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Direct integration of CSTR-UASB reactors for two-stage hydrogen and methane production from sugarcane syrup

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ABSTRACT

This study attempted to directly integrate a continuous stirred tank reactor (CSTR) with an up-flow anaerobic sludge blanket (UASB) reactor for two-stage hydrogen and methane production. CSTR was used to produce hydrogen from a 25 g-COD/L sugarcane syrup. The hydrogenogenic effluent from CSTR was directly fed to UASB for methane production. The working volumes of CSTR and UASB reactors were 1 and 24 L, respectively. Optimization of hydraulic retention times (HRT) for two-stage hydrogen and methane production after an integration were examined. A maximum hydrogen production rate of 17.5 L/L.d and methane production rate of 2.25 L/L.d were achieved at optimal HRTs of 3 h in the CSTR and 3 d in the UASB with a total energy production rate of 270 kJ/L.d. The two-stage reactors performed well in producing hydrogen and methane over 200 days with a total COD removal of 97.5%. The natural microflora in sugarcane syrup was greatly affected by HRT but HRT did not affect the archaea community. The volatile fatty acids to alkalinity ratio in the UASB reactor was below the critical value of 0.4 at every HRT, indicating stability of a long term methane production process with direct feeding of non-pretreated hydrogenogenic effluent.

Introduction

With increasing energy demands and concerns about environmental impacts, hydrogen has been proposed as a promising renewable energy [1]. Several technologies could be used to produce hydrogen from various sources including electrolysis of water, partial oxidation of methane, and steam reforming of hydrocarbon substances. However, biological hydrogen production processes are more attractive than other technologies since they are more environmentally friendly and less energy intensive. Hydrogen can be produced biologically using a variety of materials such as organic and agricultural wastes. Among biological hydrogen production

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processes, dark fermentation is known to be economically viable with a high production rate and is suitable for industrial scale [2]. Nevertheless, hydrogen production by dark fermentation processes has some drawbacks including their low energy recovery and low efficiency of chemical oxygen demand (COD) removal [3]. Generally, less than 20% of a COD in a substrate can be converted to hydrogen by dark fermentation [1,4]. To overcome these drawbacks, a two-stage fermentation process has been proposed to improve energy recovery and COD removal. Based on an anaerobic digestion process, a two-stage fermentation consists of acidogenesis (or dark fermentation) and methanogenesis phases. In the acidogenesis phase, organic compounds are converted to hydrogen, carbon dioxide and volatile fatty acids (VFAs) by acidogenic bacteria. The effluent is primarily composed of VFAs from the first phase, which are further converted to methane and carbon dioxide in the methanogenesis phase. The main differences between the acidogenic and methanogenic phases are the optimum pH and the growth rate of the microbial communities. The microbes in the acidogenic phase are fast-growing bacteria that prefer a pH range of 5.5–6.5 [5,6] whereas 6.8–7.2 is a suitable pH for the slower-growing methanogens [7]. Therefore, it is necessary to independently optimize the reactors for hydrogen and methane production in order to achieve a successful two-stage fermentation system [8]. Moreover, the operation of two-stage fermentation using two separate reactors could efficiently control acidification in the first reactor. Consequently, VFAs accumulation in the second reactor could be controlled. Hence, the stability of the whole process could be increased. Previous research indicated that the overall energy recovery from the two-stage process is generally higher than one-stage process since hydrogen is produced in the first phase in addition to the methane produced in the second phase. Additionally, the two-stage process demonstrated a greater process stability than one-stage process. For example, Massanet-Nicolau et al. [9] found that the two-stage digestion of grass gave an overall energy recovery 13% higher than one-stage digestion. Besides, the two-stage digestion could be operated at a shorter hydraulic retention times (HRT) (12 days instead of 20 days in a single stage) without any effects on gas yields and process stability. Schievano et al. [10] reported that the two-stage anaerobic digestion process of four different substrates resulted in significantly higher overall energy recovery (8–43%) than one-stage process. The comparative performance of one- and two-stage fermentation of food waste was conducted by Nathao et al. [11]. They observed that the first stage fermentation plays significant role in the overall substrate degradation efficiency and energy recovery. The two-stage fermentation improved 18% of total energy recovery from food waste compared to the one-stage fermentation. However, there was a report on the drawbacks of the separation of acidogenic and methanogenic phase into two reactors due to an adverse effect on the diversity of microbial community in each rector. Schievano et al. [12] found that by separation of the fermentation into two reactors, the microbial community in each reactor was less diverse and consequently resulted in less substrate degradation efficiency.

Continuous stirred tank reactors (CSTR) have been primarily used in anaerobic fermentation processes due to their design simplicity and ease of monitoring important parameters [13]. Complete mixing is good for substrate-inoculum contact, but less efficient in biomass retention at short HRT [14]. HRT is defined as the time that liquid (fermentation broth) remains in a reactor. It is related to the working volume of the reactor and the influent flow rate. The HRT can affect substrate uptake efficiency, the microbial population and metabolic pathways [15]. Since hydrogen producers are fast growing bacteria, a CSTR is a suitable reactor for these microorganisms. An up-flow anaerobic sludge blanket (UASB) reactor has been successfully used for treatment of wastewater and to produce methane owing to its high efficiency and process stability [16]. This reactor has a gas-liquid-solid separator at its top, resulting in efficient biomass retention and a large biomass accumulation at the bottom of the reactor [17]. Its ability to retain biomass makes UASB suitable reactor for slow-growing methanogens.

Normally, a buffer tank is installed between the stages of such processes to allow pH adjustment prior to feeding the methanogenic reactor [18]. The disadvantages of having a buffer tank are its increased design complexity and greater construction and operational costs [8]. Thus, the two reactors should be directly integrated to reduce costs and complexity as well as to realize continuous operation [19]. Therefore, the aim of this study was to directly integrate a CSTR with an UASB reactor, and subsequently optimized the HRTs for hydrogen and methane production in the CSTR and UASB after integration. In addition, the effect of variation of HRT on the microbial community was analyzed by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) in order to evaluate the stability of the process. The results of this study would provide a two-stage hydrogen and methane production system with a reduced design complexity. Costs of construction and operation are also reduced.

Materials and methods

Feedstock preparation

Sugarcane stalks (*Saccharum offinarum* Linn.) were obtained from a sugarcane field in Chaiyaphum Province, Thailand. They were crushed to collect sugarcane juice. To facilitate long storage time, the sugarcane juice was concentrated to sugarcane syrup and kept at 4 °C. Prior to use, sugarcane syrup was diluted to a concentration of 25 g-COD/L and supplemented with inorganic nutrients consisting of (all in mg/L): K_2HPO_4 125; $MgCl_2 \cdot 6H_2O$ 15; $FeSO_4 \cdot 7H_2O$ 25; $CuSO_4 \cdot 5H_2O$ 5; $CoCl_2 \cdot 5H_2O$ 0.125; NH_4HCO_3 5240 and $NaHCO_3$ 6720 (modified from Ref. [20]). The feedstock was kept in a storage tank at 4 °C during continuous feeding of the reactor.

Inocula preparation

Clostridium butyricum TISTR1032 was used as an inoculum for hydrogen production. The strain, TISTR1032, was purchased from the Thailand Institute of Scientific and Technological Research (TISTR), Thailand. The activation and enrichment methods followed the method described by Pattra et al. [21].

Anaerobic granular sludge was used as an inoculum for methane production. It was collected from an UASB wastewater treatment plant of a local brewery industry in Khon Kaen, Thailand. The granular sludge was used directly to produce methane without pretreatment. Total solids (TS) and volatile solids (VS) of the granular sludge were 77.6 and 60.1 g/L, respectively.

Reactor set-up and operation

Reactor size

This study attempted to directly integrate a CSTR with an UASB reactor. This direct integration means that the effluent from the CSTR is directly fed to the UASB reactor. Thus, the flow rates entering of the two reactors are the same. From our previous study [22], we found that the optimum HRTs for separate hydrogen and methane production in a CSTR and UASB were 4 h and 4 d, respectively, which was equivalent to a HRT ratio (CSTR:UASB) of 1:24. By fixing the HRT ratio (CSTR:UASB) of 1:24 with the same flow rate of the two reactors implied that the working volumes of the CSTR and UASB should be adjusted to a ratio of 1:24.

CSTR

A CSTR with a diameter of 10 cm and a height of 22 cm was used as a hydrogen reactor. A working volume of the reactor was 1 L. The reactor was equipped with a thermometer and a pH probe connected to a digital pH meter (pH 190 Series, Eutech Instruments). The reactor was fed with 850 mL of feedstock and 150 mL of enriched *C. butyricum* (cell concentration was 10^7 cell/mL) as inoculum and flushed with pure nitrogen at the bottom of the reactor for 15 min to create anaerobic conditions. It was initially operated in batch mode to allow growth of bacterial cells under mesophilic conditions (37 ± 1 °C). Temperature was controlled by a water jacket surrounding the reactor. The water jacket circulated water from water bath (Julabo TW20, Germany). The reactor was continuously stirred at 150 rpm using motor stirrer (Heidon BL1200, SHINTO Scientific, Japan). After 12 h of batch operation, flow of feedstock from the media tank was started. It was fed into the inlet port at the bottom of the reactor by a peristaltic pump with an initial HRT of 12 h. The effluent from the reactor was continuously removed from the outlet port at the same flow rate by another peristaltic pump. Resulting biogas was discharged through the gas outlet port of the reactor and measured using a wet-gas counter apparatus.

UASB

The UASB reactor for methane production used in the current study had a diameter of 14 cm and a height of 175 cm. The working volume of the reactor was 24 L. The reactor was filled with 15 L of the granular sludge and 9 L of effluent collected from the CSTR. After the reactor was closed, it was purged with nitrogen gas to create anaerobic conditions and operated in batch mode for 2 weeks in order to allow growth of microbial cells at room temperature (30 ± 2 °C). Afterwards, the effluent collected from the CSTR was continuously fed into the inlet port at the bottom of the reactor by a peristaltic pump with an HRT of 12 d. Wet-gas counter was used to measure volume of the biogas.

Integration of two-stage fermentation system

An integrated two-stage fermentation system was used to produce hydrogen and methane from sugarcane syrup. The CSTR and UASB reactors were started and initially operated at HRTs 12 h and 12 d, respectively, until biogas production reached a steady-state condition (less than 10% variation of biogas production rate) for 7–10 days. Afterwards, they were integrated by connecting the effluent port of the CSTR into the inlet port of the UASB. The system was operated at the same HRT until both of reactors reached a steady-state condition. Then the HRT of CSTR was stepwise decreased from 12 to 6, 4, 3, and 2 h. The HRT of the UASB was accordingly decreased. Liquid samples were taken every 3 days from the effluent ports of the CSTR and UASB reactors to determine COD, VFAs, and total sugar concentrations. Biogases produced from the two reactors were measured separately using a wet-gas counter apparatus. A schematic diagram of the two-stage CSTR and UASB reactor is shown in Fig. 1.

Analytical methods

TS and VS of the granular sludge were analyzed according to standard methods [23]. The pH of the fermentation broth was measured using a pH meter (pH-500 Clean, USA). Biogas samples were taken daily from the gas sampling port of each reactor. The hydrogen, methane and carbon dioxide content of the gases was determined using a gas chromatography (GC-2014, Shimadzu Co. Ltd.) equipped with a thermal conductivity detector (TCD) and a 2-m stainless steel column packed with Shin carbon (50/80 mesh). The GC protocol followed the method of Pattra et al. [24]. The hydrogen and methane volumes in the collected biogas were calculated by multiplying the biogas volume by its proportion of hydrogen or methane. The hydrogen production rate (HPR) and methane production rate (MPR) were expressed as L-H_2/$L_{reactor}$.d and L-CH_4/$L_{reactor}$.d, respectively. Hydrogen yield (HY) was expressed in mol-H_2/mol-hexose$_{consumed}$. The measured volumes of hydrogen and methane were expressed at standard temperature and pressure (STP, 0 °C and 760 mmHg).

The effluents from CSTR and UASB reactors were sampled every 3 days to determine COD, VFAs, and total sugar concentrations. A volume of 2 mL of each sample was centrifuged at 10,000 rpm for 5 min (WiseSpin® CF-10). A volume of 1 mL of supernatant was removed and placed in a new microtube. It was kept at −20 °C prior to COD determination according to standard methods [23]. Total sugar concentration was measured using a phenol sulfuric method with a glucose as a standard [25]. Another 0.8 mL of supernatant was acidified by mixing it with 0.2 mL of 0.2 M oxalic acid, and filtering it through a 0.45 mm cellulose acetate membrane. It was kept at −20 °C prior to using high performance liquid chromatography (HPLC) (Shimadzu LC-10AD) to determine its VFA concentrations. The HPLC was equipped with a VertiSep™ OA 8 μm column and a refractive index detector (RID). The temperature of the column was 40 °C. H_2SO_4 at a concentration of 4 mM was used as the mobile phase at a flow rate of 0.5 mL/min.

To determine the alkalinity of the UASB reactor, liquid samples were taken during the steady-state at each HRT. The

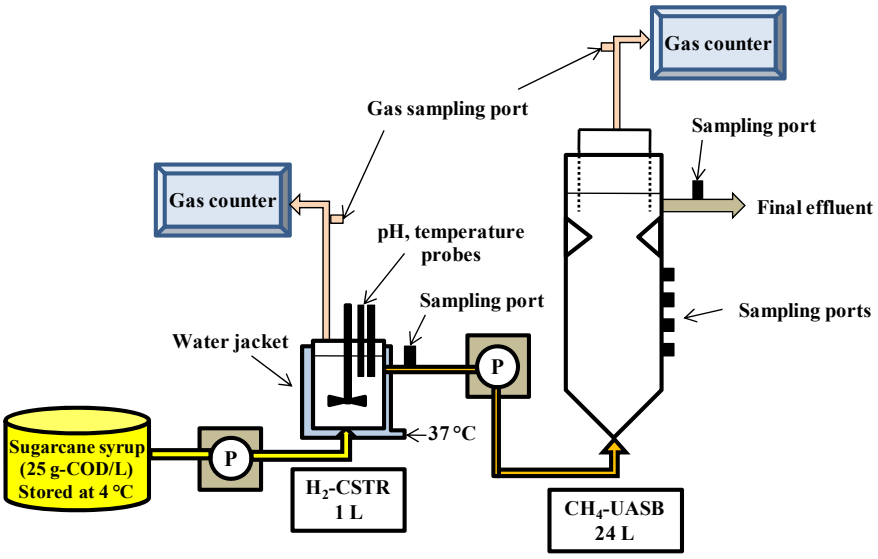

Fig. 1 – Schematic diagram of the integration of the CSTR and UASB reactors (not subjected to scale).

samples were centrifuged at 10,000 rpm for 5 min. The supernatants were collected and analyzed for alkalinity using standard methods [23].

To achieve the total energy recovery of two-stage fermentation system, HPR and MPR from the system in L-H_2/$L_{reactor}$.d and L-CH_4/$L_{reactor}$.d, respectively, were converted to energy production rate in kJ/$L_{reactor}$.d units by multiplying HPR or MPR by the energy content of hydrogen or methane, respectively. The hydrogen energy content was 10.8 kJ/L (STP) (equivalent to 121 kJ/g-H_2) and the methane energy content was 36 kJ/L (STP) (equivalent to 50 kJ/g-CH_4).

Microbial community analysis

The fermentation broth was taken from the sampling ports of the CSTR and UASB reactors at the steady-state at each HRT. The samples were centrifuged at 10,000 rpm for 5 min. The supernatant was discarded. The solids were kept in 50% sterile glycerol at −20 °C prior to analyzing the microbial communities using PCR-DGGE following the method of Kongjan et al. [26]. Most of the bands were excised from the gel and re-amplified with primer 357f without a GC clamp or the reverse primer 518r. After re-amplification, PCR products were purified and sequenced by Macrogen Inc. (Seoul, Korea). Closest matches for partial 16S rRNA gene sequences were identified by database searches in GenBank using BLAST [27].

Results and discussion

Hydrogen production from sugarcane syrup in CSTR

After start-up, the performance of the CSTR in producing hydrogen at different HRTs was investigated using stepwise decreases in the HRT from 12 to 6, 4, 3 and 2 h (Fig. 2(a)). Fig. 2 shows a HRT-dependent profile of hydrogen production in the CSTR. The experimental data under steady-state conditions at each HRT are summarized in Table 1. The reactor showed stable biogas production at every HRT and the biogas was comprised of hydrogen and carbon dioxide. Methane was not detected. The pH varied between 4.5 and 6.1 depending upon the HRT (Fig. 2(b)). The HPR was gradually increased from 1.18 ± 0.12 L/L.d to 8.67 ± 0.33 L/L.d as the HRT was decreased from 12 to 4 h (Table 1). Hydrogen content varied between 19.7 ± 1.0% to 24.1 ± 1.0% at HRTs of 12, 6, and 4 h. When the HRT was further decreased to 3 h, the HPR and H_2 content rapidly increased to 17.5 ± 0.30 L/L.d and 30.3 ± 1.1%, respectively. However, when the HRT was further decreased to 2 h, the HPR and H_2 content slightly decreased to 17.4 ± 0.63 L/L.d and 26.6 ± 0.4%, respectively. These results indicated that HRT significantly affected hydrogen production in the CSTR. At an HRT of 2 h, the reactor showed an instability in hydrogen production as a fluctuation in the HPR in the reactor was observed (Fig. 2(c)). This could be due to microorganisms in the reactor having insufficient time to hydrolyze sugar substrates at a short HRT. This was confirmed by observation of the lowest sugar consumption at HRT of 2 h (Fig. 2(d)). Subsequently, the HRT was increased to 3 h to confirm this experimental result. A similar hydrogen production profile was observed in comparison to the previous run at a HRT of 3 h. Therefore, a HRT of 3 h was chosen as the optimum HRT for hydrogen production from sugarcane syrup in the CSTR.

Soluble metabolite products (SMPs) in the hydrogenogenic effluent

The HRT not only influenced the HPR (as discussed in Section Hydrogen production from sugarcane syrup in CSTR), but also significantly affected the production of SMPs (Fig. 2(e)). The performance of the CSTR in hydrogen production can be characterized by the SMPs at each HRT. In this study, various kinds of SMPs were generated from the CSTR in different concentrations depending on the HRT as shown in Table 2. Acetic, butyric and lactic acids were found to be main components in the effluent at every HRT.

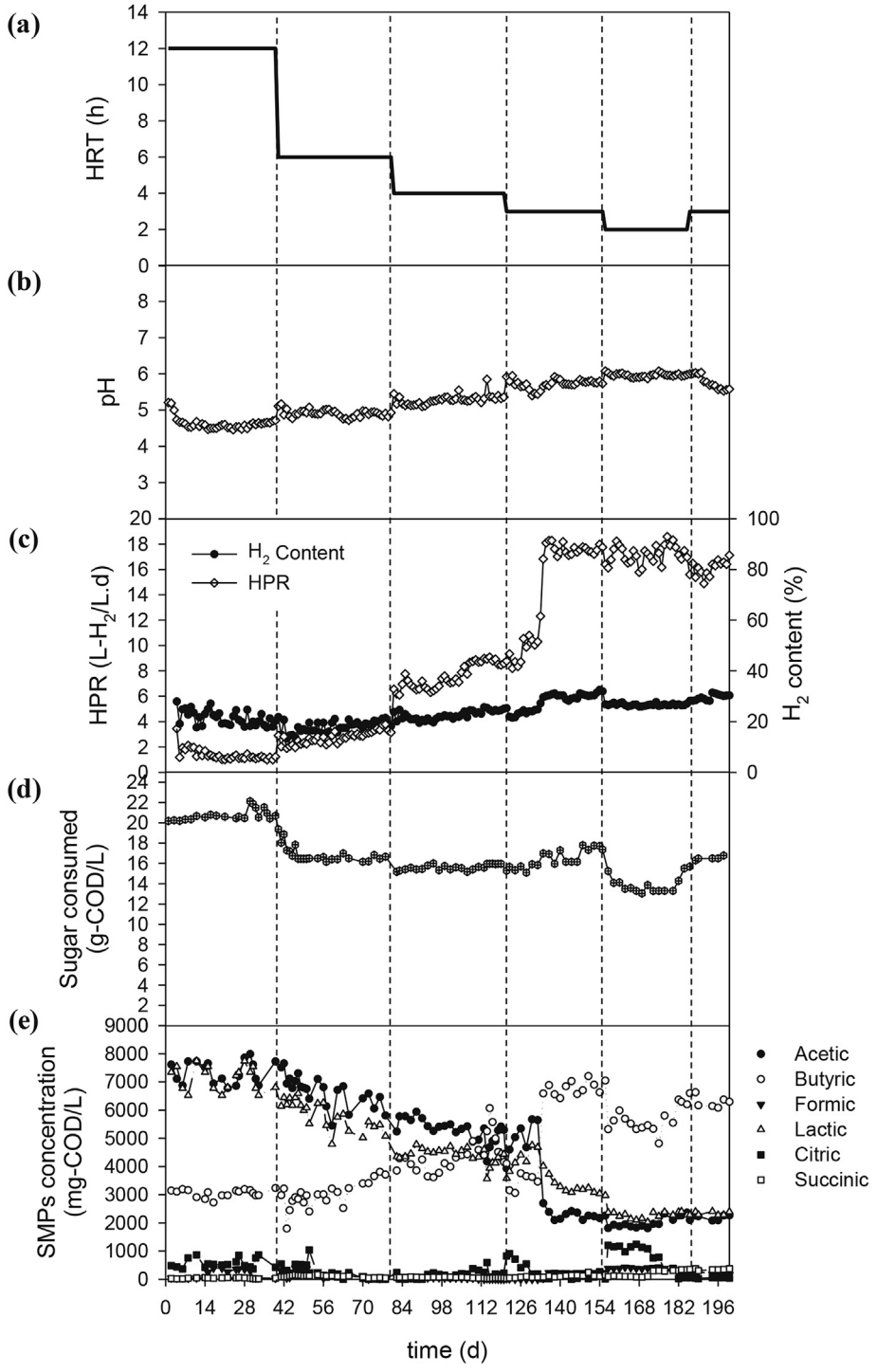

Fig. 2 – HRT-dependent profile of the H_2-CSTR: (a) HRT, (b) HPR and H_2 content, (c) sugar consumption, (d) pH in the CSTR, and (e) SMPs concentrations.

HY obtained in this experiment was directly related to the SMP distribution. HY was lowest at an HRT of 12 h and gradually increased with decreasing HRT. The highest HY of 1.32 ± 0.16 mol/mol-hexose$_{consumed}$ was obtained from the optimum HRT of 3 h (Table 1). The presence of lactic acid in fermentation broth is correlated with a low HY (as discussed in Section DGGE profile of two-stage hydrogen and methane production). This is because when lactic acid is produced, substrate is used to produce only lactic acid (Eq. (1)) as hydrogen is neither produced nor consumed. Therefore, the hydrogen production pathway competed with the lactic acid production pathway for substrate.

Table 1 – Experimental data under steady-state conditions in the two-stage hydrogen and methane production at each HRT.

Parameters	Units	Values				
Hydrogen production (first phase)						
HRT	H	12	6	4	3	2
HPR	L/L.d	1.18 ± 0.12	3.34 ± 0.25	8.67 ± 0.33	17.5 ± 0.30	17.4 ± 0.63
H_2 yield	mol/mol-hexose$_{consumed}$	0.30 ± 0.03	0.42 ± 0.09	0.82 ± 0.12	1.32 ± 0.16	1.28 ± 0.15
H_2 content	%	20.8 ± 2.6	19.7 ± 1.0	24.1 ± 1.0	30.3 ± 1.1	26.6 ± 0.4
VSS	g/L	2.70	2.43	2.33	2.87	2.89
H_2-EPR	kJ/L.d	12.74	36.07	93.64	189.00	187.92
Methane production (second phase)						
HRT	d	12	6	4	3	2
MPR	L/L.d	0.60 ± 0.04	1.38 ± 0.08	1.93 ± 0.12	2.25 ± 0.06	3.14 ± 0.16
CH_4 content	%	67.0 ± 4.1	61.3 ± 1.9	59.5 ± 2.3	63.6 ± 1.4	58.5 ± 1.1
VSS	g/L	56.16	55.67	56.14	62.60	64.44
Alkalinity	mg-$CaCO_3$/L	7500	7100	7100	6700	6600
VFA/alkalinity ratio	–	0.15	0.19	0.27	0.25	0.34
CH_4-EPR	kJ/L.d	21.6	49.68	69.48	81.00	113.04
Total (first phase + second phase)						
Total COD removal	%	98.66	98.48	98.07	97.55	96.58
Total EPR	kJ/L.d	34.34	85.75	163.12	270.00	300.96

HRT: Hydraulic retention time.
HPR: Hydrogen production rate.
MPR: Methane production rate.
EPR: Energy production rate.
VSS: Volatile suspended solid.

Table 2 – Soluble metabolite products in the hydrogenogenic effluent of the CSTR.

HRT (h)	Soluble metabolite products (mg-COD/L)						Residual sugar (g-COD/L)
	Acetic	Butyric	Formic	Lactic	Citric	Succinic	
12	7359.17 ± 461.35	3084.88 ± 87.81	348.77 ± 28.28	7212.67 ± 426.96	625.10 ± 204.08	32.01 ± 16.49	4.33 ± 1.17
6	6339.54 ± 396.07	3351.62 ± 414.45	336.00 ± 66.41	5426.18 ± 300.46	399.82 ± 160.34	51.31 ± 14.73	8.48 ± 1.05
4	5047.40 ± 372.19	4798.66 ± 579.47	42.89 ± 6.56	4286.92 ± 313.23	243.77 ± 86.91	44.10 ± 12.68	8.81 ± 1.25
3	2230.50 ± 114.07	6761.97 ± 250.87	228.89 ± 5.66	3185.72 ± 120.02	174.85 ± 60.31	140.40 ± 45.99	7.78 ± 1.04
2	2009.21 ± 183.72	5389.45 ± 326.19	399.96 ± 22.05	2237.87 ± 135.72	1063.58 ± 172.04	297.81 ± 8.57	9.69 ± 1.02

$$C_6H_{12}O_6 \rightarrow 2C_3H_6O_3 \quad (1)$$

At a HRT of 12 h, the highest concentration of lactic acid was observed, which was consistent with the lowest HY at this HRT. However, lactic acid gradually decreased when HRT was decreased from 12 to 2 h, which is consistent with an increase in HY with decreasing HRT. Acetic acid showed similar trend as that of lactic acid. The concentration of acetic acid decreased with decreasing HRT. Theoretically, when acetic acid is the only by-product from dark fermentation, a maximum HY of 4 mol-H_2/mol-hexose can be obtained (Eq. (2)). If butyric is the only by-product of the fermentation, a maximum HY of 2 mol-H_2/mol-hexose can be obtained (Eq. (3)).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2C_2H_4O_2 + 4H_2 + 2CO_2 \quad (2)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2C_4H_8O_2 + 2H_2 + 2CO_2 \quad (3)$$

However, in practice, acetic acid presence in the fermentation broth does not necessarily ensure a high HY. This is because acetic acid can also be generated by hydrogen consumers, i.e., homoacetogens (Eq. (4)). Therefore, the decreasing content of acetic acid with shortened HRTs may have resulted from washing out of slow-growing homoacetogens from the reactor. Less hydrogen was then consumed by homoacetogens resulting in increased HY.

$$4H_2 + 2CO_2 \rightarrow C_2H_4O_2 + 2H_2O \quad (4)$$

The concentration of butyric acid increased when HRT was decreased from 12 to 3 h, which correlated to an increase in HY. At the optimum HRT of 3 h, the concentration of butyric acid reached its highest concentration, 53% of the total SMPs, indicating a butyrate-type fermentation. Further decreasing the HRT to 2 h resulted in a decrease in butyric acid concentration, which correlated to a slight decrease in HY. Consequently, the butyric acid concentration in the fermentation broth was a good indicator for the level of hydrogen production in this study. The general metabolic pathway for hydrogen production by *C. butyricum* was reported to be a butyrate type fermentation [17,28,29].

Methane production from hydrogenogenic effluent in UASB

Variation in methane production of an UASB reactor as a function of HRT in the CSTR was investigated. The UASB reactor was fed directly with the hydrogenogenic effluent from the CSTR to produce methane. The methane production profile from the effluent of the CSTR in the UASB reactor is shown in Fig. 3. The HRT was stepwise decreased from 12 to 6, 4, 3 and 2 d (Fig. 3(a)). The reactor showed a high efficiency and process stability through 200 d of operation. Only methane and carbon dioxide were detected in the biogas. VFAs in the methanogenic effluent were lower than 50 mg-COD/L (data not shown), revealing an effective conversion of VFAs to methane by methanogens. The pH of the effluent was found to be stable in the range of 7.0–8.0 (Fig. 3(b)), indicating a well-buffering reactor. Results showed that MPR gradually increased from 0.60 to 2.25 L/L.d when the HRT was decreased from 12 to 3 d (Table 1). The methane content in biogas varied in a range of 60–67%.

The stability of methanogenic reactor was assessed by the VFAs/alkalinity ratio. In general, the VFAs/alkalinity ratio should be kept lower than 0.4 to avoid process instabilities [30]. The UASB reactor was stable at every HRT as evidenced by the observation that VFAs/alkalinity ratios were between 0.15 and 0.27 (Table 1). When the HRT was further decreased to 2 d, biogas production sharply increased and a MPR of 3.14 L/L.d was achieved (Fig. 3(c)). This sharp increase in biogas and methane production might have been caused by a decrease in the HRT (or an increase in the organic loading rate). Even though the highest MPR was achieved at a HRT of 2 d, the methanogenic reactor was unstable. At a HRT of 2 d, methane content in the biogas was decreased from 63.6 to 58.5%, the sludge flocculated and washed out of the reactor. Moreover, the VFAs/alkalinity ratio observed at this HRT was 0.34, which was near the critical value of 0.4. Therefore, using an HRT of 2 d risked failure of the methanogenic reactor. Subsequently, the HRT was increased to 3 d and operated at this HRT for 2 weeks to recover the stability of the reactor and confirm the optimum HRT. The MPR and CH_4 content at this HRT was similar to when the system was earlier operated with a HRT of 3 d. The sludge flocculation and washout decreased demonstrating that the stability of reactor was recovered. Therefore, a HRT of 3 d was chosen as the optimum HRT at which the MPR and CH_4 content were 2.25 L/L.d and 63.6%

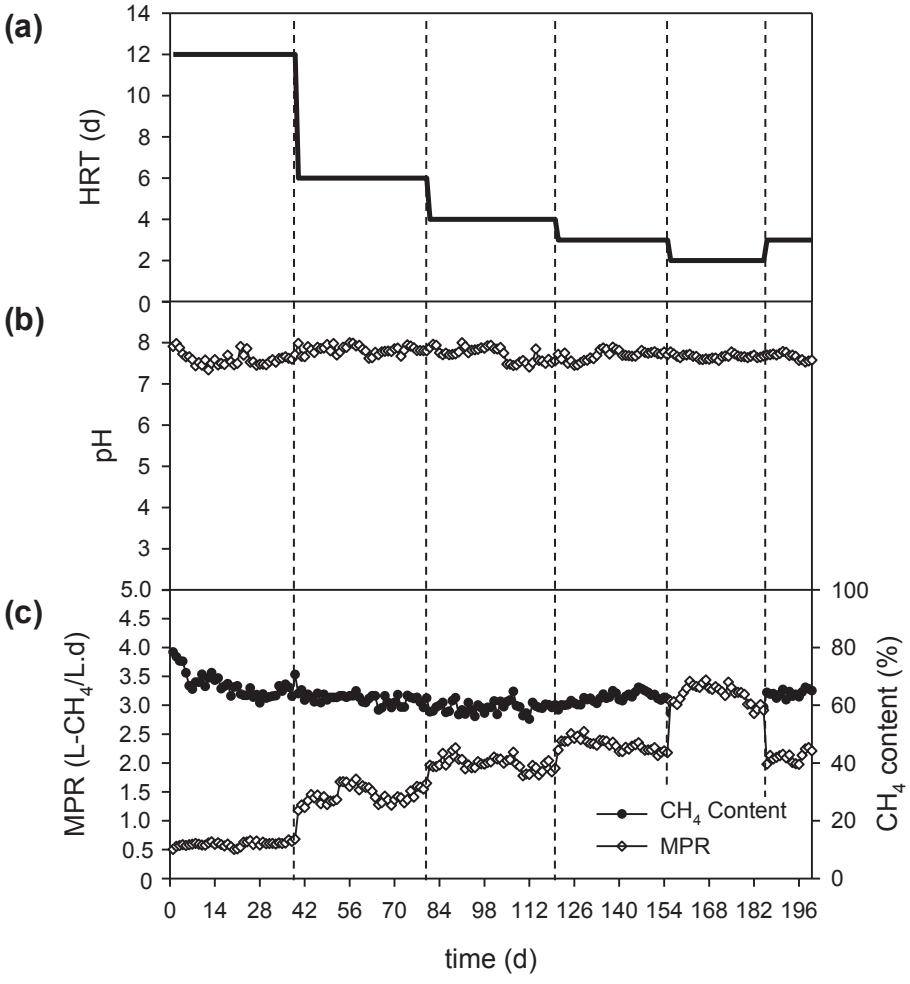

Fig. 3 – HRT-dependent profile of the CH_4-UASB reactor: (a) HRT, (b) MPR and CH_4 content, and (c) pH of the methanogenic effluent.

respectively obtained. COD removal of the UASB reactor at this optimum HRT was 97.55% (Table 1).

Energy production rate (EPR) and COD balance

At the optimum HRT (3 h in CSTR and 3 d in UASB), an EPR of 189 kJ/L.d was obtained from the fermentation of sugarcane syrup in the CSTR. The effluent from this process was converted to methane in the UASB reactor with an EPR of 81 kJ/L.d. The total EPR obtained from the two-stage fermentation system was 270 kJ/L.d. Table 3 shows the HPR, MPR, and EPR values of two-stage fermentation process using carbohydrate-rich wastes as substrates. The EPRs were different depending on the nature of substrate. This is not surprising since the hydrolysis of complex substrates into solubilized products is recognized as the rate-limiting step of anaerobic digestion processes [35]. Therefore, the highest total EPR was achieved in this study since the main composition of the sugarcane syrup was readily-usable sugars. Table 4 tabulated the comparison of overall energy yield from various types of substrates by one- and two-stage fermentation process. The data indicated that the overall energy yields of two-stage fermentation process were greater than one-stage process.

The COD balance of this two-stage hydrogen and methane production at steady-state of each HRT is shown in Table 5. The missing COD value was in the range of 8.36–13.70%. It may have resulted from unmeasured biomass. In general, approximately 10% of the biodegradable organic matter is utilized for bacterial growth in an anaerobic fermentation [38]. Thus, the results of the COD balance indicate the accuracy of the experimental data.

Comparison of one-stage and two-stage fermentation process

Our previous study [39] indicated that methane could not be produced from sugarcane syrup at 25 g-COD/L by one-stage fermentation without pH control. This is because the sugars in sugarcane syrup were rapidly consumed by acidogenic bacteria and converted to a large amount of VFAs. Subsequently the methanogenic activity was inhibited by acidic conditions in the fermentation broth. Our results are coincided with the work of Luo et al. [40] who found that one-stage methane production from a mixture of effluents (i.e. cake, glycerol, stillage) produced from rapeseed biodiesel failed due to a drop of pH caused from the build-up of VFAs while the two-stage hydrogen/methane remained stable. Therefore, the benefits of using two-stage fermentation process are not only high overall energy production, but also high stability of the whole process caused by a separation of the fermentation process into two stages.

DGGE profile of two-stage hydrogen and methane production

Fig. 4 shows DGGE analysis of microbial community in the CSTR at different HRT values. At a HRT 12 h, *Clostridium* sp. (band 17) and *Acotostipes* sp. (band 18) were dominant,

Table 3 – Comparison of the two-stage fermentation system with previous studies.

Substrate	Reactor type H_2 / CH_4	Temp. (°C)	HRT	Hydrogen			Methane			Total EPR (kJ/L.d)	Ref.
				HY (mL/g-COD)	HPR (L/L.d)	EPR (kJ/L.d)	MY (mL/g-COD)	MPR (L/L.d)	EPR (kJ/L.d)		
Molasses	PBR	35	6 h	25[a]	2.80	30.24	317[a]	1.94	69.84	100.08	[31]
	PBR	35	6 d								
Food waste	CSTR	37	2 d	104.1	4.80	51.84	278.3	3.20	115.20	167.04	[32]
	CSTR	37	7 d								
Sugary wastewater	CSTR	35	5 h	105.8[a]	3.06	33.05	341.5[a]	2.01	72.36	105.41	[15]
	UASB	35	15 h								
Cassava wastewater	UASB	55	–	54.2	0.53	5.72	164.9	0.65	23.40	29.12	[33]
	UASB	55	–								
Skim latex serum	UASB	55	36 h	63.0[a]	1.50	16.2	179.4[a]	0.71	25.56	41.76	[34]
	UASB	55	9 d								
Palm oil mill effluent	ASBR	55	2 d	210	1.84	19.87[b]	315.0	2.60	93.60[b]	113.47[b]	[45]
	UASB	35	15 d								
Palm oil mill effluent	UASB	55	2 d	215	1.90	20.52[b]	320.0	3.20	115.20[b]	135.72[b]	[46]
	CSTR	37	5 d								
Sugarcane syrup	CSTR	37	3 h	88.0	17.50	189.00	271.4	2.25	81.00	270.00	This study
	UASB	30	3 d								

PBR: Packed bed reactor; CSTR: Continuous stirred tank reactor; UASB: Up-flow anaerobic sludge blanket.
HRT: Hydraulic retention time.
HY: Hydrogen yield; HPR: Hydrogen production rate; EPR: Energy production rate; MY: Methane yield; MPR: Methane production rate.
[a] Converted from the original data and expressed in mL/g-COD unit.
[b] Calculated from the original data.

Table 4 – Comparison of hydrogen and methane energy yield from one- and two-stage fermentation process.

Substrate	Fermentation system	Hydrogen		Methane		Total energy yield (kJ/g-VS)	Substrate removal efficiency (%)	Ref.
		HY (mL/g-VS)	EY (kJ/g-VS)	MY (mL/g-VS)	EY (kJ/g-VS)			
Agave tequilana bagasse hydrolysate	One-stage (acid hydrolysate)	–	–	163[a]	5.84[a]	5.84	NA	[36]
	Two-stage (enzymatic hydrolysate)	48[a]	0.61[a]	356[a]	12.74[a]	13.35		
Pelletized grass	One-stage	–	–	310	10.36	10.36	63.88 (VS)	[9]
	Two-stage	6.7	0.07	349	11.67	11.74	67.17 (VS)	
MS + SM	One-stage	–	–	431	15.17	15.17	NA	[10]
	Two-stage	100	1.27	504	17.71	18.98		
RF + SM	One-stage	–	–	295	10.38	10.38		
	Two-stage	116	1.48	305	10.74	12.22		
FV + SM	One-stage	–	–	293	10.30	10.30		
	Two-stage	124	1.58	373	13.12	14.70		
Food waste	One-stage	–	–	82	2.95[b]	2.95	NA	[11]
	Two-stage	55	0.59[b]	94	3.38[b]	3.97		
Sun flower stalk	One-stage	–	–	191	6.88[b]	6.88	49.6 (VS)	[37]
	Two-stage	6.3	0.07[b]	196	7.06[b]	7.13	50.6 (VS)	
Palm oil mill effluent	One-stage	–	–	227[c]	8.17[c]	9.08[c]	84.0 (COD)	[45]
	Two-stage	210[c]	2.72[c]	315[c]	12.62[c]	15.34[c]	95.0 (COD)	
Co-digestion of ES + CW + LCM	Two-stage	12.5[a]	0.14[a]	223	8.03[a]	8.17[a]	84.8 (COD)	[47]
Sugarcane bagasse hydrolysate	Two-stage	93.4	NA	222	NA	8.40	58.0 (TS)	[48]
Sugarcane syrup	Two-stage	88[c]	0.95[c]	271[c]	9.76[c]	10.71[c]	97.5 (COD)	This study

TS: total solid; VS: volatile solid; COD: chemical oxygen demand.
NA: No data available.
MS: maize silage; SM: swine manure; RF: waste rice flour; FV: waste fruit/vegetable; ES: ensiled sorghum; CW: cheese whey; LCW: liquid cow manure.
[a] Calculated from the original data and expressed in mL/g-COD and kJ/g-COD units.
[b] Converted from the original data and expressed in kJ/g-VS unit.
[c] Expressed in mL or kJ per g-COD unit.

Table 5 – COD mass balance in the two-stage hydrogen and methane production at various HRTs.

COD balance (%)					
HRT	Initial	Hydrogen	Methane	Final	Balance
12	100.00	1.79	87.20	1.34	−9.67
6	100.00	2.21	87.91	1.52	−8.36
4	100.00	3.89	83.03	1.93	−11.15
3	100.00	6.29	77.56	2.45	−13.70
2	100.00	4.76	82.63	3.42	−9.20

indicating that augmented *C. butyricum* at the start-up phase competed with *Acotostipes* sp.. *Acotostipes* sp. is a gram positive lactic acid bacterium (LAB) that can produce lactic, acetic and formic acids from sugar [41]. The presence of LAB along with the lactic acid detected in the fermentation broth could explain why the HPR and HY were lowest at this HRT (discussed in Section Soluble metabolite products (SMPs) in the hydrogenogenic effluent). Additionally, LAB can excrete bacteriocins, which can inhibit other microorganisms including hydrogen producing bacteria [42]. *Acotostipes* sp. was found to disappear when the HRT was decreased from 12 to 6 h. This is consistent with the gradual increase in the HPR and HY observed when decreasing the HRT. *Olsenella* sp. (band 12) was another LAB present in the CSTR at every HRT. It was not a dominant species since the intensities of the bands were low, suggesting that this bacterium was present at low levels in the CSTR [43]. *Clostridium* sp. was found to be the dominant species at every HRT, revealing that the *Clostridium* sp. were not affected by shortening the HRT from 12 to 2 h. The normal flora in sugarcane juice consisted of *Hydrogenimonas* sp. (band 9), *Tetrasphaera* sp. (band 16), *Flammeovirga* sp. (band 13), *Tissierella* sp. (band 6), *Butyrivibrio* sp. (band 8), and *Desulfobulbus* sp. (band 3). Even though they did not inhibit hydrogen producers, they competed for sugar and formed no hydrogen. The low HPR and HY may also have been caused by the natural microflora in sugarcane juice. Therefore, running the reactor with a short HRT favored hydrogen producing bacteria, especially *Clostridium* sp., since they prefer a short HRT to produce hydrogen and VFAs in the exponential growth phase [44].

Fig. 5 shows the archaea community in the UASB reactor at different HRT values. *Methanoregula* sp. (bands 2, 4, and 8), *Methanosarcina* sp. (bands 1, 5, and 10) and *Thermococcus* sp. (bands 3 and 7) were the dominant species at every HRT. These microorganisms are methanogens that play an important role in methane production. The results clearly show that

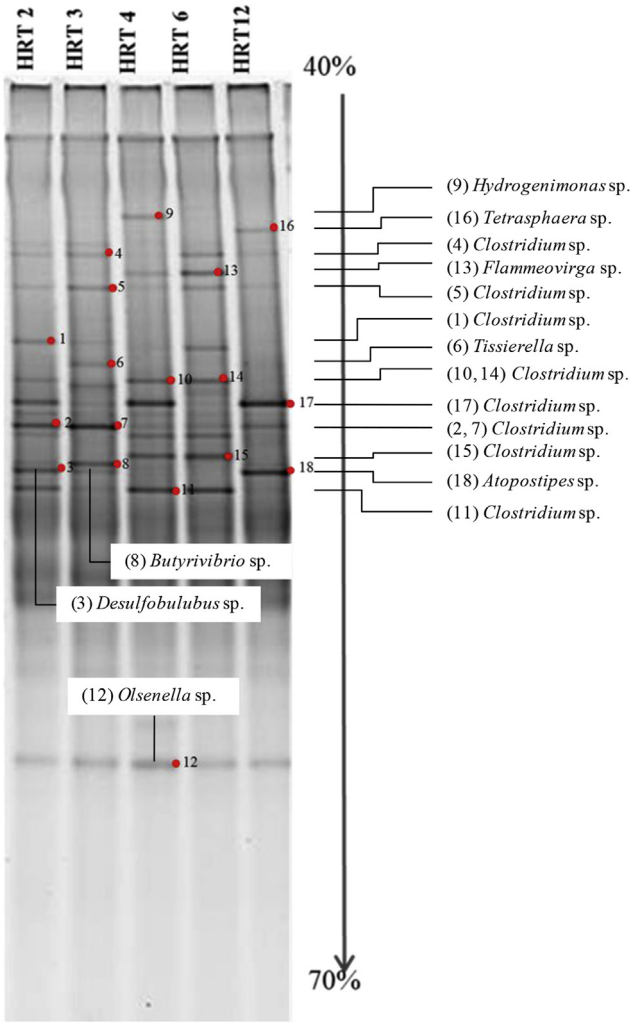

Fig. 4 – PCR-DGGE analysis of microbial community in the CSTR at different HRTs.

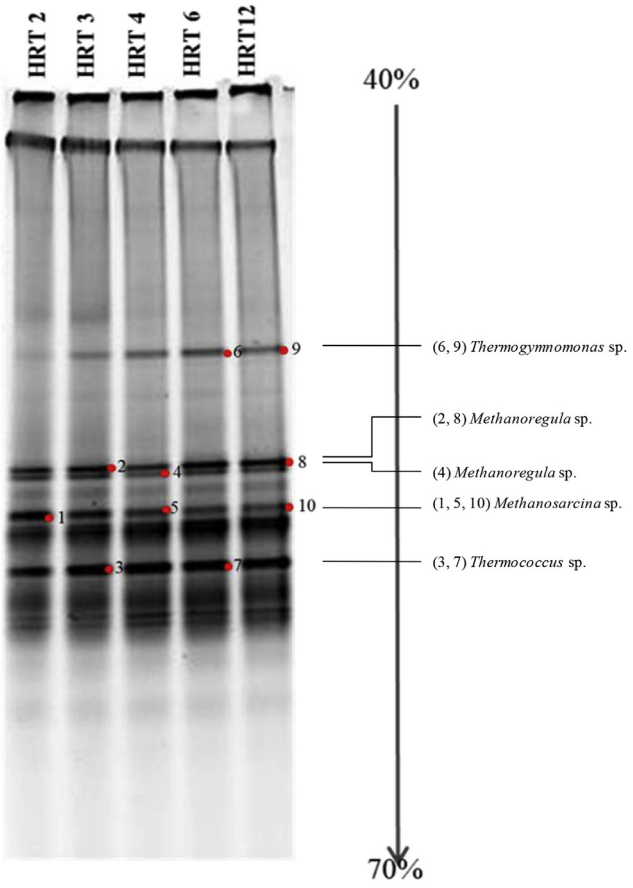

Fig. 5 – PCR-DGGE analysis of archaea community in the UASB reactor at different HRTs.

the archaea community was unchanged when the HRT was varied, indicating stability of the process at every HRT. However, *Thermogymnomonas* sp., thermophilic archaea, were washed out when the HRT was decreased since the conditions in the reactor, i.e., temperature and HRT, might not have been suitable for this species.

Conclusions

Direct integration of CSTR and UASB reactors to design a two-stage hydrogen and methane production system was successful. With no pH control in these two reactors, the system exhibited stable hydrogen and methane production for over 200 days of operation. The HRT significantly affected hydrogen and methane production. At the optimum HRTs, 3 h in the CSTR and 3 d in the UASB, a HPR of 17.5 L/L.d and the MPR of 2.25 L/L.d were obtained with a total EPR of 270 kJ/L.d. The natural microflora in sugarcane syrup was greatly affected by HRT. However, HRT did not affect the archaea community. The methane production in the UASB reactor was stable at every HRT indicated by the VFAs/alkalinity ratio was not above the critical value of 0.4.

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