Renewable Energy 86 (2016) 1224–1231

Contents lists available at ScienceDirect

Renewable Energy

journal homepage: www.elsevier.com/locate/renene

Methane production from acidic effluent discharged after the hydrogen fermentation of sugarcane juice using batch fermentation and UASB reactor

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ARTICLE INFO

Article history:
Received 8 April 2015
Received in revised form
10 September 2015
Accepted 23 September 2015
Available online 6 October 2015

Keywords:
Two-stage
Hydrogen
Methane
Sugarcane juice
Potential

ABSTRACT

Acidic effluent discharged after the hydrogen fermentation of sugarcane juice was used to produce methane by batch fermentation and UASB reactor. Significant parameters affecting methane production including substrate to biomass (S/X) ratio, nickel (Ni) concentration, and cobalt (Co) concentration were optimized by response surface methodology with central composite design in batch mode. A maximum methane yield (MY) of 305.4 mL CH_4/g-volatile solid (VS) $_{substrate\ (sub)\text{-}added}$ was achieved at an S/X ratio of 0.83 g-VS_{sub}/g-$VS_{inoculum}$, a Ni concentration of 0.53 mg/L, and a Co concentration of 0.06 mg/L. Continuous methane production was conducted at various hydraulic retention times (HRT) using the optimum conditions obtained from the batch experiments. The optimum HRT of 4 days in a UASB reactor resulted in a maximum methane production rate (MPR) and MY of 1.27 ± 0.05 L-CH_4/L-culture day and 348 ± 13 mL-CH_4/g-COD, respectively. Total energy generated was 219.23 kJ/L-substrate or 8.77 kJ/g-COD, and COD removal efficiency was 75.60%.

1. Introduction

Biohydrogen production via the dark fermentation process has received considerable attention due to its ease of operation, high rate of hydrogen production, low operation cost, and environmentally friendly nature. During the dark fermentative hydrogen production process, organic substrates such as carbohydrates, lipids, and proteins are hydrolyzed into soluble organic molecules (sugars, fatty acids, amino acids). These are subsequently converted by acidogenic bacteria to hydrogen and carbon dioxide in the gas phase, and volatile fatty acids (VFAs) in the liquid phase. The effluent discharged from the hydrogen fermentation process has a low pH and a high chemical oxygen demand (COD), and should not be disposed of in the environment without pretreatment. The effluent mainly contains acetic acid (HAc) and butyric acid (HBu) that can be further converted to methane by methanogenic bacteria. Both hydrogen and methane are very attractive alternative fuels [1]. Hydrogen is slowly being introduced into the vehicle market, and has enormous potential due to its high energy efficiency and the possibility of use in zero-emission vehicles [1], while methane is already available on the market as a gaseous biofuel and is used in combustion engines. Therefore, the utilization of the acidic effluent obtained from the hydrogen production process for methane production is not only appropriate for environmental treatment but also for energy recovery.

The important parameters controlling methane production have to be optimized to realize the maximum methane production rate (MPR) and methane yield (MY). In this study, the ratio of substrate to biomass (S/X ratio), Ni concentration, and Co concentration (mg/L) were chosen as the key parameters. The S/X ratio is an important parameter for anaerobic digestion of high solids [2]. An S/X ratio that is too high (low inoculum concentration) can be toxic to the microorganisms that secreted enzyme, while an S/X ratio that is too low can inhibit the enzyme [3]. The optimum S/X ratio is varied among the substrates [4]. For example, digesting food waste and the inoculum obtained from mesophilic anaerobic digester at Guelph's wastewater treatment plant in Ontario, Canada, required an S/X ratio of 0.25 to achieve a maximum MY of 1400 mL CH_4/g-

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http://dx.doi.org/10.1016/j.renene.2015.09.051
0960-1481/

VSS_{sub}, while digesting food waste and the inoculum obtained from the mesophilic anaerobic digester at Dufferin Organics Processing Facility in Toronto, Canada, required an S/X ratio of 0.5 to achieve a lower maximum MY of 790 mL CH_4/g-VSS_{sub} [5]. The mixture of 50% food waste and 50% green waste required an S/X ratio of 1.6 to attain the maximum biogas yield of 716 mL/g-VS [6]. Woody feedstocks and municipal wastes required an S/X ratio of 0.5 g-VS_{sub}/g-$VS_{inoculum}$ [7]. Hence, an S/X ratio should be optimized in order to obtain a maximum MPR. Previous research suggested that at a certain time of operation, the biogas production dramatically decreased due to the lack of Ni and Co [8]. Ni and Co are essential co-factors of enzymes involved in the anaerobic digestion process [9,8–11]. Ni is a well-known cofactor of F430, which is required for catalyzing the methane formation from methyl S-CoM in the conversion of acetic acid, hydrogen, and carbon dioxide to methane by acetoclastic and hydrogenotrophic methanogens (Fig. 1) [10]. In addition, Ni helps to maintain cell wall stability in some methanogens [11]. Therefore, an appropriate Ni concentration in the fermentation medium is required for maximizing the methane production process. Co is a key component of corrinoid, which is known to bind to coenzyme M [CoM] methylase. A CoM methylase such as N^5-methyl-tetrahydromethanopterin is used in the catalysis of a methyl-transferring reaction, forming methyl-S-CoM in both acetoclastic methanogens and hydrogenotrophic methanogens (Fig. 1) [12,13]. Co is also contained in the coenzyme F420 (8-hydroxy-5-deazaflavin), which was reported to be found in hydrogenotrophic methanogens [10]. Coenzyme F420 binds to the hydrogenase that is involved in methane production from hydrogen and carbon dioxide in hydrogenotrophic methanogens (Fig. 1) [13]. Thus, in order to achieve a maximum methane production, the concentration of Co should be optimized.

According to the above mentioned information, this study aimed to optimize the significant parameters affecting methane production from the acidic effluent discharged after the hydrogen fermentation process of sugarcane juice in batch and continuous UASB reactors. The batch experiments were designed by using the response surface methodology (RSM) with central composite design (CCD). Optimum conditions were further applied for producing methane by using the UASB reactor at various hydraulic retention times (HRT) in order to achieve a suitable HRT for continuous methane production. Finally, the maximum energy production rate was calculated. The optimum key parameters and hydraulic retention time from this study can be very useful for scaling-up the UASB reactor.

2. Materials and methods

2.1. Preparation of acidic effluent discharged after the hydrogen fermentation of sugarcane juice

The sugarcane (*Saccharum officinarum* Linn.) used in this study was harvested from a sugarcane field in Lopburi Province, Thailand. The sugarcane juice and sugarcane syrup were prepared according to the method previously described by Pattra et al. [14]. The sugarcane syrup had a final total sugar concentration of 800 g/L (856 g-COD/L) and was kept at −20 °C until being used. The frozen sugarcane syrup was thawed by placing it at room temperature, prior to use as a substrate for hydrogen production. The hydrogen production medium was prepared by diluting sugarcane syrup with distilled water to a concentration of 25 g-COD/L, and supplemented with sufficient inorganic nutrients for bacterial growth, including (all in mg/L): NH_4HCO_3 5240, K_2HPO_4 125, $MgCl_2 \cdot 6H_2O$ 15,

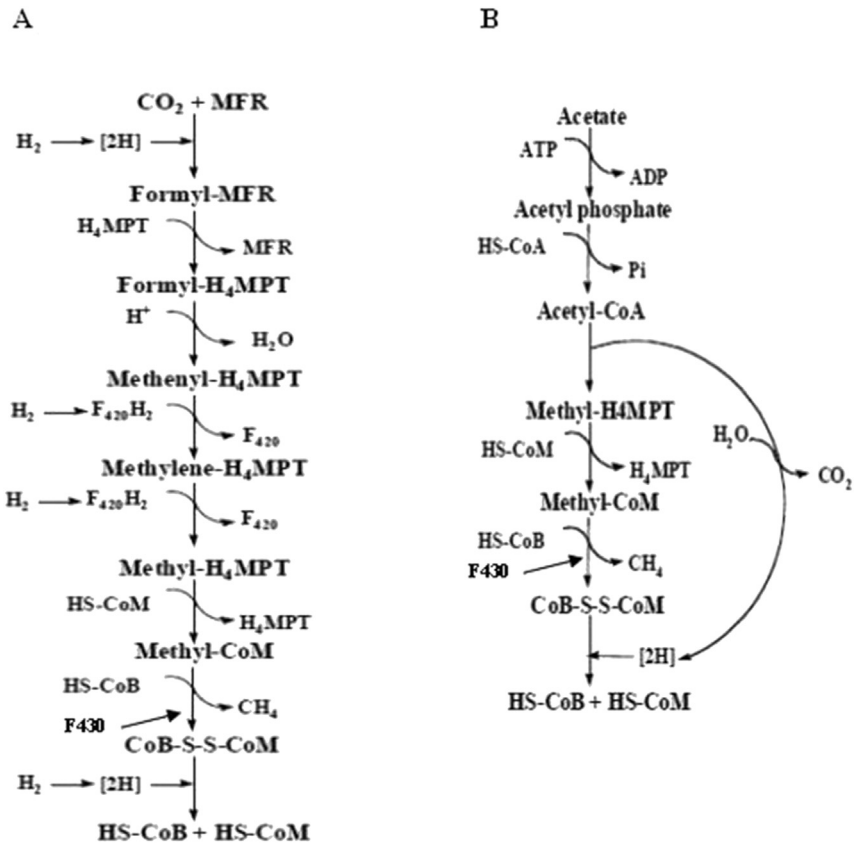

Fig. 1. The methanogenic pathway (A) The pathway used by hydrogenotrophic methanogens; (B) The pathway used by acetoclastic methanogens (Z. Mashhadi, 2010 [9]).

$FeSO_4 \cdot 7H_2O$ 25, $CuSO_4 \cdot 5H_2O$ 5, $CoCl_2 \cdot 5H_2O$ 0.125, and $NaHCO_3$ 6720 [15]. Then, the hydrogen production was conducted in a 5.5 L continuous stirred tank reactor (CSTR) with a working volume of 5 L. The CSTR was operated at the optimum HRT of 4 h following the method of Pattra et al. [14]. After reaching a steady state, the acidic effluent discharged after the hydrogen fermentation was used as the substrate for methane production. The chemical characteristics of the effluent are presented in Table 1. The main VFAs in the effluent is HBu. The COD value of the effluent was 22.50 g-COD/L, and the pH was 5.72. The VS of the effluent was 23.43 g/L. The acidic effluent was kept at 4 °C before being used in the experiment.

2.2. Preparation of the inoculum

Granules from the UASB reactor of a cassava starch manufacturing company in the northeastern part of Thailand were directly used as the seed inoculums for methane production without enrichment. The initial VS and pH of the inoculums were 26.54 g/L and 7.8, respectively.

2.3. Optimization of the key parameters affecting methane production in the batch experiment

RSM with CCD was used to optimize the key factors affecting simultaneous methane production and biodegradability. The investigated parameters included the ratio of substrate to biomass (S/X ratio) (X_1) (g-VS_{sub}/g-$VS_{inoculum}$), Ni concentration (mg/L) (X_2), and Co concentration (mg/L) (X_3). The response variables were MY and biodegradability (Table 2). The MY was calculated by dividing the methane production (mL-CH_4/L) by the substrate concentration (g-VS_{sub}/L). Biodegradability was calculated as follows:

$$\text{Biodegradability (\%)} = [\text{Methane yield at STP (mL-CH}_4\text{/g-COD)}/\text{Theoretical methane yield (350 mL-CH}_4\text{/g-COD)}] \times 100 \quad (1)$$

Design-Expert (Demo version 7.0, Stat-Ease, Inc., Minneapolis, MN, USA) was employed for experimental design, modeling, and graphical display of experimental results.

Production of methane was conducted in 120-mL serum bottles each having 70-mLof working volume. The medium used to produce methane contained acidic effluent, and Ni and Co, as well as the inoculum. Table 2 shows the S/X ratio and concentration of Ni and Co used in the fermentation. The initial pH of the medium was adjusted to 7.0 using 2 mol/L of HCl or 2 mol/L of NaOH. The serum bottles were capped with rubber stoppers and aluminum caps. They were subsequently flushed with nitrogen gas to ensure anaerobic conditions. All bottles were incubated at room temperature (30 ± 2 °C) on a shaker operating at 150 rpm. All experiments were done in triplicates.

Table 1
Composition of the acidic effluent discharged after hydrogen production process at steady state condition.

Composition	Concentration	
	g/L	g-COD/L
Sugarcane juice	3.12 ± 0.14	3.24 ± 0.13
Formic acid	0.10 ± 0.03	0.04 ± 0.01
Acetic acid	1.78 ± 0.06	1.90 ± 0.06
Propionic acid	0.00 ± 0.00	0.00 ± 0.00
Lactic acid	0.87 ± 0.08	0.93 ± 0.09
Butyric acid	4.72 ± 0.12	8.59 ± 0.22
Ethanol	2.10 ± 0.23	4.39 ± 0.48

2.4. UASB reactor setup and operation

The 30-L UASB reactor was used to produce methane. An acrylic material was used to make the reactor. It had dimensions of 14 cm (diameter) by 165 cm (height). This was large enough to allow a 25-L working volume and 5-L head space. Fig. 2 gives a schematic of the UASB reactor. The UASB reactor contained UASB granules as inoculums and acidic effluent as substrate. The acidic effluent from hydrogen production process was used as the substrate and supplemented with the optimum Ni and Co concentration obtained from batch fermentation step (Section 2.3). The reactor was started up using the optimum S/X ratio obtained from batch fermentation step (Section 2.3) for 24 h. The reactor was operated at a temperature of 30 ± 2 °C in the continuous mode at the highest HRT of 6 days (equivalent to the organic loading rate (OLR) of 3.41 ± 0.35 kg-COD/m^3 d) until it reached a steady state. The steady state of each HRT was reached when the variation in the MPR and substrate utilization was ±10%. After reaching a steady state, the HRT was decreased to 5, 4, 3, and 2 days, equivalent to the OLR of 4.20 ± 0.14, 5.25 ± 0.05 and 7.05 ± 0.13 kg-COD/m^3 d, respectively. Optimal HRT coincided with maximal methane generation.

2.5. Analytical methods

The volume of biogas produced in the batch experiment was measured using a wetted-glass syringe method [15]. The volume of biogas produced in the reactor experiment was measured using gas counter (Fig. 2). Hydrogen and methane production were determined by gas chromatography (GC, Shimadzu 2014, Japan) equipped with a thermal conductivity detector (TCD) and a 2-m stainless column packed with Unibeads C (60/80 mesh). The GC-TCD conditions followed those of Saraphirom and Reungsang [16]. The hydrogen and methane volume in the biogas was calculated using a mass balance equation [17]. The VFAs were measured by high performance liquid chromatography (HPLC) according to the method of Saraphirom and Reungsang [16]. The COD, VS, and VSS were measured according to the APHA method [18].

3. Results and discussion

3.1. Batch fermentation of methane

The MY produced in all treatments was within the ranges of 14.94–321.17 mL CH_4/g-$VS_{-sub-added}$, while the biodegradability efficiency ranged from 4.00 to 86.09% (Table 2). The highest MY and biodegradability of 321.17 mL CH_4/g-$VS_{sub-added}$, and 86.09%, respectively, were obtained at an S/X ratio, Ni and Co concentrations of 0.80 g-VS_{sub}/g-$VS_{inoclum}$, 0.60 mg/L and 0.06 mg/L, respectively (Run 2). The lowest MY and biodegradability of 14.94 mL CH_4/g-VS_{-added} and 4.00% were obtained at an S/X ratio, Ni and Co concentrations of 0.47 g-VS_{sub}/g-$VS_{inoclum}$, 0.90 mg/L, and 0.03 mg/L, respectively (Run 19). The multiple regression analysis of experimental data (Table 2) resulted in the quadratic equation shown below (Eqs. (2) and (3)). Our results showed that the microorganisms in the UASB granules effectively converted the VFAs in the acidic effluent to produce methane.

$$MY = 296.08 + 17.61X_1 - 33.01X_2 + 25.74X_3 + 1.42X_1X_2 + 8.36X_1X_3 - 25.88X_2X_3 - 77.87X_2^2 - 61.40X_3^2 \quad (2)$$

Table 2
Central composite experimental design matrix defining substrate to inoculum ratio S/X ratio (X_1) (g-VS_{sub}/g-$VS_{inoculum}$), Ni concentration (mg/L) (X_2), and Co concentration (mg/L) (X_3) and results on methane yield (MY) and biodegradability.

Run	Parameters						MY (mL CH_4/g-$VS_{sub\text{-}added}$)	Biodegradability (%)
	S/X ratio (X_1)		Ni concentration (X_2)		Co concentration (X_3)			
	Code	Actual (g-VS_{sub}/g-$VS_{inoculum}$)	Code	Actual (mg/L)	Code	Actual (mg/L)		
1	0	0.80	0	0.6	0	0.06	296.98	79.61
2	0	0.80	0	0.6	0	0.06	321.17	86.09
3	0	0.80	1.68	1.1	0	0.06	38.41	10.30
4	0	0.80	0	0.6	0	0.06	312.63	83.81
5	1	1.10	−1	0.3	1	0.09	218.74	58.64
6	0	0.80	−1.68	0.1	0	0.06	159.69	42.81
7	0	0.80	0	0.6	−1.68	0.01	109.90	29.46
8	1.68	1.31	0	0.6	0	0.06	86.43	23.17
9	1	1.10	1	0.9	−1	0.03	68.29	18.31
10	−1	0.47	−1	0.3	1	0.09	137.64	36.90
11	1	1.10	−1	0.3	−1	0.03	61.89	16.59
12	−1.68	0.26	0	0.6	0	0.06	71.13	19.07
13	0	0.80	0	0.6	1.68	0.11	140.84	37.76
14	−1	0.47	1	0.9	1	0.09	34.86	9.34
15	0	0.80	0	0.6	0	0.06	269.60	72.27
16	0	0.80	0	0.6	0	0.06	279.55	74.94
17	−1	0.47	−1	0.3	−1	0.03	41.26	11.06
18	0	0.80	0	0.6	0	0.06	295.56	79.23
19	−1	0.47	1	0.9	−1	0.03	14.94	4.00
20	1	1.10	1	0.9	1	0.09	94.61	25.36

$$\text{Biodegradability} = 79.37 + 4.72X_1 - 8.84X_2 + 6.90X_3 + 0.387X_1X_2 + 2.24X_1X_3 - 6.93X_2X_3 - 20.87X_1^2 - 18.95X_2^2 - 16.46X_3^2 \quad (3)$$

This model shows a high determination coefficient ($R^2 = 0.97$) which indicates a statistically significant model. The ANOVA with quadratic regression model demonstrates that the model is significant; this is indicated by a low probability ($P < 0.0001$ and < 0.0001 for MY and biodegradability, respectively). The main factors of S/X ratio, Ni concentration, and Co concentration all had individual significant influences on the MY and biodegradability (Table 3).

Based on the regression analysis from the CCD experiment, the optimum point for obtaining a maximum simultaneous MY and biodegradability was 0.83 g-VS_{sub}/g-$VS_{inoclum}$ S/X ratio, 0.53 mg/L Ni concentration, and 0.06 mg/L Co concentration. Under the optimum conditions, a maximum MY and biodegradability of 305.43 mL CH_4/g-$VS_{sub\text{-}added}$ and 81.88%, respectively, were obtained.

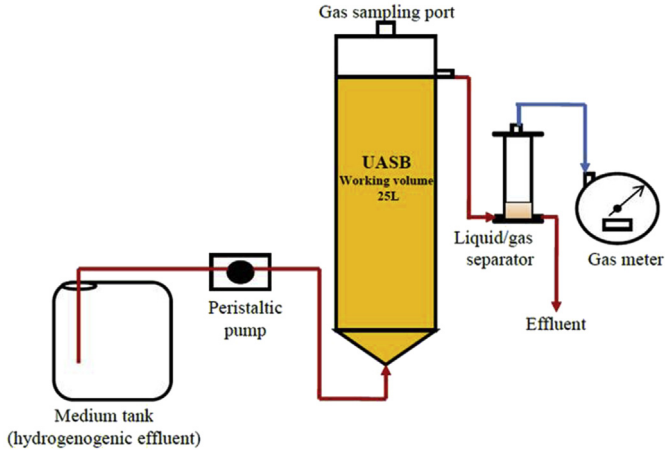

Fig. 2. Schematic diagram of UASB for methane production from the acidic effluent discharged after hydrogen production process.

Fig. 3 shows the impact of the S/X ratio upon MY generated from the acidic effluent by mixed anaerobic cultures in UASB granules. MY increased with increasing S/X ratio in the range of 0.47–0.80 g-VS_{sub}/g-$VS_{inoculum}$. There was a significant decrease in MY when the S/X ratio was greater than 0.80 g-VS_{sub}/g-$VS_{inoculum}$ as shown in Fig. 3 a, b. This indicates that an S/X ration was inappropriately high. When the S/X ratio was outside of its optimal range, there was a large impact upon hydrogen production. At high S/X ratios, the substrate inhibition occurs due to more substrate than needed [19,20]. Too high an S/X ratio (low inoculum concentration) can be toxic to the microorganisms that secreted enzymes used in the biodegradation while too low an S/X ratio can inhibit the enzyme [3]. Our findings of the optimum S/X ratio of 0.80 g-VS_{sub}/g-$VS_{inoculum}$ is very close to the findings of Owen et al. (1979) [15], who proposed that a standard S/X ratio was 1 g- VS_{sub}/g-$VS_{inoculum}$.

MY increased proportionally with increasing Ni concentration over the range of 0.30–0.60 mg/L (Fig. 3 a, c). Increasing the concentration of Ni past 0.60 mg/L decreased MY. Within the appropriate range, an increase in Ni concentration could improve the bacterial activity because Ni is a cofactor of F430, which is required for catalyzing the methane formation from methyl S-CoM in the conversion of acetic acid, hydrogen, and carbon dioxide to methane

Table 3
Model coefficients estimated by multiple linear regression (significance of regression coefficients).

Factor	Methane yield (mL CH_4/g-$VS_{sub\text{-}added}$)		Biodegradability (%)	
	Coefficient estimate	Probability	Coefficient estimate	Probability
Model	296.08	<0.0001	79.37	<0.0001
X_1	17.61	0.0308	4.72	0.0308
X_2	−33.01	0.0008	−8.85	0.0008
X_3	25.74	0.0043	6.90	0.0043
X_1X_2	1.42	0.8797	0.38	0.8797
X_1X_3	8.36	0.3830	2.24	0.3830
X_2X_3	−25.87	0.0180	−6.94	0.0180
X_1^2	−77.87	<0.0001	−20.87	<0.0001
X_2^2	−70.70	<0.0001	−18.95	<0.0001
X_3^2	−61.40	<0.0001	−16.46	<0.0001

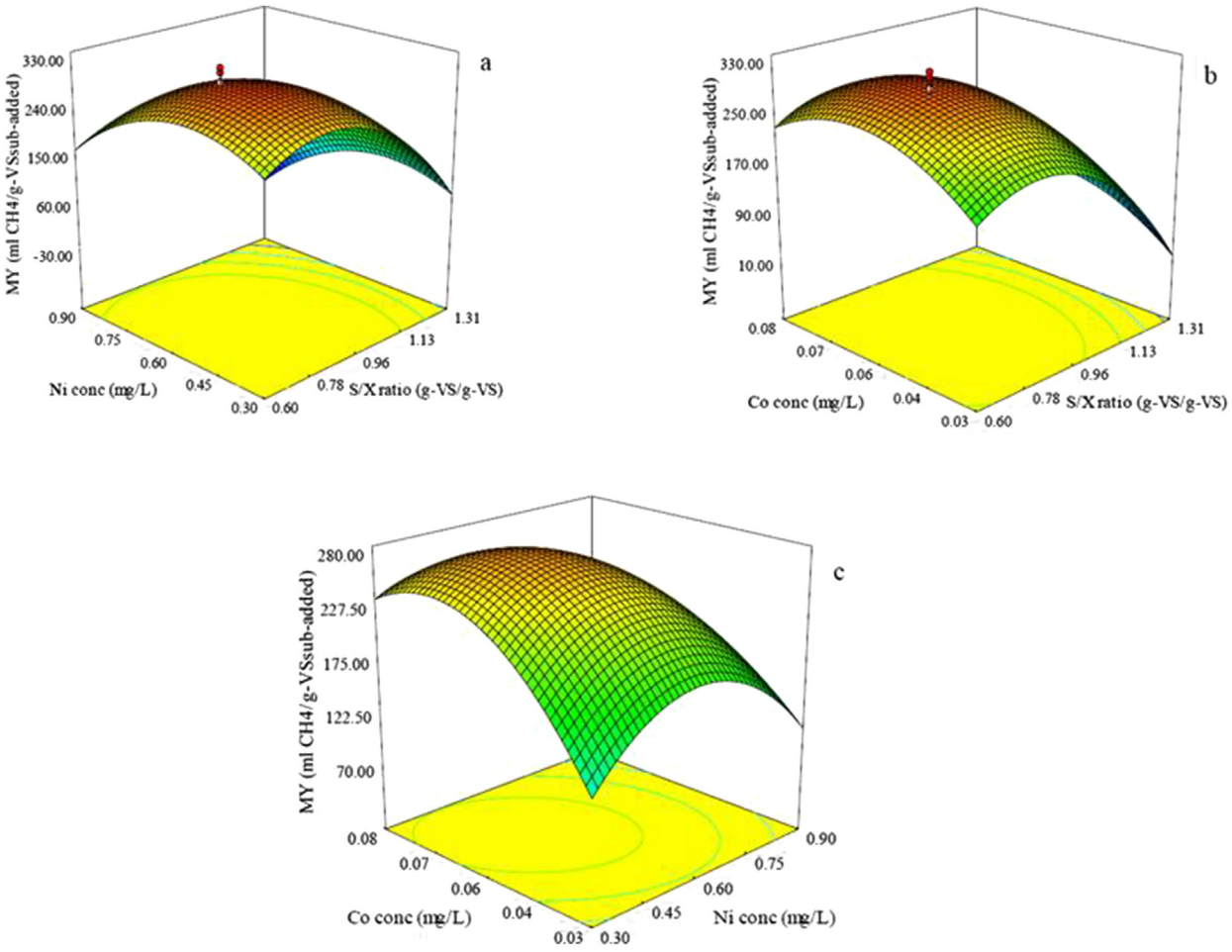

Fig. 3. Response surface plots showed the interactive effect on methane yield (MY) (a) the interactive effect of substrate to inoculum ratio (S/X) and Ni concentration at fixed Co concentration of 0.06 mg/L; (b) the interactive effect of Co concentration and S/X ratio at fixed Ni concentration of 0.53 mg/L; (c) the interactive effect of Ni and Co concentrations at fixed S/X ratio of 0.83 g-VS_{sub}/g-$VS_{inoclum}$).

by acetoclastic and hydrogenotrophic methanogens [10]. In addition, it was reported that Ni helps to maintain cell wall stability in some methanogens [11]. Previous research reported inconsistent data on the effect of Ni on anaerobic degradation. Hassan Dar and Tandon (1987) [20] found that a high concentration of Ni, greater than 3.5 μM (equivalent to 205 μg/L of Ni in $NiCl_2$), was toxic to the growth of methane-producing bacteria, and inhibited biogas yield. However, Raju et al. (1991) [21] reported that Ni concentrations up to 4 mM (equivalent to 234 mg/L of Ni in $NiCl_2$) enhanced biogas yield. Whereas, Jones et al. (1982) [22] reported that Ni does not have a stimulatory effect on methane production. Therefore, in order to maximize the methane production, there is a need to optimize the Ni concentration so that it corresponds to the requirements of the inoculum used in the study. In this study, the optimum Ni concentration that gave a maximum MY was 0.53 mg/L.

MY increased as the concentration of Co was increased from 0.01 to 0.06 mg/L as shown in Fig. 3 b, c. MY decreased when the Co concentration was higher than 0.06 mg/L. Our results indicated that a maximum MY of 321.17 mL CH_4/g-$VS_{sub\text{-}added}$ was obtained at Co concentration of 0.06 mg/L. Co is a key component of corrinoid, which is known to bind to coenzyme M [CoM] methylase. A CoM methylase such as N^5-methyl-tetrahydromethanopterin is used in the catalysis of a methyl-transferring reaction forming methyl-S-CoM in both acetoclastic methanogens and hydrogenotrophic methanogens [12,13]. Co is also contained in the coenzyme F420 (8-hydroxy-5-deazaflavin), which is reported to be found in hydrogenotrophic methanogens [10]. Coenzyme F420 binds to the hydrogenase that is involved in methane production from hydrogen and carbon dioxide in hydrogenotrophic methanogens [13].

The biodegradability ranged from 4.00% to 86.09%, which indicated that the microorganisms in a methane production system could efficiently degrade the effluent whilst simultaneously producing methane. Biodegradability is a key indicator in evaluating the conversion efficiency of substrate to methane in anaerobic digestion processes in comparison to the theoretical yield. The substrate conversion efficiency in the methane production process depends on the type of substrate, type of microorganisms, and operational conditions, as well as microbial activity [20,23,24].

The maximum MY obtained from the optimum conditions (321.17 mL CH_4/g-$VS_{sub\text{-}added}$) was approximately seven fold higher than that from the low condition (Run 17) (41.26 mL CH_4/g-$VS_{sub\text{-}added}$) (Table 4). The results indicate a significant enhancement of MY under the optimum S/X ratio, and concentration of Ni and Co. The maximal MY of the current study was low in comparison to other studies looked at the methane production from the acidic effluent of hydrogen production process by anaerobic mixed cultures [20,24,25]. This is not surprising because of the difference in

Table 4
Confirmation experiments, and methane yield (MY) and biodegradability at the end of fermentative methane production in confirmation experiment.

Run	S/X ratio (g-VS_{sub}/g-$VS_{inoclum}$)	Ni concentration (mg/L)	Co concentration (mg/L)	MY (mL CH_4/g-$VS_{sub\text{-}added}$)	Biodegradability (%)
Low	0.47	0.30	0.03	65.16	17.47
Medium	0.80	0.60	0.06	299.12	80.18
High	1.10	0.90	0.09	83.04	22.26
Optimum	0.83	0.53	0.06	308.04	82.57

inoculum types, VFA types, nutrients used, and operational conditions.

The maximum simultaneous methane production and biodegradability from the acidic effluent was obtained at an S/X ratio of 0.83 g-VS_{sub}/g-$VS_{inoclum}$, a Ni concentration of 0.53 mg/L, and a Co concentration of 0.06 mg/L. Under these conditions, a MY of 308.04 mL CH_4/g-$VS_{sub\text{-}added}$ and a biodegradability of 82.57%, were achieved.

Confirmation of the model was conducted using the data from the regression analysis at the optimum, low, high, and medium levels of each factor (Table 4). Under the optimum conditions, the actual MY of 308.04 mL CH_4/g-$VS_{sub\text{-}added}$ was almost identical to the predicted value of 305.43 mL CH_4/g-$VS_{sub\text{-}added}$ while the actual biodegradability (82.57%) was only 1.00% different from the predicted values (81.88%). These results reflected the validly of the model.

3.2. Continuous production of methane in the UASB reactor

We investigated the effect of the HRT on methane production using acidic effluent as the substrate. Fig. 4 gives the time-course profiles of the biogas production rate (BPR), MPR, methane content, HRT, and OLR. The results show that the HRT affects the BPR, MPR, methane content, and COD removal. The biogases produced in this fermentation system were carbon dioxide and methane. Table 5 gives the influent and effluent concentrations, COD removal, BPR, MPR, methane content, and MY at several HRTs in a UASB reactor obtained under continuous steady state conditions. The maximum BPR, methane content, MPR, and MY of 2.20 ± 0.03 L-biogas/L-culture day, 57.94%, 1.27 ± 0.05 L-CH_4/L-culture day, and 348 ± 13 mL-CH_4/g-COD, respectively, were obtained at the HRT of 4 d, which indicated that the HRT of 4 d was suitable for methane production from acidic effluent in the UASB reactor (Table 5). At HRT values above or below the optimal level, methane production was adversely affected. At lower HRTs, the concentration of substrate was higher than necessary which inhibited microorganisms [26–28]. At higher HRTs, substrate concentration was lower than at the optimum HRT. This reduced the activity of microorganisms [26–28]. In addition, a higher HRT could reduce the contact between substrate and microorganisms due to a low up-flow velocity.

3.3. Biogas recovery, COD removal efficiency and total energy recovery from one-stage hydrogen or methane production process versus two-stage hydrogen and methane production process

The compositions of hythane (a mixture of hydrogen and methane gases) from the two-stage hydrogen and methane production process, calculated based on the data from this study and Pattra et al. [14], were 52.92% carbon dioxide, 28.32% methane, and 18.75% hydrogen (Table 6). The results show that the two-stage hydrogen and methane production processes increased the COD removal efficiency (75.60% COD removal efficiency) compared to the one-stage hydrogen (16.08% COD removal efficiency) [14] and one-stage methane production processes (59.52% COD removal efficiency) (Fig. 5). Our results imply that the one-stage hydrogen production process cannot achieve an effective utilization of the substrate. Therefore, it should be combined with an ancillary treatment method such as methane fermentation in order to obtain the complete utilization and/or treatment of the acidic effluent.

The data from our previous research was used to calculate the total energy production of the one-stage hydrogen production process [14] in order to compare this with the energy produced from one-stage hydrogen production, one-stage methane production and two-stage hydrogen and methane production. Sugarcane juice can be converted into hydrogen and methane. The energy value of the resulting gas mixture can be determined from their volumes (mL-H_2/L-substrate and mL-CH_4/L-substrate) and their relative densities (0.089 kg-H_2/m^3-H_2 and 0.72 kg-CH_4/m^3-CH_4) [29]. One also needs to account for the heating values of these gases, which are 0.089 kg-H_2/m^3-H_2 and 0.72 kg-CH_4/m^3-CH_4 [30]. The total energy production from sugarcane juice can be calculated based on the hydrogen production and methane production (mL-H_2/L-substrate and mL-CH_4/L-substrate, respectively), relative density of hydrogen and methane (0.089 kg-H_2/m^3-H_2 and 0.72 kg-CH_4/m^3-CH_4, respectively) [29], and the heating values of hydrogen and methane (120 MJ/kg-H_2, 50 MJ/kg-CH_4, respectively) [30].

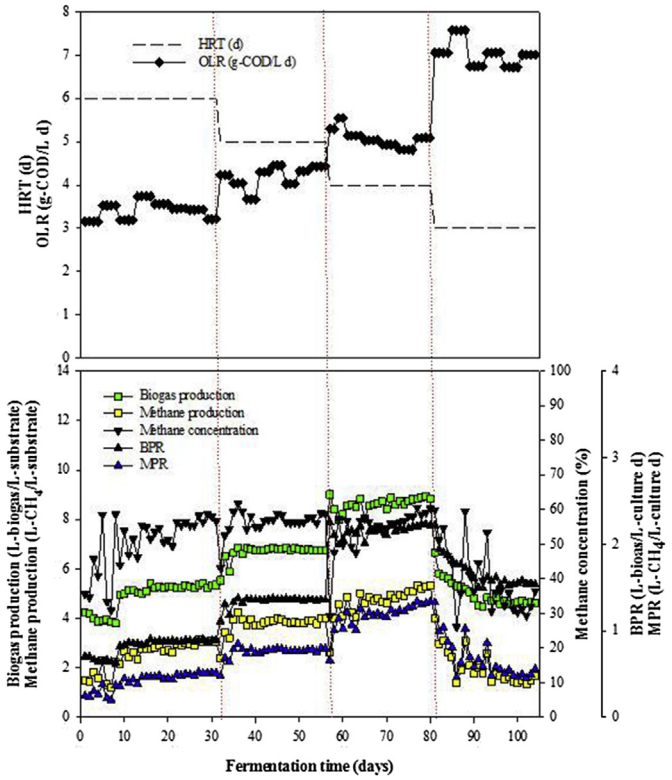

Fig. 4. Biogas production, methane production, methane concentration, biogas production rate (BPR) and methane production rate (MPR) at different hydraulic retention time (HRT) and different organic loading rate (OLR).

Table 5
Effect of hydraulic retention time (HRT) and organic loading rate (OLR) on process performance of UASB reactor for methane production.

HRT (days)	OLR (kg-COD/m^3 d)	Influent conc. (g-COD/L)	Effluent conc. (g-COD/L)	COD removal (%)	BPR[a] (L-biogas/ L-culture d)	Methane content (%)	MPR[b] (L-CH_4/ L-culture d)	MY[c] (mLCH_4/g-COD)
6	3.41 ± 0.35	20.45 ± 1.75	4.88 ± 0.97	76.13 ± 4.16	0.88 ± 0.01	56.74 ± 1.20	0.50 ± 0.01	192 ± 8
5	4.20 ± 0.14	21.02 ± 1.89	5.35 ± 1.89	74.56 ± 2.72	1.36 ± 0.01	57.05 ± 1.13	0.77 ± 0.01	246 ± 11
4	5.25 ± 0.05	20.98 ± 2.08	6.10 ± 1.12	69.46 ± 3.65	2.20 ± 0.03	57.94 ± 1.73	1.27 ± 0.05	348 ± 13
3	7.05 ± 0.13	21.16 ± 1.43	7.93 ± 0.94	63.80 ± 2.14	1.55 ± 0.03	44.46 ± 1.83	0.51 ± 0.06	113 ± 8

[a] BPR = biogas production rate.
[b] MPR = methane production rate.
[c] MY = methane yield.

Table 6
Biogas production, composition of hythane (mixture of hydrogen and methane) and COD removal efficiency from one-stage hydrogen, one-stage methane and two-stage hydrogen and methane production processes.

Reactor	Biogas production (L-biogas/ L-substrate)	Composition of hythane						COD removal efficiency (%)	References
		Hydrogen		Carbon dioxide		Methane			
		Production (L-H_2/L-substrate)	Content (%)	Production (L-CO_2/L-substrate)	Content (%)	Production (L-CH_4/L-substrate)	Content (%)		
CSTR (one-stage H_2)	9.18	3.37	36.66	5.81	63.34	0	0	16.08	[12]
UASB (one-stage CH_4)	8.79	0	0	3.70	42.06	5.09	57.94	59.52	This study
Two-stage H_2 and CH_4	17.97	3.37	18.75	9.51	52.92	5.09	28.32	75.60	Calculated from the data of [12] and this study

Thus, the energy produced from the stage fermentation of sugarcane juice is as follows:

Energy from H_2 = 3.37 L-H_2/L-substrate × [0.089 kg-H_2/m^3-H_2 × 1 m^3-H_2/1000 L-H_2] × [120 MJ/kg-H_2] = 0.036 MJ = 35.99 kJ/L-substrate

Energy from CH_4 = 5.09 L-CH_4/L-substrate [0.72 kg-CH_4/m^3-CH_4 × 1 m^3-CH_4/1000 L-CH_4] × [50 MJ/kg-CH_4] = 0.18 MJ = 183.24 kJ/L-substrate

Total energy generated from the two-stage fermentation = 35.99 + 183.24 = 219.23 kJ/L-substrate/25 g-COD/L-substrate] = 8.77 kJ/g-COD. Hence, the total energy generated from the two-stage fermentation process was 219.23 kJ/L-substrate or 8.77 kJ/g-COD. Our results suggest that the two-stage hydrogen and methane production processes provide a higher total energy than the one-stage hydrogen or methane production process. The maximum total energy (8.77 kJ/g-COD), obtained in this study was much lower than the total energy obtained from co-digestion of activated sludge and food waste (10.57 kJ/g-COD) when using 100%

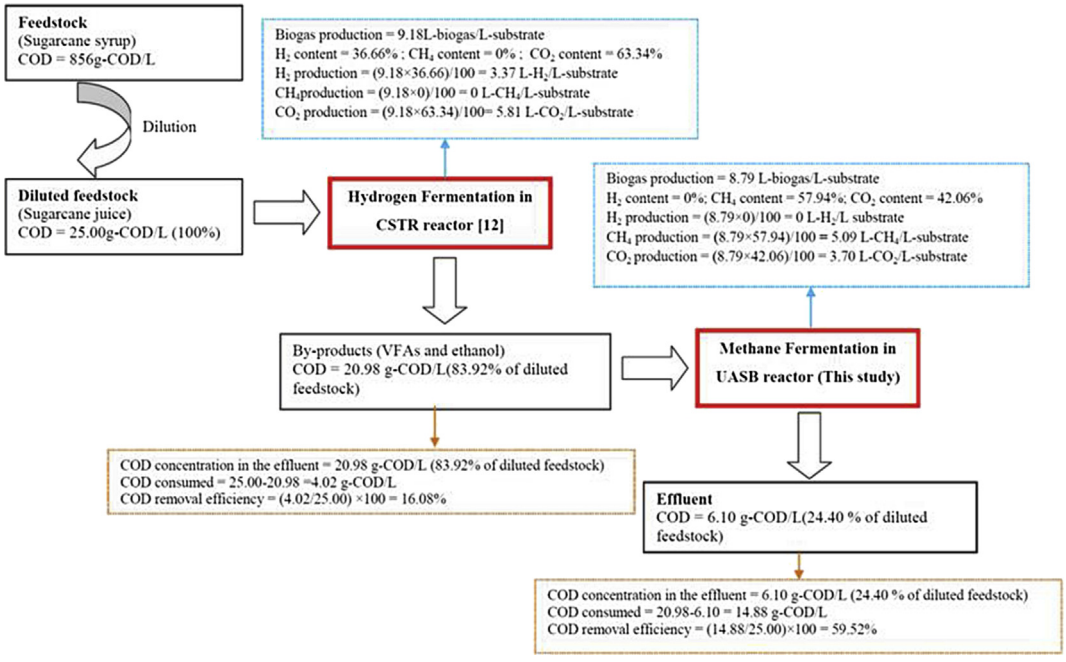

Fig. 5. Biogas recovery and COD removal efficiency of a two-stage hydrogen and methane production process.

of food waste as the substrate [31]. This discrepancy might be due to the types of substrate, microbial consortium and operational conditions.

4. Conclusions

The acidic effluent obtained after the hydrogen fermentation of the sugarcane juice was used as the substrate to produce methane. The optimum conditions that maximized a MY of 305.43 mL CH_4/g-$VS_{sub\text{-}added}$ were at an S/X ratio of 0.83 g-VS_{sub}/g-$VS_{inoculum}$, a Ni concentration of 0.53 mg/L, and a Co concentration of 0.06 mg/L. The optimum conditions were further used to produce methane in a 30 L UASB reactor. The optimum HRT was found to be 4 d in which the maximum MPR and MY of 1.27 ± 0.05 L-CH_4/L-culture day and 348 ± 13 mL-CH_4/g-COD, respectively, were obtained. Our results indicated that the two-stage hydrogen and methane production process proved to be a reliable and efficient way for energy recovery, as well as reduction of the COD load of the waste water, with a total energy production of 219.23 kJ/L-substrate or 8.77 kJ/g-COD and a COD removal efficiency of 75.60%.

Acknowledgments

This research was supported by Agricultural Research Development Agency, Thailand (ARDA) (Grant number 25/2554) and the Promoting Science and Technology, Ministry of Education, Culture Sports Science and Technology, Japan. Fermentation Research Center for Value Added Agricultural Products is much appreciated for the facilities used in this study.

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