In-depth observations of fermentative hydrogen production from liquid swine manure using an anaerobic sequencing batch reactor

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Abstract
In this study, experiments were designed to reveal in-depth information of the effect of pH and hydraulic retention time (HRT) on biohydrogen fermentation from liquid swine manure supplemented with glucose using an Anaerobic Sequencing Batch Reactor (ASBR) System. Five values of HRT (8, 12, 16, 20, and 24 h) were first tested and the best HRT determined was further studied at five pH levels (4.4, 4.7, 5.0, 5.3, and 5.6). The results showed that for HRT 24 h, there was a dividing H2 content (around 37%) related to the total biogas production rate for the ASBR System running at pH 5.0. When the H2 content went beyond 37%, an appreciable decline in biogas production rate was observed, implying that there might exist an H2 content limit in the biogas. For other HRTs (8 through 20 h), an average H2 content of 42% could be achieved. In the second experiment (HRT 12 h), the highest H2 content (35%) in the biogas was found to be associated with pH 5.0. The upswing of pH from 5.0 to 5.6 had a significantly more impact on biogas H2 content than the downswing of pH from 5.0 to 4.3. The results also indicated good linear relationships of biogas and H2 production rates with HRT (r=0.9971 and 0.9967, respectively). Since the optimal ASBR operating conditions were different for the biogas/H2 production rates and the H2 yield, a compromised combination of the running parameters was determined to be HRT 12 h and pH 5.0 in order to achieve good biogas/H2 productions.

Keywords: biohydrogen fermentation, swine manure, hydraulic retention time, pH values, anaerobic sequencing batch reactor

1. Introduction
Currently, hydrogen is produced exclusively from fossil fuels through energy intensive processes, which themselves are not clean technologies from the perspective of sustainability. For being used as a major energy source, hydrogen must be produced via sustainable means (Benemann 1996; Dunn 2002), among which biological pathways have come to the center stage due to its low energy needs and environment-friendly nature. Furthermore, biological conversion normally works with waste materials, so it can achieve both waste reduction and energy recovery. In view of these benefits, a considerable amount of research effort has been dedicated to biological production of hydrogen in the last two decades (Chen et al. 2008; Bičáková and Straka 2012; Zhao et al. 2013; Rai et al. 2014), among which fermentative
hydrogen production from organic compounds (especially carbohydrates) by anaerobic bacteria is generating profound interests among researchers due to its unique advantages over other technologies (Liu et al. 2013). In this process, high hydrogen production rates can be achieved with an active dark-fermentative consortium without the assistance of a light source (Das and Veziroglu 2001). In addition, the majority of the substrates used for dark fermentation consist of waste materials that otherwise need to be treated before disposal, which incurs extra costs. The investigated waste streams so far for hydrogen fermentation include tofu processing wastewater (Zhu et al. 2002), rice winery wastewater (del Campo et al. 2012; Yu et al. 2002), starch manufacturing wastewater (Yokoi et al. 2002), potato processing wastewater (Yokoi et al. 2001), beer processing wastewater (Lay et al. 2005), sugar refinery wastewater (Won et al. 2013), sugarcane bagasse (Rai et al. 2014), dairy wastewater (Gadhe et al. 2013), cheese whey wastewater (Kargi and Uzunçar 2012), fruit juice wastewaters (Fernández et al. 2011), and pineapple wastes (Wang et al. 2006). Obviously, the fermentative pathway for hydrogen production can be an ideal vehicle to not only produce hydrogen but also reduce the volume of these wastes, thus saving the treatment costs and paving the way for building a sustainable economy.

One of the waste materials that have not been studied extensively in hydrogen fermentation is liquid swine manure, despite a few publications existing in the literature, almost all of which, however, were coming from the work conducted by the authors (Wu et al. 2009; Zhu et al. 2009). The results from these reports, in general, evidenced the feasibility of using swine manure as substrate for hydrogen fermentation, but without elaborating on some intrinsic characteristics of the fermentation process. Given the fact that swine manure contains all the necessary components for hydrogen fermentation by microorganisms such as Clostridia and the tremendous volume generated in the world every year, it is worthwhile to further our understanding of the process by providing in-depth information on the characteristics of the process for biogas/hydrogen production. Therefore, in this study, new information that had not been reported before related to fermenting swine manure supplemented with glucose to produce hydrogen was collected and reported using an Anaerobic Sequencing Batch Reactor (ASBR) System running on different hydraulic retention time (HRT) and pH values. Such information might provide insight on improving the hydrogen fermentation efficiency of liquid swine manure.

2. Materials and methods

2.1. Seed sludge and pretreatment

A running anaerobic digester treating dairy manure, located in St. Peter, Minnesota, USA, was the source for the seed sludge for this study. After collection, the sludge was pretreated using a prepared nutrient medium under room temperature for 24 h. The nutrient medium (1 L) contained 10 g glucose, 1.5 g KH₂PO₄, 0.5 g NH₄Cl, 0.18 g MgCl₂-6H₂O, 0.05 g FeSO₄·6H₂O, 5 g polypeptone and 2 g yeast extract (Fang et al. 2006). The pH of the medium was also lowered from 7.1 to 5.0 with hydrochloric acid. After incubation, the sludge was boiled at 100°C for 30 min to inactivate non-H₂-producing bacterial species in the sludge.

2.2. Liquid swine manure source and preparation

The main substrate, liquid swine manure, was collected from a finishing building at the University of Minnesota Southern Research and Outreach Center at Waseca, USA. Preliminary treatments of the collected manure included dilution with tap water to a solid content of 0.5% followed by freezing in a freezer, if not placed immediately in the feeding tank. According to our preliminary trials (data not shown), swine manure alone was found to be ineffective in H₂ fermentation, and a sugar source, such as glucose, was needed in the culture media due to the lack of sufficient carbohydrates in the manure for the fermentative bacteria. To that end, to assist the growth of H₂ producing bacteria with sufficient carbohydrates, the manure in the feeding tank was supplemented with 10 g L⁻¹ glucose, 500 mg L⁻¹ KH₂PO₄, and 400 mg L⁻¹ peptone. The characteristics of the raw liquid swine manure and the prepared substrate were presented in Table 1. The adjusted pH, which was slightly higher than 5.0, took into account the potential pH drop caused by the fresh influent fed into the reactor at the beginning of each ASBR cycle that would normally reduce the liquid pH as a result of quick production of organic acid.

2.3. Reactor setup and operation

The lab-scale ASBR System was presented in Fig. 1. A polyethylene jar, 20.3 cm in diameter and 45.0 cm in height, was employed as the bioreactor, which had a total volume of 8 L with a working volume of 4 L. The reactor was heated by a hot plate stirrer to maintain the mixed liquor temperature inside the reactor. Complete mixing of the reactor was obtained using a centrifugal water pump circulating the liquid through an outside loop where a T connector was installed with a pH probe (Cole-Parmer, USA) connected to it to simultaneously record the real-time pH. A pH controller (Cole-Parmer, USA) was used to take feedback from the probe, based on which two microtube-pumps were operated to add either base (1.0 mol L⁻¹ NaOH) or acid (1.0 N HCl) to the reactor for pH adjustment. The feeding tank was a 20-L water bottle equipped with a mixer that ran for 10 s to
homogenize the content in the tank before the feeding pump started to move liquid into the reactor.

The ASBR System was operated automatically by a set program of cycle operation following a time sequence. The control of the influent and effluent movements, including all the mixers and pumps, were achieved using peristaltic pumps (Barnant Company, USA), which were controlled through a programmable data board (Campbell Scientific, USA) using the software (Campbell Scientific, USA) installed on a computer. The time for each cycle was set at 4 h, and during each cycle, the liquid was circulated continuously in all phases but the filling, settling, and withdrawal (total about 30 min) to achieve thorough mixing and rapid diffusion of H₂. At the end of each cycle, a certain amount of reactor content (based on the HRT used) was discharged into an effluent tank. Sampling ports at different locations were installed on the bioreactor to collect both biogas and liquid samples as needed.

2.4. Experimental design and sample analysis

The experiment was carried out in two stages. First, five HRTs of 8, 12, 16, 20, and 24 h were examined for their impact on biogas/H₂ production rates and H₂ content with pH controlled at 5.0. In the second stage, the best HRT identified in the first stage was used as a fixed parameter with five varying pH values (4.4, 4.7, 5.0, 5.3, and 5.6) to investigate the pH effect on the same biogas production characteristics. For each new test, after inoculation, the reactor was first filled with the prepared liquid swine manure to the working volume of 4 L, and then sealed air-tight. To create a completely anaerobic environment, the headspace of the reactor was purged with nitrogen gas for 1 min. For tests in each stage, a break-in period of 24 h was used to operate the reactor in batch mode to establish biogas production before starting the normal fed-batch mode. For HRT experiments, the reactor started with HRT 24 h, which was then progressively reduced at 4 h increments (20, 16, 12, 8 h) by increasing the organic loading rate (i.e., hexose loading rate from 40, to 48, 60, 80, and 120 g d⁻¹). The reactor temperature was maintained at (37±1)°C for all the experiments. Evaluation of the system performance for each parameter (HRT and pH) would not start until the reactor entered the steady-state condition, which was defined as the

<table>
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<th>Parameters</th>
<th>Values of original manure</th>
<th>Values of prepared substrate</th>
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<tr>
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<td>TKN (mg N L⁻¹)</td>
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<tr>
<td>BOD₅ (mg L⁻¹)</td>
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<td>9220</td>
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<tr>
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</tr>
<tr>
<td>VFAs (mg L⁻¹)</td>
<td>3547</td>
<td>854</td>
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1) TS, total solids; TVS, total volatile solids; TSS, total suspended solids; Ortho-P, ortho-phosphate; TKN, total Kjeldahl nitrogen; BOD₅, five-day biochemical oxygen demand; COD, chemical oxygen demand; VFAs, volatile fatty acids.

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Fig. 1 Experimental set-up of Anaerobic Sequencing Batch Reactor (ASBR) System.
variations of biogas production and glucose conversion rates falling within 5% for five consecutive cycles. The volume of biogas produced was measured by a wet gas meter (GCA/Precision Scientific Inc., Chicago), and the biogas samples were analyzed using a gas chromatography (GC) (Varian 3800; Agilent Technologies, CA, USA) to determine H₂ and CO₂ content at preset time intervals. The GC had a thermal conductivity detector (TCD) installed with a Varian CP7429 column. Helium was used as the carrier gas at a flow rate of 30 mL min⁻¹. The temperatures for the oven, injector, and detector were, respectively, maintained at 50, 120 and 150°C. Each experiment (HRT or pH) lasted 3 wk after the reactor was running in steady-state.

Standard methods were used to analyze liquid samples for total solids (TS), volatile solids (VS), total suspended solids (TSS), five-day biochemical oxygen demand (BOD₅), chemical oxygen demand (COD), and dissolved ortho-phosphate (Ortho-P) (APHA 1998). A Foss Kjeldahl analyzer was used to analyze total Kjeldahl nitrogen (TKN). For dissolved parameters analysis (COD, ortho-P, and hexose concentration), each sample was centrifuged at 4500 r min⁻¹ for 10 min and then filtered through a paper filter (GVWP02500; Fisher, USA) with a pore size of 0.22 μm. All samples were generally analyzed promptly or stored by freezing and thawed to room temperature before analysis. Wherever applicable, the student’s t-test was used to compare different treatments at a significance level of α<0.5.

3. Results and discussion

3.1. Hydrogen content affected by biogas production rate

Fig. 2 presented information on the variations of biogas production rate associated with the H₂ content after the ASBR System entered the steady state under the HRT of 24 h and pH 5.0 (similar situations were not observed for the other four HRTs). The H₂ content in the biogas produced was relatively stable over a range from 32 to 37%, with the biogas production rate fluctuating between 12 and 15 L d⁻¹. However, when the H₂ content moved upwards from 37 to 41%, a drastic drop in biogas production rate occurred from about 13.8 to 3.8 L d⁻¹. This observation is interesting and appears to suggest that when everything else in terms of running conditions is unchanged, the maximum operational H₂ content in the biogas produced will not exceed 38% without causing an appreciable reduction in biogas production rate. The reason for this phenomenon is not well understood. One of the possibilities could be attributed to the rising H₂ partial pressure in the headspace as the concentration of H₂ increased in the biogas generated (Chang and Lin 2004), which has been reported by a number of past researchers (Kim et al. 2005; Mandal et al. 2006; Jin 2007). High hydrogen partial pressure can lead to accumulation of higher molecule volatile fatty acids (VFAs) than acetate, indicating a potential shift of biological pathway from production of H₂ to organic acids (Kaparaju et al. 2009). However, this still does not sufficiently explain the plunging reduction of biogas production rate, although H₂ production rate is part of it. It is known that, in an H₂ fermentation environment, acidogenesis is a major process for producing H₂ by acetogenic bacteria with acetate as the by-product (Mu et al. 2006). In the meantime, CO₂ is also produced along with H₂ as a result of anaerobic respiration. The loss of biogas production rate associated with the rise in H₂ content suggested that the production rates for both H₂ and CO₂ were severely hindered, leading to the overall reduction in biogas production rate. The scenario observed here may thus imply that there could exist an upper limit of H₂ content in the biogas with respect to the particular setups used in this experiment such as HRT, pH, and temperature. Besides, it also indicates that high biogas production rate may not guarantee a high H₂ content in the biogas, and vice versa. More research is thus needed to determine the intrinsic relationships between the biogas production rate and its H₂ content, as well as the underlying mechanisms.

Another observation with the ASBR System running at the HRT 24 h and pH 5.0 was related to the formation of biomass granules. The occurrence of granules was detected about 23 days after the inception of the ASBR operation including the startup period, which was much faster than reported in other studies (Lee et al. 2004; Zhang et al. 2008), indicating that the ASBR System investigated in this study was able to shorten the time for the granulation process, a critical step to develop an H₂-producing consortium responsible for effective H₂ production (Wang and Chang 2008). This was, as a matter of fact, proved by the uptick of biogas generation when the formation of granules was positively identified. This information has not been reported elsewhere in the literature.
3.2. Effect of hydraulic retention time and pH on biogas $H_2$ content

The effect of HRT on the ASBR System was evaluated by progressively reducing the HRT from 24 to 8 h at 4-h intervals, which was achieved by increasing the loading rate of hexose from 40, 48, 60, 80 and 120 g d$^{-1}$. At least three comments can be made about the information generated. First, the results indicated that HRT had a profound impact on the biogas $H_2$ content (Fig. 3). Generally speaking, for HRTs of 8, 12, 16 and 20 h, the $H_2$ content in biogas was similar ranging from 40 to 43%, despite that it was significantly lower for HRT 16 h as compared to the other three. As the HRT increased to 24 h, the biogas $H_2$ content dropped to a much lower level (35%) with a much larger variation. Thus, it may be concluded from the data obtained that the biogas $H_2$ contents for HRTs of 8, 12, 16 and 20 h did not appear to be significantly different for the ASBR System examined in this study, while HRT 24 h experienced a significant reduction in $H_2$ content.

Second, the data revealed the high conversion efficiency of sugar to $H_2$ of the ASBR System examined. Theoretically, if all the hexose added was converted to $H_2$, the $H_2$ content in the biogas produced would reach 67% ($C_6H_{12}O_6 \to 2CH_3OH+4H_2+2CO_2$). In this study, the average $H_2$ content in biogas for HRTs from 8 to 20 h was found to be around 42% (Fig. 3), leading to an overall conversion efficiency of 63%, which was almost on par with the upper limit of typical conversion efficiencies (48–67%) of the strict anaerobic hydrogen producers, mainly *Clostridium* species, reported by Lay (2001) and Ueno et al. (2001). Therefore, it may be inferred that the ASBR System developed in this study could effectively produce $H_2$ at a nearly optimal level from swine wastewater supplemented with glucose. Further research is thus warranted to scale up the system for real applications.

Third, it was posited that the biogas produced usually contained not only hydrogen but also carbon dioxide as the other major component. The presence of CO$_2$ might prevent the biogas from being used in many conventional fuel cells due to the potential toxic effect of the impurities on the fuel cell electrodes which were primarily made of precious metals. Although a most recent study showed a limited effect of CO$_2$ on the fuel cell electrodes (del Campo et al. 2014), it is ideal to keep the CO$_2$ content in the fermentation biogas at the minimum if at all possible, which is reflected by the CO$_2$/H$_2$ ratio in the biogas. The results obtained from this study evinced a CO$_2$/H$_2$ ratio ranging from 1.33 to 1.96, which was better than those observed by other researchers (Lee et al. 2004; Yang et al. 2007). Also, previous reports showed that biogas with 70% $H_2$ and 30% CO$_2$ (CO$_2$/H$_2$ ratio=0.43) could be successfully used as fuel for proton exchange membrane (PEM) fuel cells (Mann et al. 2000). Therefore, the biogas produced from the ASBR tested herein has the potential to be used in PEM fuel cells only after moderate purification to further reduce the CO$_2$ content.

The changes of $H_2$ content in the biogas in relation to pH is presented in Fig. 4 (HRT 12 h was selected for this batch of tests) for the ASBR System operating in the steady state with standard error bars also provided. It appeared that the spread of the $H_2$ content data increased with increasing pH (longer error bars). When pH was 5 or below, the variation in $H_2$ content was small, but increased as the pH value went beyond 5. This indicated that the ASBR System could become unstable at higher pH values. Han et al. (2012) reported that at pH 4.4, the distribution of VFAs produced was reduced with butyrate being the major acid associated with $H_2$ production, which could be one of the reasons that narrowed the variation range in $H_2$ content in the biogas. On the other hand, when pH moved higher, the metabolic products of dark fermentation for $H_2$ production started to change from $H_2$ to alcohol, and it was reported that when pH reached 6.1, the alcohol production rate would transcend that of $H_2$, leading to large variations in $H_2$ content in the headspace biogas during the transition period (Jung et al. 2011). In addition, when pH was higher than 5.7, methanogenic reactions could gain momentum, resulting in increasing $H_2$ content variations due to the unstable or reduced $H_2$ production (Pender et al. 2004). These past research results might explain the decline in $H_2$ content as well as its increased variation observed in this study.

The data also showed that the best pH for the highest $H_2$ content appeared to be 5.0, which was consistent with the results obtained by Zhu et al. (2007) and Infantes et al. (2011). Thus, it may be concluded that the optimal pH for the ASBR System to achieve the highest $H_2$ content in the
biogas should be maintained around 5.0. Also, an interesting observation from Fig. 4 needing notice was the unequal impact that lower or higher pH values than 5.0 had on the H₂ content. Apparently, as pH decreased, the H₂ content in the biogas decreased as well, but only slightly (from around 37 to 31% according to Fig. 4). However, it was not the case if pH went to the opposite direction, i.e., increasing from 5.0 to 5.6, in which a surprisingly sharp decrease in H₂ content was seen (from 37 to 22% at pH 5.3, and to 8% at pH 5.6). As pointed out by Jung et al. (2011), pH has been widely accepted as having the most significant impact on dark fermentation of H₂ production among various operational parameters because of its direct effects on the hydrogenase activity, metabolic pathway, and dominant species. The fall in H₂ content in the biogas could thus be attributed to the digression of pH from its optimal values, i.e., 5.0. More interestingly, a strong inversely linear relationship between H₂ production rate and the H₂ production rate were affected by pH and H₂ content over the range from pH 5.0 to 5.6 was clearly shown in Fig. 4, with the correlation coefficient of 0.9983. This information has not been reported in the existing literature, and it emphasizes once again the importance of pH in achieving good H₂ fermentation. According to the linear equation, it appeared that the H₂ content in the biogas would arrive at zero at pH 5.78, which might not happen in real operations; however, there were reports showing that when pH was greater than 5.7, methanogenic reactions would come to dominance with the H₂-producing microflora being severely outnumbered as a result (Ting and Lee 2007). Based on the data from this study and the literature information, it may be concluded that maintaining pH below 5.3 is critical for the ASBR System experimented to achieve good H₂ production.

3.3. Relationships between biogas/H₂ production rates, HRT, and H₂ yield

Fig. 5 documented the relationships of biogas and H₂ production rates as well as H₂ yield with the HRT. Apparently, two good linear relationships were observed, i.e., biogas production rate vs. HRT (r=0.9971) and H₂ production rate vs. HRT (r=0.9967). The reduction of HRT from 24 to 8 h was accompanied with the increase in biogas production rate from 15 to 34 L d⁻¹. A similar trend was seen for H₂ production rate (from 5 to 14 L d⁻¹) over the same HRT reduction period. These observations indicated that both the biogas production rate and the H₂ production rate were affected by HRT in a linear manner, and the lower the HRT, the higher these two rates would be. This is expected because the inverse relationships between biogas/H₂ production rates and HRT have been commonly encountered in bihydrogen research. Antonopoulou et al. (2008) identified similar characteristics of biogas/H₂ production rates vs. HRT, and they found that the highest production rates for biogas and H₂ occurred at HRT 4.4 d among the three HRTs tested (20, 10, and 4.4 d) for a periodic anaerobic baffled reactor treating cheese whey. Other researchers reported even much shorter HRTs (0.5–1 h) when the biogas/H₂ production rates reached the highest (Chang et al. 2002; Lee et al. 2006). Since the adjustment of HRT in this study was achieved by increasing the organic loading rate (OLR) from 40 to 120 g d⁻¹ (hexose), it was thus inferred that a higher OLR (a lower HRT) was beneficial in improving biogas/H₂ production rates. However, one caveat worths to be mentioned here is that there is a limit for increasing OLR with any biohydrogen production system because it has been recognized by previous workers that too high an OLR would adversely impact H₂ production (Tawfik and El-Qelish 2012) due to the structural changes of the hydrogenic microbial community (Han et al. 2012). The shortest HRT used in this study (8 h) appeared to have not come across the inhibitory limit yet. And, as a matter of fact, the highest H₂ production rate of over 0.16 L h⁻¹ L⁻¹ obtained from this study with reduced nutrients added in the substrate was largely as good as the reported values (typically 0.1–0.3 L h⁻¹ L⁻¹) in the literatures (Chang et al. 2002; Lee et al. 2006), rendering the ASBR System developed in this study more economically attractive. The results clearly suggested that HRT 8 h was the best for biogas/H₂ production.

However, there is another observation on Fig. 5 that cannot be ignored, i.e., H₂ yield. It seemed that the best HRT (8 h) for biogas/H₂ production did not coincide with the best HRT for H₂ yield (HRT 16 h). In fact, the three H₂ yields (1.58, 1.63, and 1.61 mol H₂ mol⁻¹ hexose, respectively) for the middle three HRTs (12, 16, 20 h) were fairly similar,
and significant falls in H₂ yield were seen either when HRT went down to 8 h (1.2 mol H₂ mol⁻¹ hexose) or went up to 24 h (1.26 mol H₂ mol⁻¹ hexose), indicating that at these two HRTs, the ASBR System were inefficient in converting hexose to H₂. A close look at Figs. 3 and 5 might provide some answers for the low H₂ yield at HRT 24 h. According to Fig. 3, the H₂ content at HRT 24 h was low, which could be the reason for the low H₂ yield observed in Fig. 5 for the same HRT. And this certainly indicated that 24 h was not a suitable HRT for the ASBR System investigated herein for efficient H₂ production. This observation may be verified by the ratios of VFAs to soluble microbial products (SMP) because for HRT 24 h, the VFA/SMP ratio was 91% (but for the rest HRTs, it was above 95%), indicating that less H₂ was produced (Pattra et al. 2011). Another possible reason for the low H₂ yield and content at HRT 24 h could be the development of methanogenic activities, which weakened H₂ production (Park et al. 2010). For the shortest HRT, i.e., 8 h, the reason for the observed low H₂ yield needed some elaboration because comparing Figs. 3 and 5 did not yield the same inference as for HRT 24 h. Normally, shorter HRTs meant quick turnovers of the biomass in the ASBR, and for good H₂ yields, biomass concentration was considered an important factor (Argun et al. 2008). Although the loss of biomass during operating cycles were not quantified in this study, the washout of H₂ producing biomass to some extent was suspected for HRT 8 h when half of the reactor content was removed and refilled for each cycle. Analogous observations were reported by Chang and Lin (2004), in which they found drastic reductions in H₂ yield for HRT at both 4 and 24 h using an up-flow ASBR to treat sewage sludge. In comparison, it was interesting to note that many previous workers came to conclusions from their studies that higher H₂ yields were obtained at lower HRTs (Chang et al. 2002; Lee et al. 2004; Van Ginkel and Logan 2005), which was inconsistent with the results from this study that showed a bell shape of H₂ yield against HRT with the highest H₂ yield occurring at the center HRT (16 h) and the lower H₂ yields located at the both ends of the HRT spectrum (8 and 24 h). Reviewing their work revealed that they either employed bioreactors with high biomass retention capability (fixed bed reactors) (Chang et al. 2002; Lee et al. 2004) or reduced the organic loading rate (Van Ginkel and Logan 2005). All these strategies certainly helped improve the retention and activity of H₂-producing consortium, leading to the upkeep of high H₂ yields at lower HRTs. Therefore, it may be concluded that considering the experimental design used, the data obtained from this study relatively accurately reflected the trend of H₂ yield associated with the changes in HRT. And the best HRTs for higher H₂ yields included 12, 16, and 20 h according to Fig. 5.

Fig. 5 also revealed another interesting phenomenon that a high biogas/H₂ production rate did not occur in concurrence with a high H₂ yield, which was not uncommon in biohydrogen production because the metabolic pathway of the H₂ producers was not designed to achieve multiple optimums for products production (García-Peña et al. 2009). Factors, such as substrate concentration, biomass concentration, etc., all have impact on the metabolite products of H₂ producers. For instance, at low substrate concentrations, Clostridium acetobutylicum produces organic acids, but solvents otherwise (Argun et al. 2008; García-Peña et al. 2009). Therefore, to achieve acceptable biogas/H₂ production rates and H₂ yields at the same time, compromises are
necessary when it comes to selecting the HRT for the ASBR System. In this case, an HRT of 12 h could be a good choice for which biogas/H\textsubscript{2} production rates of 26 and 13 L d\textsuperscript{-1} and an H\textsubscript{2} of 1.58 mol H\textsubscript{2} mol\textsuperscript{-1} hexose can be obtained.

4. Conclusion

The results showed that for the HRT tests (pH 5.0), high biogas production rate might not guarantee a high H\textsubscript{2} content in the biogas, and vice versa. HRTs of 8, 12, 16, and 20 h generated good H\textsubscript{2} content (42% on average) in the biogas, while 24 h achieved much lower (35%). The ASBR System demonstrated an overall conversion efficiency of 63%. For the pH tests (HRT 12 h), the optimal pH for the ASBR System to achieve the highest H\textsubscript{2} content in the biogas appeared to be 5.0; however, reducing pH to below 5.0 would not affect production rate and H\textsubscript{2} production rate.

Good linear relationships were observed between biogas pH to above 5.0 (31% at pH 4.4, but only 8% at pH 5.6). The optimal HRT for the ASBR System studied to achieve good biogas/H\textsubscript{2} and 0.9967, respectively). The optimal HRT for the ASBR System was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR), USA.

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