suspended-cell systems [10,11]. At present, a variety of high-rate bioreactor systems, including a fixed-bed reactor [12], packed bed reactor [13,14], fluidized bed reactor [15] and up-flow anaerobic

* Corresponding author.

sludge blanket reactor [11], were developed for hydrogen production. Although many studies have reported that relatively high unit volumetric production rates were found in these process systems, there have some limitations. Firstly, the HPR decreases due to the development of methanogenic biofilms on the carrier media, which could adversely affect process stability that is critical for sustained hydrogen production [16]. Secondly, some of the processes easily exit mass transfer resistance. Due to lack of shear force to assist the separation of the biogas and sludge particles. Bubbles would make the particles float and cover the liquid surface on top of the bioreactor, resulting in inefficient, unstable hydrogen fermentation or even a reactor shutdown [17]. Finally, the formation of granule sludge needs a long period to start-up the granular-sludge bed reactor, which generally requires a few months for the development of hydrogen-producing granules [11,18–20].

On the other hand, the approaches for overcoming the problem of cell washing-out from CSTR via the recycling of the H_2-production bacteria in the effluent were universally adopted [16]. In Harbin Institute of Technology (HIT), a CSTR had an internal gas–liquid–solid three-phase separator (ICSTR) and was successfully developed for high-rate hydrogen production by a biohydrogen research team [5,21]. However, hydrogen production efficiency did not improve when the HRT was shortened to 4 h due to bacteria washing-out [5]. Therefore, to retain sufficient H_2-producing bacterial population in the reactor, another novel CSTR configuration, the anaerobic contact reactor (ACR) was invented by authors for hydrogen production at HIT in 2007 [22]. The usage of this process for H_2 production was reported by Hafez et al. [23] in the past few years. In their studies, they used glucose and corn-syrup as substrates for H_2 production with butyrate-type fermentation. In addition, they compared the performance of hydrogen production with conventional CSTR at two different start-up organic load rates (OLRs). However, the direct comparison of the ACR with the internal gas–liquid–solid three-phase separator installed (ICSTR) for hydrogen production is not yet available in current literature. In this present study, the effect of ICSTR and ACR configuration on the performance of H_2 production from molasses was assessed by varying the influent chemical oxygen demand (COD) concentration range from 2 to 8 g COD/L/d and varying HRT range from 8 to 4 h. The characteristics of biohydrogen production from ethanol-type fermentation of molasses were evaluated.

2. Materials and methods

2.1. Bioreactors configuration

Experiments were performed in two systems as shown in Fig. 1. For the ICSTR, the total volume of ICSTR is 26 L and it had a working volume of 12 L (Fig. 1a). A gas–liquid–solid three-phase separator was installed inside it to promote retention of sludge, which is an integrated structure of

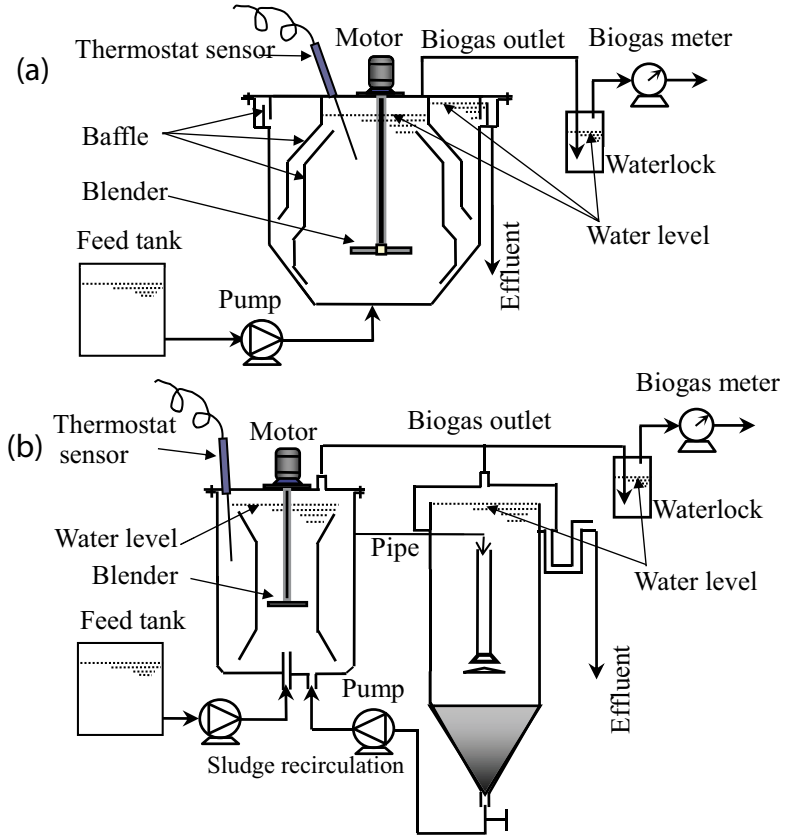

Fig. 1. Schematic diagram of the continuous reactor systems for hydrogen production (a) CSTR with inner three-phase separator and (b) anaerobic contact reactor.

reaction zone and sedimentation zone. The mixture was separated in the sedimentation zone. After the sludge was precipitated between the reflux seams, the sludge entered the reaction zone again and participated in the reaction through the stirring of the motor. For the ACR, it was comprised of a CSTR (12 L working volume), and followed by a sedimentation tank (volume 14 L) (Fig. 1b). The sludge of the sludge bucket was returned from the peristaltic pump to the reaction unit through the sludge recirculation. The wastewater was pumped into the bottom of the system reaction unit by the metering pump. After the reaction, the mixed liquid was separated from the reaction goes into the gas–liquid–solid three-phase separation and through the connecting pipe between the two main units of the reactor. Effluent left the overflow Weir in the upper part of the gas–liquid–solid three-phase separation unit and went through the *u*-tube.

Two reactors were wrapped in electrothermal wire to keep a consistent operating temperature of 35°C ± 1°C, and the paddle agitator of both reactors were run at 160–180 rpm. The influent flow rate was controlled by a feed pump to regulate the HRT and organic loading rate in the reactor. The evolved biogas was collected and led into a waterlock. The biogas volumes were measured using a wet gas meter (Model LML-1, Changchun Filter Co. Ltd.). The waterlock and wet gas meter were filled with acidified saturated salt solution to prevent the biogas from dissolution.

2.2. Feeding solution and seed sludge

Molasses in this study were collected from a local beet sugar refinery, and their characteristics have been reported previously [24]. Molasses were diluted with tap water to a certain concentration (2,000–8,000 mg COD/L) and the COD:nitrogen:phosphorus ratio was maintained at 500–1,000:5:1 by the addition of synthetic fertilizer to ensure the growing demand of microorganisms.

The reactors were inoculated with the same microbial inoculum. The seed sludge was obtained from a secondary settling tank in a local municipal wastewater treatment plant. It was first sieved through a mesh with a diameter of 0.5 mm to remove waste materials that could cause pump failure. The sludge was allowed to settle at room temperature for 8 h and was then transferred into the reactors. At the beginning of the start-up period, the biomass in the reactor were all approximately 6.5 g/L in terms of mixed liquid volatile suspended solids.

2.3. Startup and reactor operation

The two reactors were seeded with 6 L of sludge and started up in a continuous mode at an OLR of 8 L COD/L-d, with initial influent COD of 2,000 mg/L. OLR was increased step by step, by increasing the COD concentration from 2,000 to 8,000 mg/L or shortening HRT from 8 to 4 h. The reactor was run for at least 24 cycles of corresponding HRT to ensure that the steady state was reached. Steady-state conditions were based on constant products and biomass concentration with a variation of <10%. It must be emphasized that there was no sludge wastage from the reactors throughout the operation, and the values of sludge retention times (SRTs) presented in Tables 1 and 2 represent the average ± standard deviation (SD) during steady-state operation. It is noteworthy that the reactor operation was consistent over time and accordingly, the average SRT with SD of less than 10% of the mean SRT is representative of the overall SRT during the run. As expected the effluent volatile suspended solid (VSS) was substantially lower than the reactor VSS and remained unchanged during the steady-state operation. During the whole process, no alkali was added to control the pH value. The detailed operation strategy for the two reactors is shown in Tables 1 and 2, respectively.

2.4. Analytical methods

The biogas was measured daily using a wet-gas meter at a standard temperature (0°C) and pressure (760 mm Hg). The COD, total suspended solids and VSS were determined according to the procedures described in Standard Methods [25]. Oxidation–reduction potential (ORP) and pH were measured by a pHS-25 acidity voltmeter. Gas component was measured using a gastight syringe (0.1 mL injection volume) and a gas chromatograph (Model SC-7, Shandong Lunan Instrument Factory, China) equipped with a thermal conductivity detector with nitrogen as the carrier gas. The determinations of volatile fatty acid (VFA) and ethanol were analyzed by another gas chromatography (Model GC-112, Shanghai Analytical Apparatus Corporation, China) with a hydrogen flame ionization detector. The detailed analysis procedures were the same as described in our previous study [24]. The total sugar was measured by the phenol-sulfuric acid method using glucose as standard [26]. The HPR

Table 1
Operational conditions of the ICSTR

Phase	Time (d)	HRT (h)	SRT (d)	COD (mg/L)	OLR (g COD/L-d)
Start-up	1–8	8	–	2,000	6
1	9–20	8	47 ± 2.0	4,000	12
2	21–34	8	40 ± 1.5	6,000	18
3	35–38	8	–	8,000	24
4	39–55	6	29 ± 0.5	6,000	24
5	56–66	4	–	6,000	36

Note: values represent average ± standard deviation.

Table 2
Operational conditions of the ACR

Phase	Time (d)	HRT (h)	SRT (d)	COD (mg/L)	OLR (g COD/L-d)
Start-up	1–8	8	–	2,000	6
1	9–21	8	83 ± 3.6	4,000	12
2	22–34	8	79 ± 3.2	6,000	18
3	35–45	8	73 ± 3.1	8,000	24
4	46–59	6	47 ± 2.2	6,000	24
5	60–74	4	30 ± 0.6	6,000	36

Note: values represent average ± standard deviation.

calculation method is the volume of hydrogen produced per day per cubic meter of reactor.

3. Results and discussion

3.1. Performance of H_2 production in the ICSTR

Fig. 2 shows the ICSTR throughout 66 d of operation, and the variation of the characteristic parameters of the reactor. Figs. 2a and b show the diurnal variation of HPR and hydrogen content for the ICSTR. After 8 d of operation at the OLR of 6 g COD/L-d, the HPR reached 0.62 L/L-d, the HRT was kept constant at 8 h, while the influent COD was increased to 8,000 mg/L step by step with an increment of 2,000 mg/L, which was aimed to evaluate the effect of substrate concentration on the hydrogen produced during the initial 3 phases (9–37 d). It can be seen in Fig. 2a that the HPR was increased proportionately to the increase of

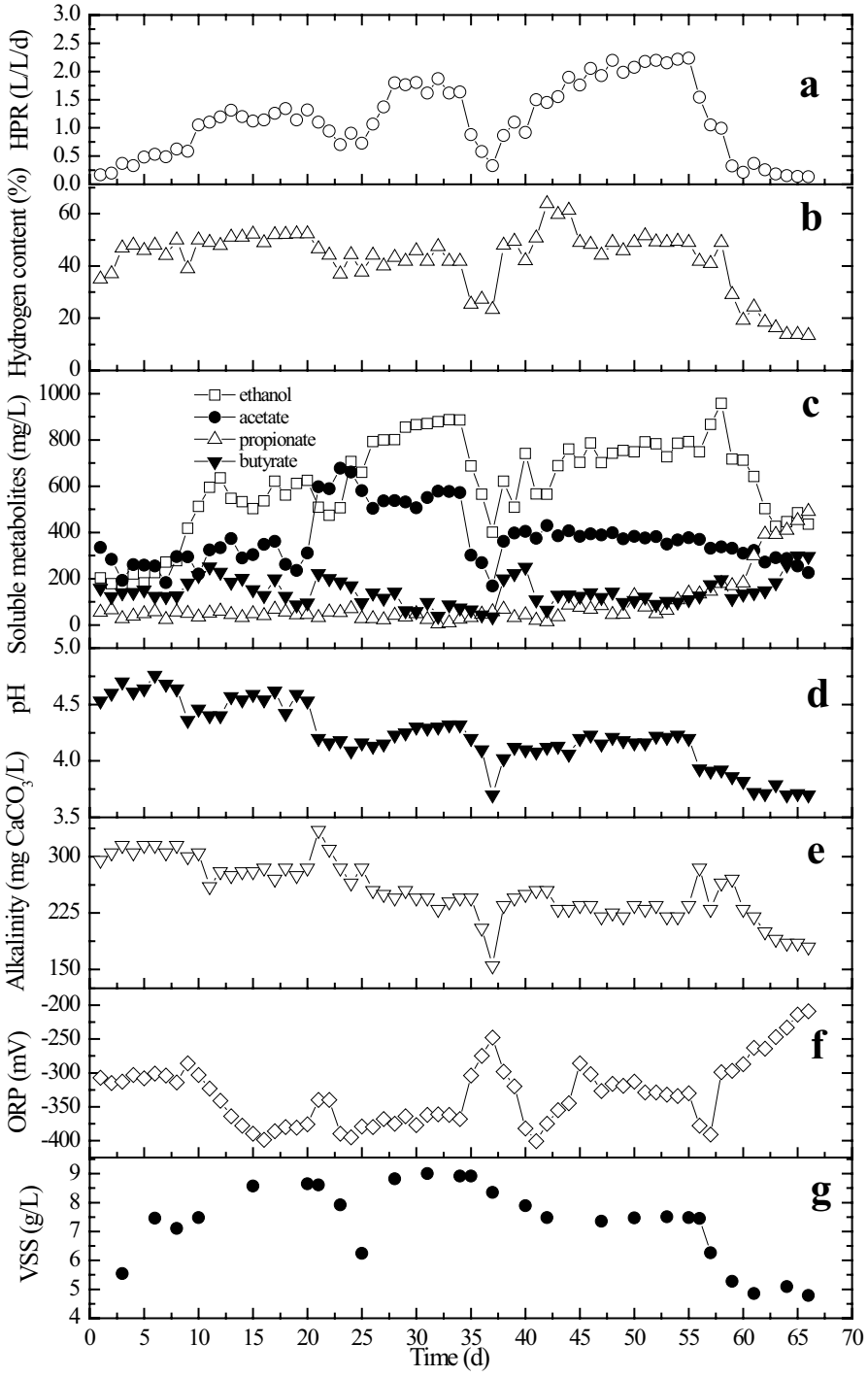

Fig. 2. Performance of hydrogen production in the ICSTR.

OLR. The system steadily produced hydrogen at the rates of 1.22 ± 0.09 and 1.73 ± 0.10 L/L-d at the OLRs of 12 and 18 g COD/L-d, respectively. But when the influent COD was increased to 8,000 mg/L, the HPR decreased rapidly to a very low level (0.33 L/L-d) on the 37th day, with the hydrogen content decreasing from 43% ± 2% to 23.3%. This should be attributed to the low buffer ability of the reactor to the shock loads. When the COD increased from 6,000 to 8,000 mg/L, the pH dropped from 4.3 to 3.7, while the acidogenic bacteria could be inhibited when the pH was below 4.0 [27]. Thus, VFAs and ethanol concentration dropped largely (Fig. 2c). To recovery the HPR of the reactor, only after 3 d of operation, the OLR was adjusted to 24 g COD/L-d with the COD of 6,000 mg/L and HRT of 6 h as phase 4 of operation. After 8 d of operation, the ICSTR reached the steady-state again, as the average HPR and hydrogen content of the biogas were 2.12 ± 0.10 L/L-d and 48% ± 2%, respectively. For the last 10 d of operation (phase 5), the OLR was increased to 36 g COD/L-d by reducing the HRT from 6 to 4 h. The HPR decreased rapidly due to the washing out of a large quantity of biomass from the reactor.

As depicted in Fig. 2, the ICSTR system exhibited three steady states during the operation of phase 1, phase 2 and phase 4, respectively. The HPR increased from 1.22 to 2.11 L/L-d with the increase of OLR from 12 to 24 g COD/L-d. Simultaneously, the operation parameters of pH, ALK and ORP also underwent fluctuation and achieved three steady states along with the adjustment of the OLR (Figs. 2d–f). The values of pH, ALK and ORP ranged from 4.2–4.5, 280–320 mg $CaCO_3$/L and –320–380 mV during the 3 steady-state operations, which were the optimal scopes for hydrogen production with ethanol-type fermentation [5]. During the stable operation of the reactor, the detection results of the liquid end products of the fermentation system also confirmed that the reactor was ethanol-type fermentation. As apparent from Fig. 2g, when the COD increased from 2,000 to 6,000 mg/L, the average concentration of VSS in the reactor increased from 7.10 to 8.91 g VSS/L, while it reduced to 7.45 and 4.8 g VSS/L when the HRT was shortened to 6 and 4 h, respectively.

The concentration and components of aqueous products could reflect the metabolism of hydrogen-producing anaerobes, which have considerable effects on hydrogen production. The dominant soluble metabolites included ethanol, acetate, propionate, and butyrate. It can be seen from Fig. 2c that the concentration of ethanol and acetate dramatically increased along with the increase of OLR from 6 to 18 g COD/L-d, while the propionate and butyrate remained almost kept unchanged. In phase 2, the ethanol and acetate concentration reached 92.7% of the total liquid end products, indicating that typical ethanol-type fermentation was formed in this reactor. Although the system was affected by the OLR overload from the 35th day to 37th day, the VFAs and ethanol concentrations, as well as the HPR, recovered on the 46th day by self-adjustment after about one week. When the HRT was shortened from 8 to 6 h in phase 4, some biomass was washed out from the reactor in the initial days and the VSS concentration dropped from 8.35 to 7.35 g/L. Thus, the substrate conversion was limited and the amount of the soluble metabolites reduced a little, compared to phase 2 with the same substrate concentration. Nevertheless, when the HRT was further shorted to 4 h, the amount of the soluble products dropped significantly and the hydrogen production almost ceased at the end. The results indicated the ability of maintaining biomass of this CSTR, which had an inner three-phase separator installed in it was limited, and the maximum OLR at the system COD of 6,000 mg/L in terms of hydrogen production was 24 g COD/L-d.

3.2. Performance of H_2 production in the ACR

To systematically compare the effects of the two different reactor configurations (ICSTR and ACR systems) on hydrogen production, these two reactors were operated under identical conditions. As illustrated in Fig. 3, the ACR system exhibited five steady states during the 74 d of operation. During the entire operation, the biogas only contained H_2 and CO_2, with no detectable methane. The H_2 content of the biogas was between 35% and 46%. The HPR increased linearly with increasing OLR, from 0.93 to 3.77 L/L-d as the OLR increased from 12 to 36 g COD/L-d (Fig. 3a). It is noteworthy that the HPR obtained under the steady-state of phase 4 was slightly higher than that of phase 3. This should be attributed to the different microbial communities and metabolite pathways under different operation parameters (HRT and COD), which corresponded to the different ethanol and acetate concentration.

In the hydrogen production system of the ACR process, pH, ALK and ORP all underwent fluctuation as the adjustment of the OLR, and they were all reached steady-state at each phase. As depicted in Fig. 3, pH, ALK and ORP ranged from 4.2–4.9, 210–500 mg $CaCO_3$/L and –354–492 mV during the whole operation (Figs. 3d–f). In addition, it could be found that the fluctuation of pH and ALK in the ACR system is not as prominent as that in the ICSTR system. Because the ACR had a better ability to enhance the biomass concentration and had a better buffer capability to the shock loads. As apparent from Fig. 3g, when the COD was increased from 6,000 to 8,000 mg/L, the biomass of the reactor was increased from 10.39 to 11.48 g/L. It also increased from 10.55 to 11.70 g/L when the HRT was shortened from 6 to 4 h. Thus, the ACR could be obtained a steady state under the operation of phase 3 and phase 5, while the ICSTR could not.

As shown in Fig. 3c, the concentration of ethanol and acetate both increased linearly as the influent COD concentration increased during the initial 3 phases. However, when the HRT was shorted from 8 h (phase 3) to 4 h (phase 5), the acetate concentration decreased rapidly from 945 to 452 mg/L, whereas the ethanol concentration only decreased slightly. In addition, ethanol and acetate were the major soluble metabolites, together accounting for 77%–90% of the total soluble metabolites during the whole operation period. Like the ICSTR, the ACR is an ethanol type of fermentation.

3.3. Comparison of hydrogen production between ICSTR and ACR

The COD mass balance for the two systems under different OLRs is shown in Table 3, computed considering their measured influent and effluent CODs, and the equivalent CODs for both gas and biomass. The closure of the COD balance at 93%–107% validates the reliability of the data.

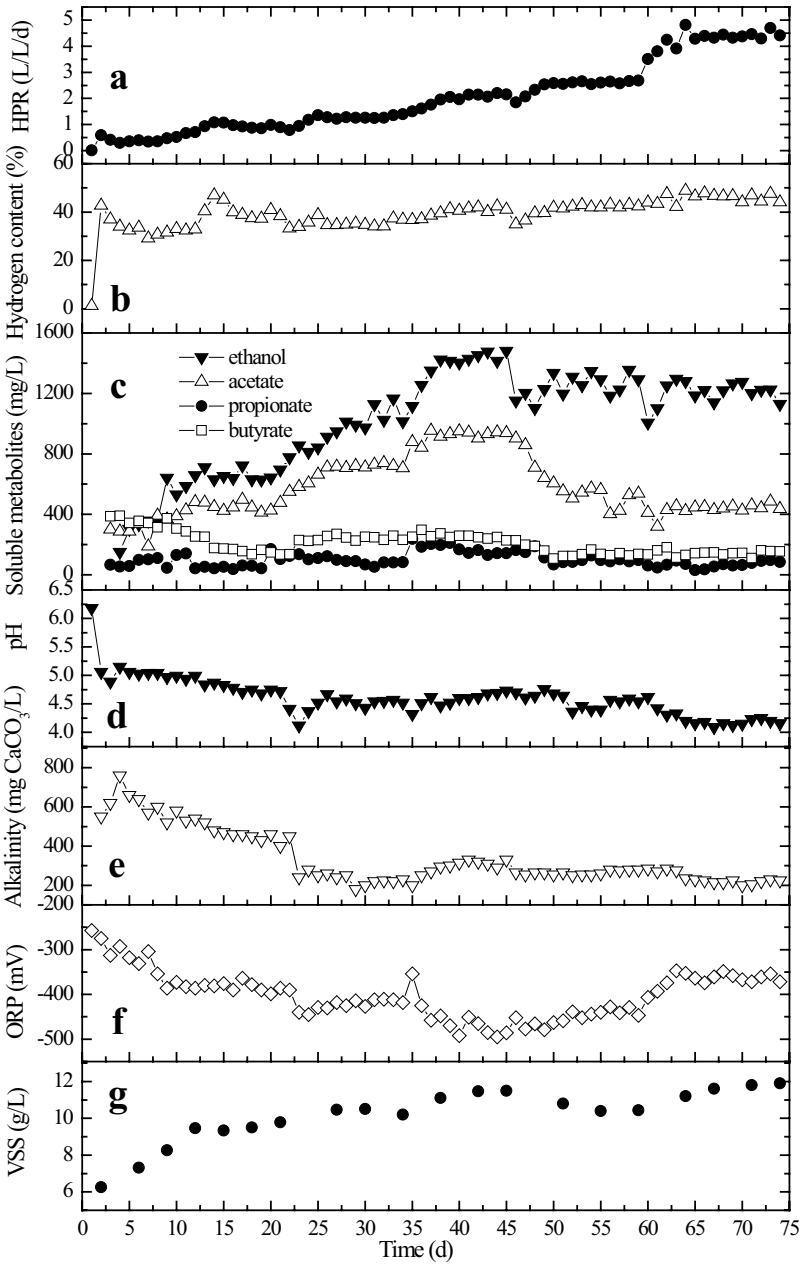

Fig. 3. Performance of hydrogen production in the ACR.

The characteristics of the ICSTR and ACR at each steady-state, including the concentration of metabolic products (ethanol and acetate) measured, are presented in Table 3. For the ICSTR, when the influent COD increased from 4,000 to 6,000 mg/L, the substrate degradation efficiency decreased from 98% to 91.3%, and when the HRT was shorted to 6 h, only 82.3% of the substrate was converted. However, the carbohydrate in the diluted molasses substrate was completely converted at OLR from 12–36 g COD/L-d (above 93%) in the ACR operation mode. This should be due to the higher biomass concentration of the ACR than that of the ICSTR because high biomass concentration could ensure a high level of substrate utilization for hydrogen production [16]. As seen in Tables 1 and 2, the SRT in the ACR mode was larger than that of the ICSTR mode, thus, for the same OLR, the biomass concentration (g VSS/L) was significantly higher in the ACR mode than in the ICSTR mode. In the ACR mode, the biomass did not significantly decrease as the HRT was shorted from 8 to 4 h in the ICSTR mode, the biomass decreased rapidly and resulted in the system failure. Hence, the ACR could be achieved a steady state at the OLR of 36 g COD/L-d as the operation phase 5.

As illustrated above, the ACR could be reached a HPR of 3.77 L/L-d at the OLR of 36 g COD/L-d, whereas the maximum HPR of 2.11 L/L-d was obtained in the ICSTR operation mode in this present study, which indicated that in the ACR process could improve the hydrogen production, compared to the ICSTR. However, when the ICSTR was operated at a lower

Table 3
Comparison of the hydrogen production performance in the ICSTR and ACR under steady-state conditions for each phase

OLR (g COD/L-d)	12		18		24		24		36	
	ICSTR	ACR	ICSTR	ACR	ICSTR	ACR	ICSTR	ACR	ICSTR	ACR
H_2 content (%)	51	40	43	35	–	41	48	42	–	46
HPR (L/L-d)	1.22	0.93	1.73	1.28	–	1.92	2.11	2.19	–	3.77
Substrate conversion (%)	98	99	91.3	98	–	95	82.3	93.7	–	93.2
VSS reactor (g/L)	8.61	9.52	8.91	10.39	–	11.48	7.45	10.55	–	11.70
VSS out (mg/L)	1,457	923	1,764	1,054	–	1,262	2,032	1,349	–	1,556
VSS out (mg COD/L)[a]	2,069	1,311	2,505	1,497	–	1,792	2,885	1,916	–	2,210
$SCOD_{out}$ (mg/L)	1,933	2,307	2,754	3,954	–	5,220	3,061	3,807	–	3,789
H_2 (gCOD/d)[b]	10.5	8	14.8	11	–	16.5	18.1	18.8	–	32.3
COD balance (%)[c]	107	96	95	96	–	93	102	102	–	107
Ethanol (mmol/L)	12.5	14.4	18.8	22.5	–	31.4	16.5	27.7	–	26.5
Acetate (mmol/L)	5.2	7.6	7.7	12	–	15.6	6.3	8.7	–	7.5
Ethanol/acetate (mmol/mol)	2.4	1.89	2.43	1.88	–	2.01	2.62	3.18	–	3.55
F/M (g COD/g VSS-d)	1.39	1.26	2.02	1.73	–	2.09	3.22	2.27	–	3.08
SHPR (L/g VSS-d)	0.14	0.10	0.19	0.12	–	0.17	0.28	0.21	–	0.32
H_2 yield (mol/mol)	1.69	1.27	1.71	1.18	–	1.37	1.74	1.59	–	1.83
r_{Hthe} (mol/d)[d]	0.74	0.9	1	1.4	–	1.85	1.17	1.83	–	2.62
r_{Hexp} (mol/d)[e]	0.65	0.49	0.93	0.69	–	1.03	1.13	1.17	–	2.02

[a]Based on 1.42 g COD/gVSS.
[b]Based on 8 g COD/gH_2.
[c]COD balance (%) = ((VSS_{out} (g COD/d) + H_2(g COD/d) + $SCOD_{out}$ (g COD/d))/($TCOD_{in}$ (g COD/d)).
[d]r_{Hthe}-theoretical H_2 production.
[e]r_{Hexp}-experimental H_2 production.

OLR (12–18 g COD/L-d), it can be found from Table 3 that the HPR was higher than that of the ACR, and with a higher hydrogen content in the biogas. Besides, the biomass specific hydrogen production rate (SHPR) presented in Table 3 was 45%–57% higher at the ICSTR compared to the ACR in the initial two phases. Furthermore, it can be seen from Table 3 that in the ICSTR operation mode, the hydrogen yield of 1.69 and 1.71 mol/mol glucose-consumed were obtained at the OLR of 12 and 18 g COD/L-d, respectively, while the hydrogen yield was lower in the ACR mode under these two OLRs, and they decreased from 1.27 to 1.18 mol/mol glucose-consumed when the COD was increased from 4,000 to 6,000 mg/L. Understanding the relevance of biomass and hydrogen yield, previous studies have been reviewed and indicate that the higher biomass concentration in the reactors could improve the hydrogen yield. Oh et al. [28] achieved a hydrogen yield of 0.4 mol/mol at a biomass concentration of 2.2 g/L in a CSTR and Wu et al. [10] using a CSTR seeded with silicone-immobilized sludge realized a hydrogen yield of 1.6 mol/mol at 3.5 g/L of biomass compared to a hydrogen yield of 2.1 mol/mol achieved by Zhang et al. [29] at a similar OLR with a higher biomass concentration (4.6 g/L). Moreover, comparing the biomass concentration in two studies with CSTRs utilizing agricultural soil as the seed and glucose as a substrate under approximately same OLRs, Van Ginkel and Logan [30] achieved much higher hydrogen yield (2.2 mol/mol) at a biomass concentration of 8 g/L compared to Zhang et al. [29] who reported 0.72 mol H_2/mol hexose with 0.9 g/L biomass. They contrast with the results of this present study. After analysis, the lower hydrogen yield that was obtained in the ACR with higher biomass concentration should be attributed to the homoacetogenesis in the bioreactor in this present study.

In general, acetate and butyrate production favor hydrogen production according to Eqs. (1) and (2) [31]:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (1)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (2)$$

In the propionate production pathway, 1-mole hydrogen gas was consumed when 1-mole propionate was produced (Eq. (3)). In the ethanol production pathway no hydrogen is consumed or produced (Eq. (4)). However, in a hydrogen production system with ethanol-type fermentation, ethanol production pathways exist (Eq. (5)) with a theoretical production of 2 mol hydrogen per mol of glucose [24].

$$C_6H_{12}O_6 + H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \quad (3)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (4)$$

$$C_6H_{12}O_6 + 2H_2O + 2NADH \rightarrow 2CH_3CH_2OH + 2HCO_3^- + 2NAD^+ + 2H_2 \quad (5)$$

Thus, in a hydrogen production system with ethanol-type fermentation, ethanol, acetate, and butyrate production are favored. But as apparent from Table 3, the ethanol concentration of the ACR was slightly higher than the ICSTR, whereas the acetate concentration was larger than the ICSTR under the same operating conditions when the OLR were 12 and 18 g COD/L-d. Furthermore, when the ICSTR was operated under the OLR of 12 and 18 g COD/L-d, the experimental hydrogen production value is in accordance with the theoretical hydrogen production which was calculated based on the metabolite products, that is, 2 mmol of hydrogen per 1 mmol of acetate produced, 2 mmol of hydrogen per 1 mmol of ethanol produced and 2 mmol of hydrogen per 1 mmol of butyrate produced. The experimental hydrogen production of ACR was much lower than the theoretical value (Table 3). Therefore, the assumption that 1 mmol of acetic and butyric acids are followed by 2 mmol of hydrogen does not seem to be the case in the ACR reactor. It is possible that microorganisms producing acetic acid from glucose with no hydrogen production (Eq. (6)) or producing acetic acid with hydrogen consumption (Eq. (7)) [32] have been established in the ACR system, probably due to the attached growth and the resulting long solids retention times.

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \quad (6)$$

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \quad (7)$$

Although the hydrogen yield of the ACR was lower than the ICSTR under the OLRs of 12–18 g COD/L-d, they achieved the same level when the OLR was increased to 24 at the operation of phase 4, and the hydrogen yield of the ACR reached 1.83 mol/mol glucose consumed when the OLR increased to 36 g COD/L-d. The higher yields observed at higher OLRs in this study were consistent with previous studies [17,33,34]. Lin et al. [17] reported hydrogen yields of 2.17 mol/mol glucose consumed at an OLR of 160 g COD/L-d compared to 1.34 mol/mol glucose consumed at an OLR of 20 g COD/L-d. This should be due to the suppression of homoacetogenic bacteria at higher OLRs. It can be found that the SRTs were in the range of 72–82 h on average in phase 1 to 3 (Table 2), SRTs were only 47 and 30 h in phase 4 and 5 due to the biomass washout as clarifier limitations, so the slower-growing microorganism (homoacetogenic bacteria) could not be retained [35]. Moreover, the ratio of ethanol/acetate (mol/mol) was both increased as accompanied by the increasing hydrogen yield in the two systems. Fig. 4, which shows the correlation of molar hydrogen yield with ethanol/acetate molar ratios, corroborates that indeed low molar hydrogen yields are associated with low ethanol-to-acetate ratios in the range of 1.9–2.0, whereas ethanol-to-acetate ratios of 2.4–3.5 results in high molar hydrogen yields. It indicated that the increased acetate production was not associated with increased hydrogen production due to homoacetogenesis, whereby CO_2 and H_2 are converted to acetic acid [11,36]. Finally, the inhibition of homoacetogenesis should be due to the decrease of the pH. When the OLR was increased from 24 to 36 g COD/L-d, the pH dropped from 4.5 to 4.2 (Fig. 3d). According to the previous study by Calli et al. [37], acetogenic H_2 consumption

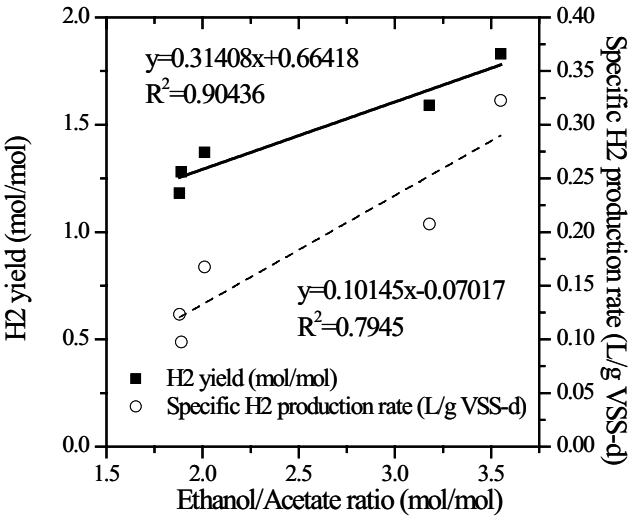

Fig. 4. Hydrogen yield and specific hydrogen production rate at different ethanol/acetate ratios.

was found to be more favorable at higher pH levels than low pH levels. Simultaneously, the SHPR increased along with the ratios of ethanol/acetate (Fig. 4). Despite the homoacetogenesis has been attracted more and more interesting in the hydrogen production system at present, they were all reported in the butyrate-type fermentation. The result of this present study found that the homoacetogenesis was also exited in the ethanol-type fermentation and it could significantly influence the hydrogen production, which was a contrast to the study of Kraemer and Bagley [38]. They reported that H_2 consumption may be of minor concern for continuous biohydrogen systems with butyrate-type fermentation.

In summary, when the OLRs were below 18 g COD/L-d, the ICSTR provided better hydrogen production than the ACR mode, since the homoacetogenesis took a significantly impacted the ACR mode. But the ACR mode did provide a distinct benefit over the ICSTR mode when the OLR was near the overloaded level. In the ACR, the HPR and hydrogen yield continued to increase linearly as the OLR increased from 12 to 36 g COD/L-d. In the ICSTR, however, the OLR became significantly overloaded (substrate conversion <90%) above 24 g COD/L-d (COD > 8,000 mg/L, HRT < 6 h). In addition, for the two biohydrogen production reactors, the hydrogen yields at the higher OLRs were all significantly greater than those at the lower OLRs, and the hydrogen yield of ACR reached 1.83 mol/mol glucose converted at an OLR of 36 g COD/L-d. So the ICSTR was preferable for hydrogen production when the OLRs were below 18 g COD/L-d, while the ACR was recommended when the OLR was above 24 g COD/L-d since ACR configuration combines shorter HRT (and therefore lower capital cost) with higher HPRs compared to ICSTR technology.

4. Conclusions

The influence of OLRs on the performance of fermentative hydrogen production bioreactors operating in ICSTR and ACR modes was investigated. Five OLRs were examined,

ranging from 12 to 36 g COD/L-d, with influent diluted molasses concentrations ranging from 4,000 to 8,000 mg COD/L and HRT ranging from 8 to 4 h. For the ICSTR, when the OLR was below 24 g COD/L-d, the substrate was almost completely utilized and the hydrogen yield remained at about 1.89 mol/mol glucose converted. The system failed to operate when the COD was increased to 8,000 mg/L or the HRT was shorted to 4 h, since the low buffer ability and washing out of biomass. However, for the ACR system, the HPR and hydrogen yield increased linearly along with the increasing of OLR from 12 to 36 g COD/L-d due to the elevating enhancement of biomass. The HPR and hydrogen yield increased from 0.93 to 4.4 L/L-d and 1.18 to 2.14 mol/mol glucose converted. It was found that the ICSTR gave higher HPR and hydrogen yield than the ACR when the OLRs were at 12 and 18 g COD/L-d, which should be due to the significant hydrogen consumption by homoacetogenic bacteria in the ACR system. Moreover, the results showed that the molar hydrogen yield correlated linearly with the ethanol-acetate ratios when the OLR increased, which indicated that the homoacetogenesis was inhibited.

Acknowledgment

This work was financially supported by the National Natural Science Foundation of China (Grant No. 51508539).

References

[1] F.R. Hawkes, R. Dinsdale, D.L. Hawkes, I. Hussy, Sustainable fermentative hydrogen production: challenges for process optimization, Int. J. Hydrogen Energy, 27 (2002) 1339–1347.
[2] D. Das, T.N. Veziroglu, Advances in biological hydrogen production processes, Int. J. Hydrogen Energy, 33 (2008) 6046–6057.
[3] D.B. Levin, L. Pitt, M. Love, Biohydrogen production: prospects and limitations to practical application, Int. J. Hydrogen Energy, 29 (2004) 173–185.
[4] H. Fang, H. Liu, T. Zhang, Bio-hydrogen production from wastewater, Water Sci. Technol. Water Supply, 4 (2004) 77–85.
[5] N.Q. Ren, J.Z. Li, B.Q. Li, Y. Wang, S.R. Liu, Biohydrogen production from molasses by anaerobic fermentation with a pilot-scale bioreactor system, Int. J. Hydrogen Energy, 31 (2006) 2147–2157.
[6] K.Y. Show, Z.P. Zhang, J.H. Tay, D.T. Liang, D.J. Lee, W.J. Jiang, Production of hydrogen in a granular sludge-based anaerobic continuous stirred tank reactor, Int. J. Hydrogen Energy, 32 (2007) 4744–4753.
[7] C.C. Chen, C.Y. Lin, Using sucrose as a substrate in an anaerobic hydrogen-producing reactor, Adv. Environ. Res., 7 (2003) 695–699.
[8] C. Li, H. Fang, Fermentative hydrogen production from wastewater and solid wastes by mixed cultures, Crit. Rev. Env. Sci. Technol., 37 (2007) 1–39.
[9] H.Q. Yu, Z.H. Hu, T.Q. Hong, Hydrogen production from rice winery wastewater by using a continuously stirred reactor, J. Chem. Eng. Jpn., 36 (2003) 1147–1151.
[10] S.Y. Wu, C.H. Hung, C.N. Lin, H.W. Chen, A.S. Lee, J.S. Chang, Fermentative hydrogen production and bacterial community structure in high-rate anaerobic bioreactors containing silicone-immobilized and self-flocculated sludge, Biotechnol. Bioeng., 93 (2006) 934–946.
[11] F.Y. Chang, C.Y. Lin, Biohydrogen production using an up-flow anaerobic sludge blanket reactor, Int. J. Hydrogen Energy, 29 (2004) 33–39.
[12] J.S. Chang, K.S. Lee, P.J. Lin, Biohydrogen production with fixed-bed bioreactors, Int. J. Hydrogen Energy, 27 (2002) 1167–1174.
[13] M.A. Rachman, Y. Nakashimada, T. Kakizono, N. Nishio, Hydrogen production with high yield and high evolution rate by self-flocculated cells of *Enterobacter aerogenes* in a packed-bed reactor, Appl. Microbiol. Biotechnol.,49 (1998) 450–454.
[14] K.S. Lee, Y.S. Lo, Y.C. Lo, P.J. Lin, J.S. Chang, H_2 production with anaerobic sludge using activated-carbon supported packed-bed bioreactors, Biotechnol. Lett., 25 (2003) 133–138.
[15] Z.P. Zhang, J.H. Tay, K.Y. Show, D.T. Liang, D.J. Lee, W.J. Jiang, Biohydrogen production in a granular activated carbon anaerobic fluidized bed reactor, Int. J. Hydrogen Energy, 32 (2007) 185–191.
[16] H. Hafez, B. Baghchehsaraee, G. Nakhla, D. Karamanev, A. Margaritis, H.E. Naggar, Comparative assessment of decoupling of biomass and hydraulic retention times in hydrogen production bioreactors, Int. J. Hydrogen Energy, 34 (2009) 7603–7611.
[17] C.N. Lin, S.Y. Wu, J.S. Chang, Fermentative hydrogen production with a draft tube fluidized bed reactor containing silicone-gel-immobilized anaerobic sludge, Int. J. Hydrogen Energy, 31 (2006) 2200–2210.
[18] H. Fang, H. Liu, T. Zhang, Characterization of a hydrogen-producing granular sludge, Biotechnol. Bioeng., 78 (2002) 44–52.
[19] Y. Mu, H.Q. Yu, Biological hydrogen production in a UASB reactor with granules. I: Physicochemical characteristics of hydrogen-producing granules, Biotechnol. Bioeng., 94 (2006) 980–987.
[20] H.Q. Yu, Y. Mu, Biological hydrogen production in a UASB reactor with granules. II: Reactor performance in 3-year operation, Biotechnol. Bioeng., 94 (2006) 988–95.
[21] N.Q. Ren, J. Tang, B.F. Liu, W.Q. Guo, Biological hydrogen production in continuous stirred tank reactor systems with suspended and attached microbial growth, Int. J. Hydrogen Energy, 35 (2010) 2807–2813.
[22] J.Z. Li, S. Chang, Biological Hydrogen Production in Acidogenic Anaerobic Contact Reactor, Pending Patent, Chinese Patent Application (No.ZL200710144460.1), 2007.
[23] H. Hafez, G. Nakhla, El.H. Naggar, E. Elbeshbishy, B. Baghchehsaraee, Effect of organic loading on a novel hydrogen bioreactor, Int. J. Hydrogen Energy, 35 (2010) 81–92.
[24] H.Z. Li, B.K. Li, G.F. Zhu, N.Q. Ren, L.X. Bo, J.G. He, Hydrogen production from diluted molasses by anaerobic hydrogen producing bacteria in an anaerobic baffled reactor (ABR), Int. J. Hydrogen Energy, 32 (2007) 3274–3283.
[25] APHA, AWWA, WEF, Standard Methods for Examination of Water and Wastewater, 19th ed., 1995.
[26] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem., 31 (1959) 426–427.
[27] M.H. Hwang, N.J. Jang, S.H. Hyun, I.S. Kim, Anaerobic bio-hydrogen production from ethanol fermentation: the role of pH, Int. J. Hydrogen Energy, 111 (2004) 297–309.
[28] S.E. Oh, P. Lyer, M.A. Bruns, B.E. Logan, Biological hydrogen production using a membrane bioreactor, Biotechnol. Bioeng., 87 (2004) 119–127.
[29] J.J. Zhang, X.Y. Li, S.E. Oh, B.E. Logan, Physical and hydrodynamic properties of flocs produced during biological hydrogen production, Biotechnol. Bioeng., 88 (2004) 854–860.
[30] S.W. Van Ginkel, B. Logan, Increased biological hydrogen production with reduced organic loading, Water Res., 39 (2005) 3819–3826.
[31] P.E.P. Koskinen, C.H. Lay, J.A. Puhakka, P.J. Lin, S.Y. Wu, J.O. Rlygsson, C.Y. Lin, High-efficiency hydrogen production by an anaerobic, thermophilic enrichment culture from an Icelandic hot spring, Biotechnol. Bioeng., 101 (2008) 665–678.
[32] G. Gottschalk, Bacterial Fermentations, In: Bacterial Fermentations, Bacterial Metabolism, Springer, New York, 1986, p. 208.
[33] D.H. Kim, S.K. Han, S.H. Kim, H.S. Shin, Effect of substrate concentration on hydrogen production and 16S rDNA-based

analysis of the microbial community in a continuous fermenter, Process Biochem., 41 (2006) 199–207.
[34] H.J. Yang, P. Shao, T.M. Lu, J.Q. Shen, D.F. Wang, Z.N. Xu, Continuous bio-hydrogen production from citric acid wastewater via facultative anaerobic bacteria, Int. J. Hydrogen Energy, 31 (2006) 1306–1313.
[35] L.H. Shen, D.M. Bagley, S.N. Liss, Effect of organic loading rate on fermentative hydrogen production from continuous stirred tank and membrane bioreactors, Int. J. Hydrogen Energy, 34 (2009) 3689–3696.
[36] H.S. Shin, J.H. Youn, S.H. Kim, Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis, Int. J. Hydrogen Energy, 29 (2004) 1355–1363.
[37] B. Calli, J. Zhao, E. Nijssen, K. Vanbroekhoven, Significance of acetogenic H_2 consumption in dark fermentation and effectiveness of pH, Water Sci. Technol. Water Supply, 57 (2008) 809–814.
[38] J.T. Kraemer, D.M. Bagley, Measurement of H_2 consumption and its role in continuous fermentative hydrogen production, Water Sci. Technol., 57 (2008) 681–685.