CHAPTER IV

Thermophilic dark co-digestion of skim latex serum (SLS) and palm oil mill effluent (POME) to sequentially produce biohydrogen and biomethane

4.1 Abstract

The two-stage anaerobic process for sequential production of biohydrogen and biomethane from thermophilic dark co-digestion of skim latex serum (SLS) with palm oil mill effluent (POME) was investigated. The CSTR seed was treated by load shock method by using 30 g-Sucrose/L under thermophilic temperature to enrich thermophilic hydrogen-producing seeds from anaerobic mixed culture that were collected from mesophilic wastewater treatment pond. The production of biohydrogen in continuous process in the first stage was operated under hydraulic retention times (HRTs) between 2.25 days and 4.50 days, corresponding to organic loading rate of 20 g-VS/ $L_{reactor}$ d and 10 g-VS/ $L_{reactor}$ d, respectively. Hydrogen production was achieved under HRT of 4.50 days with the hydrogen production rate and hydrogen production yield was 341±19 mL $H_2/L_{reactor}$ d and 1533±88 mL $H_2/L_{substrate}$, respectively, which is higher than that achieved from HRT of 2.25 days. Under the operational conditions at 4.50-day HRT, soluble metabolites were dominated with lactic acid (99.1-138.1 mM), acetic acid (14.7-24.1 mM), butyric acid (15.8-28.7 mM), propionic acid (12.7-26.5 mM) and ethanol (13.2-21.0 mM). Subsequently, the homogenized effluents collected from the first stage hydrogen production which operated under the optimal conditions was further fed into the second stage under HRT of 18 days, corresponding to the organic loading rate of 2 g-VS/$L_{reactor}$ d. The average methane production rate and methane production yield was 79±12 mL $CH_4/L_{reactor}$ d. and 1517±199 mL $CH_4/L_{substrate}$, respectively. Further optimization for the second stage UASB is necessary due to acetic acid accumulated in rather high concentration (63-150 mM).

4.2 Introduction

Thailand is one of the most agricultural areas in the Southeast Asia which is various kinds of agricultural crops such as cassava, sugar cane, corn, palm oil and rubber. Among them, rubber is the major agricultural crops cultivated in Southern Thailand, corresponding with a mostly of located in rubber plant, whereof mostly are provided to produce concentrated latex. There are several methods available for concentration of natural latex is comprehensive creaming, evaporation, electro-decantation and centrifugation. Among them, centrifugation is the most method used to produce concentrated latex in Southern Thailand. An annual production capacity of concentrated latex obtained from Southern Thailand was around 1.3×10^6 metric ton which is produced in 2013 (Kongjan *et al*., 2014). In this process about 580 m^3 of skim latex serum was generated and approximately of 405 ton of concentrated latex was produced. Skim latex serum is a concentrated with a relatively high organic content in the macronutrients in carbon, nitrogen and phosphorus which are the crucial on microbial growing. Thus, skim latex serum generated cannot be discharged into environmental directly, thank to it could cause seriously environmental pollutions.

There are several biological ways to treat this organic waste generated such as aerobic and anaerobic system. Among this option, anaerobic system is a suitable approach and great interest since there are several advantages over aerobic system such as higher organic loading rate, lower operating cost, gave less excess biomass and produced biogas as a usable product. Although skim latex serum contains high nutrients content as previously mentioned, but it's also containing in a relatively high inhibitants content such as ammonia (latex preservation) and sulfate (latex coagulation) which is important to microbial growing as well as the efficiency of biogas formation. Corresponds with a study from Kongjan *et al*. (2014) used two-stage anaerobic digestion process found that a relatively low of hydrogen and methane production yield achieved from sole fermentation of skim latex serum. The hydrogen generated in H_2-UASB reactor was 59.2±2.4 mL H_2/g-VS, while methane generated in CH_4-UASB reactor was 168.6±13.8 mL CH_4/g-VS, which is just only 11 and 45% of hydrogen and methane theoretical yield (498 mL H_2/g-VS and 373 mL CH_4/g-VS). They were reported the possible reason for having low hydrogen formation because of the competition of hydrogen producing bacteria and sulfate reducing bacteria to produce neither hydrogen nor hydrogen sulfide. In additional, hydrogen sulfide (H_2S) has inhibitory effect on methanogenic archaea at even low concentration of 20-30 mM (Boe, 2006).

Thus, to enhance the biogas production potential from skim latex serum fermentation, the approach of anaerobic co-fermentation of organic wastes and two stages process was performed in this work. Apart from rubber, palm oil is one of the most agricultural crops cultivated in Southern Thailand. A study from O-Thong *et al*. (2008) found that high hydrogen production yield was achieved from sole fermentation of palm oil mill effluent was 84.2 mL H_2/g-COD and

methane production yield was 392 mL CH_4/g-VS (O-Thong *et al*., 2012). In our previous study was performed using anaerobic co-digestion of skim latex serum and palm oil mill effluent of thermophilic batch two stages process, satisfactory results of hydrogen and methane production yield was 85.7±4.9 mL H_2/g-VS and 418±10 mL CH_4/g-VS, respectively was achieved. The hydrogen and methane production yield achieved from co-digestion of SLS and POME which was 3 and 2 times greater than that achieved from sole fermentation of SLS. These results indicate that it would be possible to be applied in a commercial scale.

Deliberation of reactor types that will be used to generate biogas mostly is determined by substrate characteristics. In this work was carried out by using SLS and POME as substrate, POME is a slurry, relatively viscous fluids and high colloid suspension substrates which was necessary to treat by using continuously stirred tank reactor (CSTR). Besides substrate and biomass in the reactor was mixed well, it was provided to a more convenient method for the engineering commissioning. At the same time, organic soluble wastes which were treated in the acidogenic stage mostly are further fed into fixed film reactors such as upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB) reactors. Among them, there are several advantages of UASB over EGSB reactors such as more stable of methane production and lower of VFA accumulation in the reactor. In order to develop a two stages anaerobic digestion process to generates biohydrogen and biomethane from co-digestion of SLS and POME in a commercial scale which is necessary to be applied foreseeable future.

In the light of these concerns, in this work aims to investigate the effect of hydraulic retention time (HRT) on the sequential productivity of hydrogen and methane from the optimal mixing ratio of SLS to POME in a continuously stirred tank reactor (CSTR) and an upflow anaerobic sludge blanket reactor (UASB), respectively.

4.3 Materials and methods

4.3.1 Inoculum preparation

The anaerobic seed sludge originating employed in this research was collected from mesophilic wastewater treatment pond of Palm Pattana Southern Border Co, Ltd., Pattani Province; Southern Thailand was used as inoculum for produced hydrogen and methane. The sludge used for produced hydrogen was treated by load shock method by using basic anaerobic (BA) medium (Angelidaki and Sanders, 2004) with sucrose 30 g/L to inactivate methanogens and enrich for hydrogen producing bacteria. Meanwhile, the sludge used for produced methane was enriched for methanogens by using BA medium with sucrose 3 g/L. Both hydrogen and

methane production stages was conducted in batch reactor for 7 days and 14 days, respectively. Subsequently, the enriched sludge was further transferred into a continuously stirred tank reactor (CSTR) and an up-flow anaerobic sludge blanket (UASB) reactor for continuous processes.

4.3.2 Skim latex serum

The skim latex serum used in this experiment was collected after latex coagulation process from Chana Latex Co, Ltd., Songkhla Province; Southern Thailand. It had the following characteristics; light yellow color, pH 5.04±0.01, volatile solid content (VSC) of 40.61±0.06 g/L, chemical oxygen demand (COD) of 33600±0 mg/L, proteins content of 6417±202 mg/L, and carbohydrates content of 379±0 mg/L. The physical and chemical characteristics of the SLS are given in **Table 4.1**. The SLS was kept at 2±1°C and was used within a month in order to minimize self-biodegradation and acidification.

4.3.3 Palm oil mill effluent

The palm oil mill effluent used in this experiment was collected from the receiving tank of Palm Pattana Southern Border Co, Ltd., Pattani Province; Southern Thailand. Palm oil mill effluent has brown color, pH 4.74±0.01, COD of 76160±0 mg/L, proteins content of 5180±35 mg/L, carbohydrates content of 10340±0 mg/L, and oil and grease content of 12083±86 mg/L. The physical and chemical characteristics of the POME are summarized in **Table 4.1**. The raw POME was kept at 2±1°C and was used within a month.

Table 4.1 Physical and chemical characteristics of the skim latex serum and palm oil mill effluent used.

Parameters	Unit	SLS	POME
pH		5.04±0.01	4.74±0.01
TSC	g/L	46.94±0.13	61.44±0.11
VSC	g/L	40.61±0.06	50.82±0.13
Alkalinity	mg/L-$CaCO_3$	83±0	42±0
COD	mg/L	33600±0	76160±0
TKN	mg/L	4975±32	829±6
Protein	mg/L	6417±202	5180±35
Carbohydrate	mg/L	379±0	10340±0
Oil and grease	mg/L	191±5	12083±86
Sulfate	mg/L	233±0	-
Soluble phosphorus	mg/L	39±0	161±0

4.3.4 Experimental set-up and operation

In this experiment, the sequential productivity of hydrogen and methane was operated in a continuously stirred tank reactor (CSTR) for the first stage and an up-flow anaerobic sludge blanket (UASB) reactor for the second stage. The first phase reactor, 1350 mL working volume was maintained at 55°C with both recirculating hot water inside a water coil and assistance of the heating plate of the magnetic stirrer which was equipped with a temperature control unit and it was stirred at 90 rpm by magnetic bar. Meanwhile, the second phase reactor with 2700 mL working volume was also maintained at 55°C by recirculating hot water through a water jacket surrounding UASB reactor and control a re-circulating flow rate at 30 mL/h (Kongjan *et al*., 2014). Schematic diagram of the two-stage anaerobic process for sequential production of biohydrogen and biomethane which was operated in the lab-scale CSTR and UASB reactors is shown in **Fig. 4.1**.

The enriched hydrogen producing bacteria achieved from batch reactor for 1000 mL was directly fed into H_2-CSTR reactor. Subsequently, the rest of the reactor active volume was filled up with a substrate consisting of SLS and POME at the mixing ratio of 50:50 (%v/v) supplemented with $NaHCO_3$ 2 g/L for acclimation period was applied for three weeks. Then, the mixing ratio of SLS and POME was changed to the optimal mixing ratio which was achieved

from batch assay was 55:45 (%v/v) with the same concentration of buffer supplemented. The H_2-CSTR reactor was continuously operated at the HRTs of 2.25 days and 4.50 days, respectively. At the same time, methanogenic granular sludge used in this research was obtained from Kiang Huat Sea Gull Trading Frozen Food Public Co, Ltd., Songkhla Province; Southern Thailand. An 840 mL of methanogenic granules were directly added into CH_4-UASB reactor as cell-mass carriers (Kongjan *et al*., 2014). Afterwards, 1260 mL of the enriched methanogenic inoculum achieved from batch reactor was fed into the CH_4-UASB reactor. Then, the rest of the reactor active volume was filled up with BA medium supplemented with sucrose 3 g/L which was applied for a month. After that, substrate consisting of effluent achieved from H_2-CSTR reactor under the optimal mixing ratio of SLS to POME of 55:45 (%v/v) and BA medium at a volumetric mixing ratio of 1:1 was fed into CH_4-UASB reactor for a month. Subsequently, substrate for CH_4-UASB reactor was changed to effluent from H_2-CSTR reactor supplemented with $NaHCO_3$ 2 g/L and was continuously operated at the HRT of 18 days.

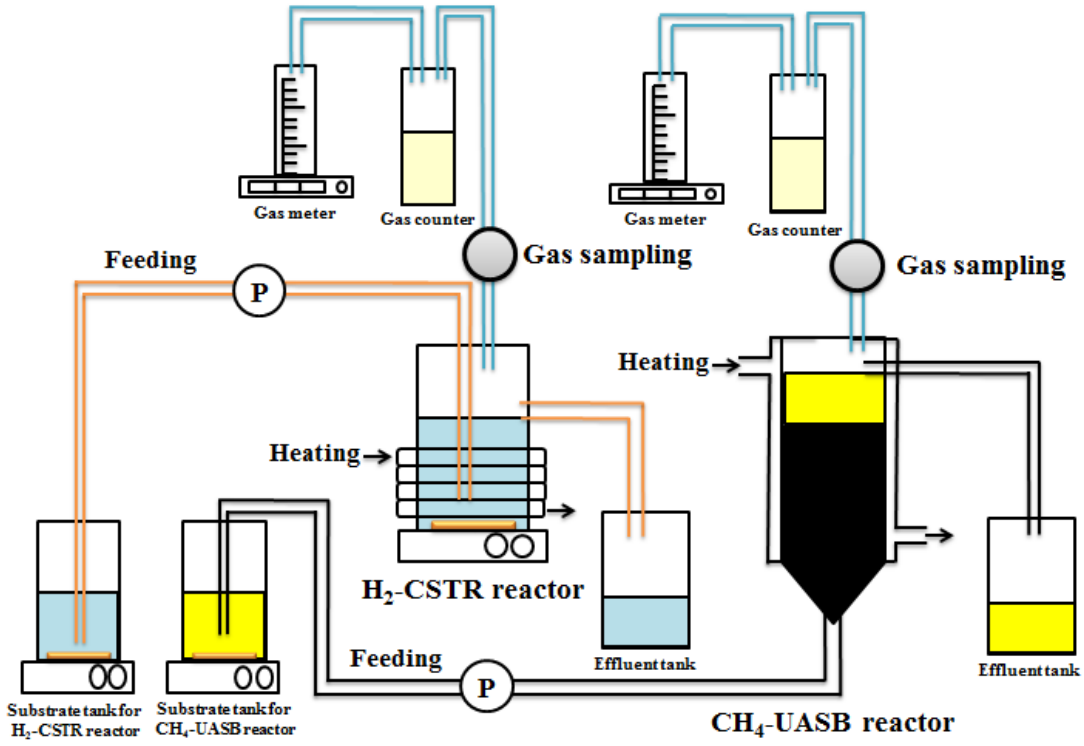

Fig. 4.1 Schematic description of lab-scale bioreactor operation for sequential production of biohydrogen and biomethane which operated under thermophilic temperature.

4.3.5 Analytical methods

The volume of biogas produced was recorded by water displacement gas meter. The hydrogen content was measured by gas chromatography (Shimadzu GC 14A equipped with thermal conductivity detector, TCD) fitted with a 1.5 m stainless steel column paced with molecular sieve 58 (80/100 mesh). Argon was used as a carrier gas at a flow rate of 15 mL/min. The temperature of the injection port, oven and detector were 100, 50, and 100°C, respectively. 0.5 mL of sampling gas was injected in triplicate (O-Thong *et al*., 2008; Akutsu *et al*., 2009).

Volatile fatty acids (VFAs) (i.e. acetic, propionic, butyric acids) and alcohol (ethanol) were analyzed by a gas chromatography (Shimadzu, GC 8A) equipped with a flame ionization detector (FID). A column capillary packed with nitroterephthalic acid-modified polyethleneglycol (DB-FFAP) and with a length of 30 m was used. The chromatography was performed using the following program: 100°C for 5 min, 100-250°C with a ramping of 10°C/min, 250°C for 12 min. The detector temperature was set at 300°C (O-Thong *et al*., 2008; Prasertsan *et al*., 2009). Lactic acid (HLa) was analyzed by a high performance liquid chromatography (HP1100, Hewlett-Packard GMGH) with the following operating conditions; Pinnacle® II C18 Columns, ultraviolet (UV) detector at 210 nm, 2.5 mM of H_2SO_4 was used as a mobile phase with a flow rate of 0.8 mL/min, and an oven temperature of 45°C (Prasertsan *et al*., 2009). The liquor samples were first centrifuged at 10,000 rpm for 10 min, and were then filtered through 0.45 µm nylon membrane. Calculation of chemical oxygen demand (COD) balance was followed the method described by Sittijunda and Reungsang (2012).

Chemical oxygen demand (COD), pH, total solid content (TSC), Volatile solid content (VSC), alkalinity, total Kjeldahl nitrogen (TKN), protein content, total organic nitrogen (TON), carbohydrate content, sulfate content, oil and grease and soluble phosphorus were determined in accordance with the procedures described in the standard methods (APHA, 1999).

4.4 Results and discussion

4.4.1 H_2-CSTR experiments

The H_2-CSTR reactor was operated at the HRTs of 2.25 days (from 1^{st} to 19^{th} day of operating time) and 4.50 days with, (from 54^{th} to 68^{th} day of operating time) and without dilution (from 20^{th} to 53^{rd} day and 69^{th} to 95^{th} day of operating time, respectively), corresponding to the organic loading rates (OLRs) of 20 g-VS/$L_{reactor}$ d, 10 g-VS/$L_{reactor}$ d, and 5 g-VS/$L_{reactor}$ d,

respectively. The pH of H_2-CSTR reactor was maintained within the optimal favorable pH for hydrogen producing bacteria ranged 5.0-5.5 (O-Thong *et al*. 2012) using $NaHCO_3$ 2 g/L as shown in **Fig. 4.2**. The optimal mixing ratio of SLS to POME at 55:45 (%v/v) supplemented with $NaHCO_3$ 2 g/L was completely mixed by magnetic stirrer at 90 rpm during semi-continuous fed into H_2-CSTR reactor.

At HRT of 2.25 days and OLR of 20 g-VS/$L_{reactor}$ d, the average hydrogen content in biogas was 30±2% (**Fig. 4.3**) with lowers of both hydrogen production rate and hydrogen production yield was 95±8 mL H_2/$L_{reactor}$ d and 231±18 mL H_2/$L_{substrate}$ were obtained under steady state conditions at 5 times of HRT as shown in **Fig. 4.4**. One explanation for low hydrogen production yield obtained under the HRT of 2.25 days is that microbial are inhibited by high substrate supplied which facilitate microbial population shift to other metabolic pathways such as lactate formation pathway. Moreover, POME is a concentrated substrate with high concentration of oil and grease which preferred longer time to degrade this substance. Thus, operating shorter HRT of 2.25 days is may be not adequate on biodegradability, resulting in low hydrogen production yield was achieved.

Meanwhile, the highest hydrogen production rate and hydrogen production yield was 341±19 mL H_2/$L_{reactor}$ d and 1533±88 mL H_2/$L_{substrate}$, respectively achieved under HRT of 4.5 days with OLR of 10 g-VS/$L_{reactor}$ d, which is just only 7% of hydrogen theoretical yield (498 mL H_2/g-VS) (Boe, 2006). Although the extension of HRT as well as decreasing of OLR, resulting increase in hydrogen production yield, even so lactic acid was still found dominated soluble metabolite products (**Fig. 4.5**). Thus, this is one of the possible reasons for lower hydrogen production yield was obtained. Moreover, the other possible reason is high hydrogen content in biogas accumulated in the headspace of the H_2-CSTR reactor which could be dissolved in the fermentation broth, causes deactivation of hydrogenase (Kongjan, 2010). A study from Batstone *et al*. (2002) found that the solubility of hydrogen gas in water is 3.3% under the temperature ranged 35-50°C.

At HRT of 4.50 days and OLR of 5 g-VS/$L_{reactor}$ d, BA medium was used to reinforce the microbial growth at the volumetric mixing ratio of substrate and BA medium of 1:1. The result shows that the hydrogen production yield is still lower (699±15 mL H_2/$L_{substrate}$) which is just only 6% of hydrogen theoretical yield. On the other hand, lactic acid produced and accumulated in the H_2-CSTR reactor is gradually decreases and a relatively stable after the 12^{nd} of operating time as shown in **Fig. 4.5**.

In final run was operated under HRT of 4.50 days with OLR of 10 g-VS/$L_{reactor}$ d to assert the performance of the H_2-CSTR reactor. The hydrogen production yield was gradually increases and eventually be similar to the hydrogen production yield achieved under the same previous operational conditions. These results indicated that the operating under the optimal conditions provides higher hydrogen production ability, the possible reasons is that the hydrogen producing bacteria could utilize the carbohydrates more efficiency to generates hydrogen. Nevertheless,

lactic acid was still found as the major soluble metabolite products of all operational conditions. Which is consistent with our previous research in batch assay that used the enriched and acclimated hydrogen producing bacteria obtained from H_2-CSTR reactor was operated under the volumetric mixing ratio of SLS to POME at 1:1 under HRT of 4.50 days. The result shows that lactic acid was the main soluble metabolite products in a relatively high concentration of all treatment. **Reactions (4.1)** and **(4.2)** shows metabolic pathway produced lactic acid by lactic acid bacteria (LAB), which distinguished between homofermentative LAB (**Reaction 4.1**) and heterofermentative LAB (**Reaction 4.2**) (Mariakakis *et al*., 2011). Although lactic acid could be transformed to acetic, propionic (**Reaction 4.3**) and butyric acids (**Reaction 4.4**) by *Clostridium propionicum* and *Clostridium tyrobutyricum* to generates hydrogen (Noike *et al*., 2002). Nevertheless, hydrogen could be generated by secondary fermentation (**Reactions 4.3 and 4.4**) which provides the biological reaction rate slower than that generated by primary reaction rate. Moreover, a study from Noike *et al*. (2002) found that the hydrogen production yield gradually decreased and ceased since high concentration of lactic acid was detected.

$$C_6H_{12}O_6 \longrightarrow 2\ CH_3CH(OH)COOH \quad (4.1)$$

$$C_6H_{12}O_6 \longrightarrow CH_3CH(OH)COOH + CH_3COOH + CO_2 \quad (4.2)$$

$$3\ CH_3CH(OH)COOH \longrightarrow 2\ CH_3CH_2COOH + CH_3COOH + CO_2 + H_2O \quad (4.3)$$

$$CH_3CH(OH)COOH + 0.4\ CH_3COOH \rightarrow 0.7\ CH_3(CH_2)_2COOH + CO_2 + 0.4\ H_2O + 0.6\ H_2 \quad (4.4)$$

Furthermore, the other possible reason for still lower of hydrogen was generated of all operational condition is that the growing of sulfate reducing bacteria in the H_2-CSTR reactor which could potentially consume hydrogen generated (**Reactions 4.5 and 4.6**) (Schink, 1997).

$$4H_2 + SO_4^{2-} \longrightarrow S^{2-} + 4H_2O \quad (4.5)$$

$$H_2 + S^0 \longrightarrow H_2S \quad (4.6)$$

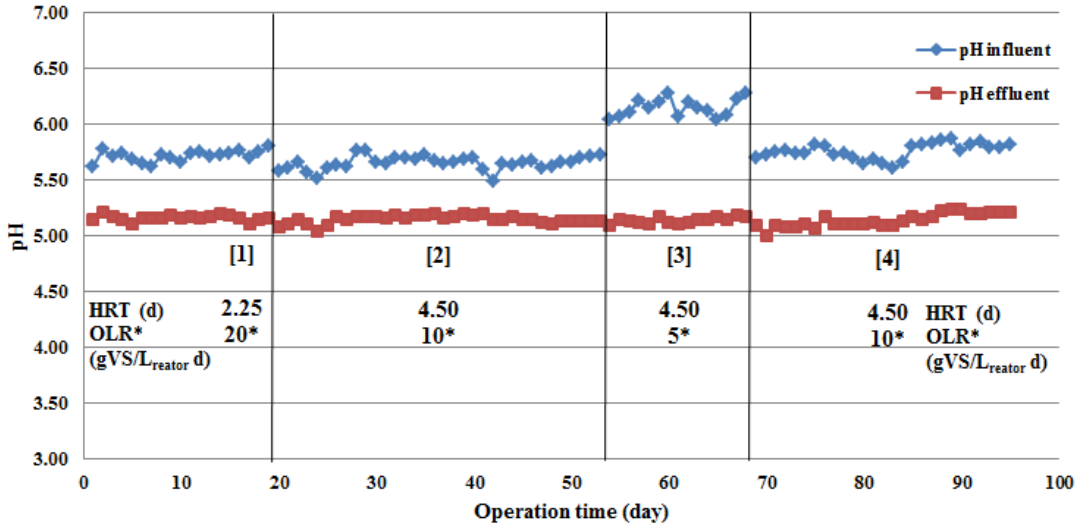

Fig. 4.2 Variation of pH of H_2-CSTR reactor which was operated at different HRTs under thermophilic temperatures; [1] HRT of 2.25 days and OLR of 20 gVS/L d, [2,4] HRT of 4.50 days and OLR of 10 gVS/L d, and [3] HRT of 4.50 days and OLR of 5 gVS/L d.

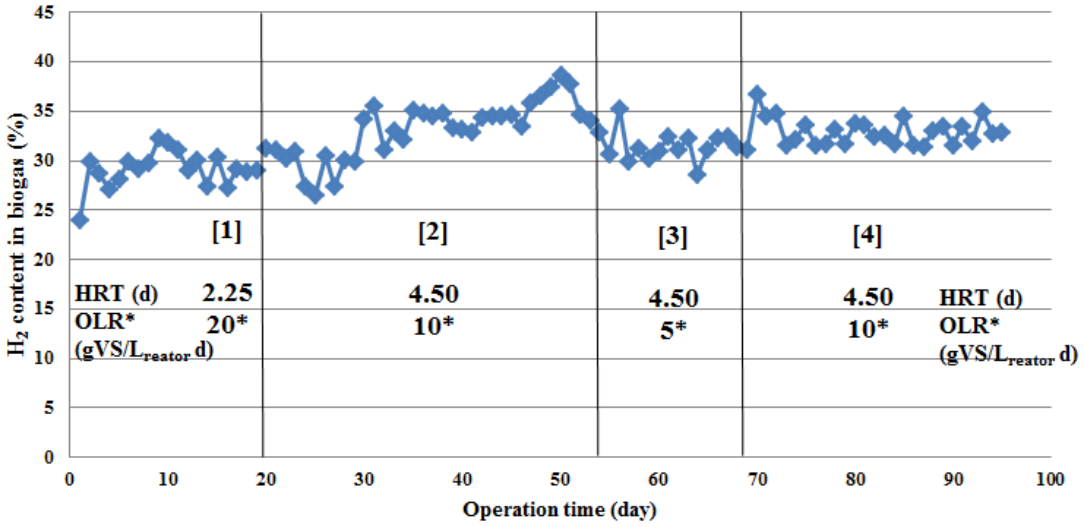

Fig. 4.3 Variation of hydrogen content in biogas at different HRTs achieved from H_2-CSTR reactor; [1] HRT of 2.25 days and OLR of 20 gVS/L d, [2,4] HRT of 4.50 days and OLR of 10 gVS/L d, and [3] HRT of 4.50 days and OLR of 5 gVS/L d.

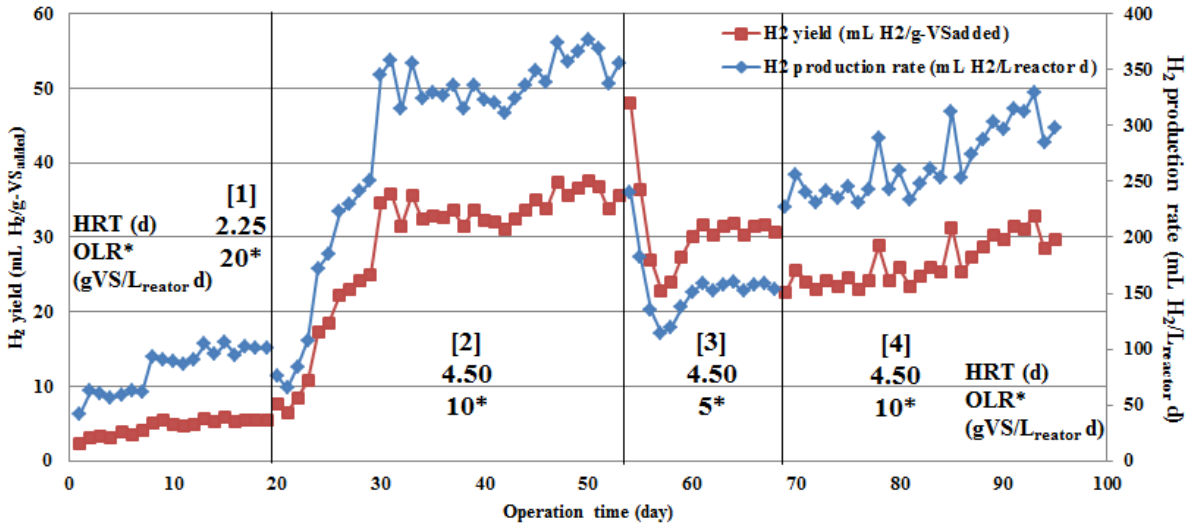

Fig. 4.4 H_2-CSTR reactor performance achieved from co-digestion of SLS to POME at different HRTs under thermophilic temperatures; [1] HRT of 2.25 days and OLR of 20 gVS/L d, [2,4] HRT of 4.50 days and OLR of 10 gVS/L d, and [3] HRT of 4.50 days and OLR of 5 gVS/L d.

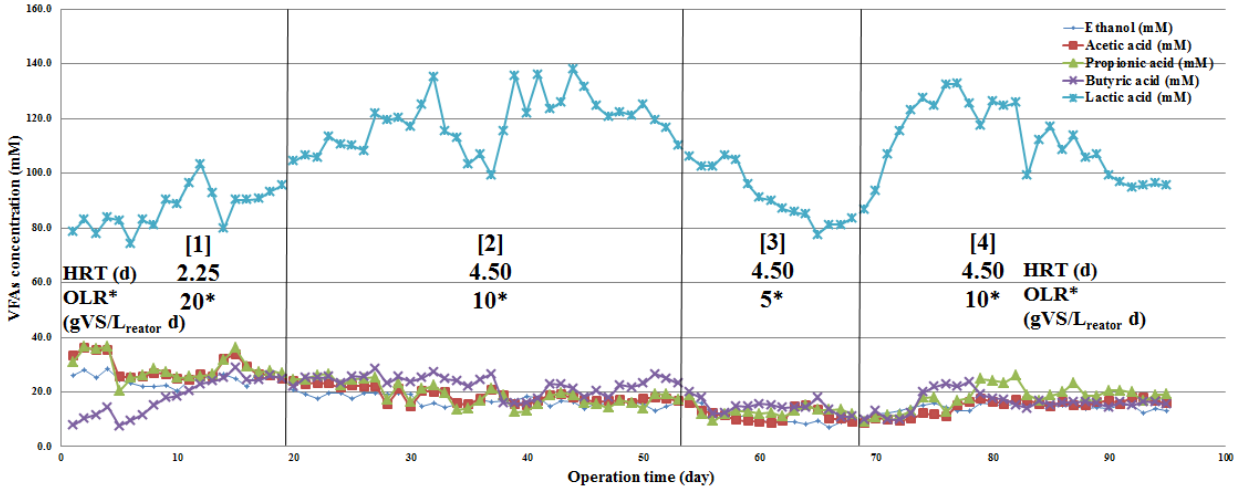

Fig. 4.5 Variation of soluble metabolite products achieved from H_2-CSTR reactor at different HRTs under thermophilic temperatures; [1] HRT of 2.25 days and OLR of 20 gVS/L d, [2,4] HRT of 4.50 days and OLR of 10 gVS/L d, and [3] HRT of 4.50 days and OLR of 5 gVS/L d.

4.4.2 CH_4-UASB experiments

During CH_4-UASB reactor start-up and acclimation period (from the 1st to the 34th days of operation), BA medium supplemented with sucrose 2 g/L was applied during the first 5 days of operating time under the HRT of 18 days in order to enrich and reinforce the methanogens growth. The result shows that high methane content in biogas of 85±3% (**Fig. 4.6**) was achieved, which indicates that the methanogens immobilized in CH_4-UASB reactor was enriched and could functioned. Then, CH_4-UASB reactor was switched to acclimate using the mixture consisting of the homogenized effluent obtained from H_2-CSTR reactor under steady state conditions which was operated under HRT of 4.50 days and BA medium supplemented with sucrose 2 g/L at a volumetric mixing ratio of 1:1 was continuously fed from the 6th to the 10th days of operating time. Under this operating, the methane content in biogas was still high ranged 84±3% and lower concentration of VFAs produced found in the reactor. Subsequently, the mixture consisting of effluent obtained from H_2-CSTR reactor and BA medium at the mixing ratio of 50:50 (%v/v) was continuously fed into CH_4-UASB reactor, resulting dramatically decreased in methane content in biogas from 87 to 29%, corresponding to VFAs produced is gradually increase (**Fig. 4.7**). The possible cause for having low methane content in the biogas is that due to the enriched methanogens was inhibited by substrate fed compositions which having high content of VFAs produced in H_2-CSTR reactor especially lactic acid. To prevent the process failure as well as to reinforce the methanogens growth, BA medium supplemented with sucrose 2 g/L was applied from days 18-34 of operating time. The result shows that the methane content in biogas is progressively increased along with gradually decreased in VFAs produced in the reactor. After 7 days of acclimation period (from days 35-41), the methane content in biogas slightly decreased, which indicates that the enriched methanogens immobilized in CH_4-UASB reactor acclimated and could tolerate under high lactate content in the substrate (**Fig. 4.5**). Corresponds to methane production yield which was 4 times greater than that achieved from the previously acclimated (from days 11-17).

After acclimation period, it was started by using the homogenized effluent achieved from H_2-CSTR reactor as previously mentioned supplemented with $NaHCO_3$ 2 g/L. Under this condition, the methane content in biogas and methane production rate is progressively decrease and stable after 7 days of operating time. Corresponding to methane production yield was 1587±140 mL $CH_4/L_{substrate}$ or 54±7 mL CH_4/g-VS_{added} (**Fig. 4.8**), whereas, acetic acid was still found in very high concentration up to 103±25 mM (**Fig. 4.7**). Although high VFA content was produced and/or accumulated in CH_4-UASB reactor as well as in the substrate fed. The CH_4-UASB reactor was still maintained pH within the optimal favorable pH for methanogenesis stage ranged 6.5-8.0 (Nielsen, 2004) (**Fig. 4.9**). Therefore, further operating under this condition would be very much risky to the process failure due to imbalance of metabolite products produced and accumulated in the reactor. Thus, the mixture consisting of effluent from H_2-CSTR reactor and BA medium at the volumetric mixing ratio of 1:1 was applied from days 66-76 of

operating time. Resulting in progressively decreased in acetate produced and/or accumulated in the reactor and pH in CH_4-UASB reactor ranged 7.43-7.90 as shown in **Fig. 4.9**. Best known some species of methanogens such as *Methanosaeta* has lower growth rate due to affected by acetate concentration. Moreover, a study from Kongjan *et al*. (2014) found that acetic acid was the major soluble metabolite products and it's produced in relatively high concentration of 51±5 mM achieved from sole fermentation of SLS in UASB reactor. These corresponded to a study from Boe (2006) reported that acetate oxidation pathway become more favorable under thermophilic temperatures. Therefore, one explanation for low methane production yield is that methanogens was inhibited by a very high concentration of acetate produced and accumulated in the reactor. Moreover, other possible reasons are: (1) low methane producing archaea in the CH_4-UASB reactor; (2) inhibitants in SLS and POME such as sulfate and phenolic compounds; (3) high hydrogen partial pressure; (4) the competition of sulfate reducing bacteria with methanogenic archaea which could occur under low concentrations of sulfate (Boe, 2006). Reduced product especially lactate was broken dawn to acetate, carbon dioxide and bicarbonate which was performed by two groups of sulfate reducing bacteria including incomplete and complete oxidizers (Chen *et al*., 2008).

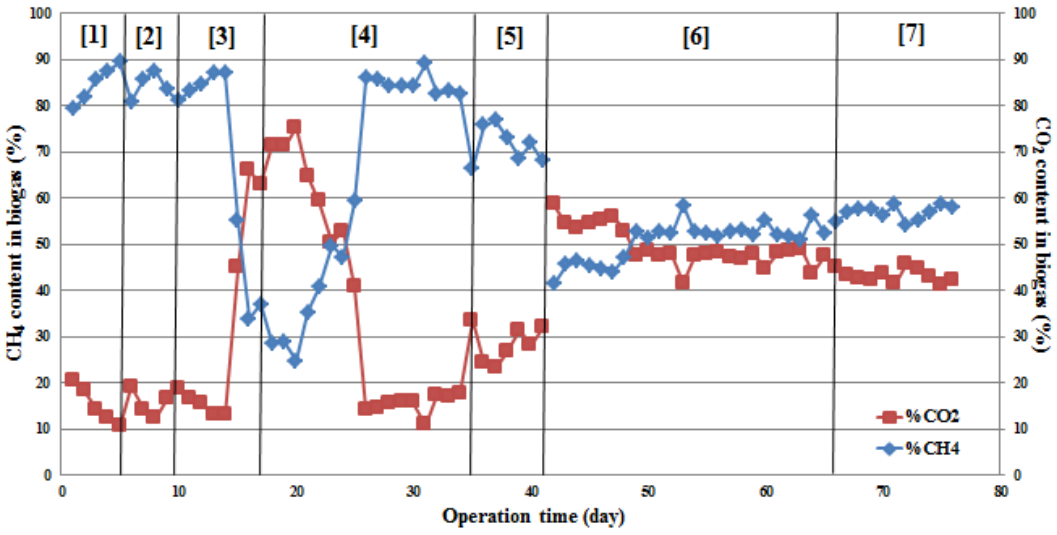

Fig. 4.6 Variation of methane and carbon dioxide content achieved from CH_4-UASB reactor at different HRTs under thermophilic temperatures; [1, 4] BA medium + sucrose 2 g/L, [2] (BA medium + sucrose 2 g/L) + effluent H_2 at 1:1 (%v/v), [3, 5, 7] BA medium + Effluent H_2 and [6] Effluent H_2 + $NaHCO_3$ 2 g/L

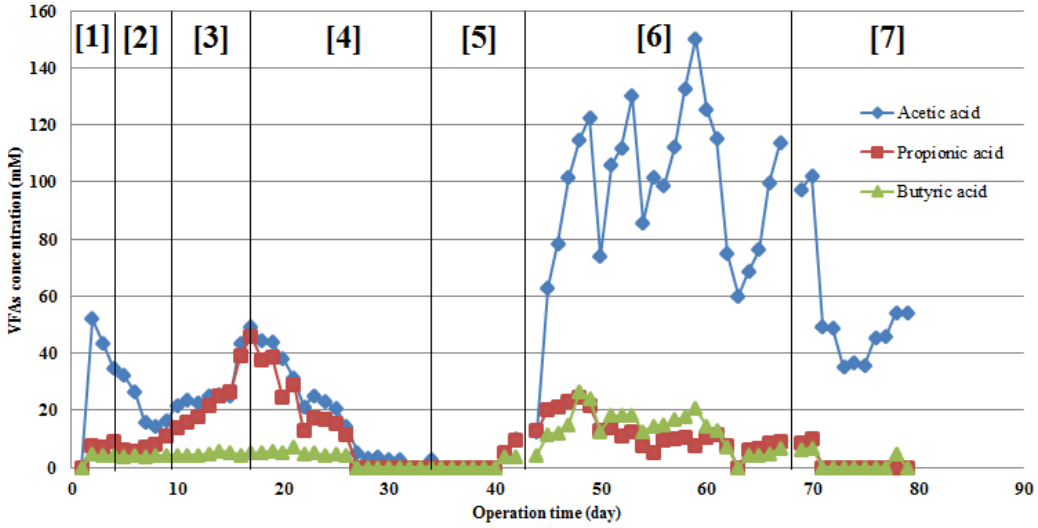

Fig. 4.7 Variation of soluble metabolite products achieved from CH_4-UASB reactor at different HRTs under thermophilic temperatures; [1, 4] BA medium + sucrose 2 g/L, [2] (BA medium + sucrose 2 g/L) + effluent H_2 at 1:1 (%v/v), [3, 5, 7] BA medium + Effluent H_2 and [6] Effluent H_2 + $NaHCO_3$ 2 g/L

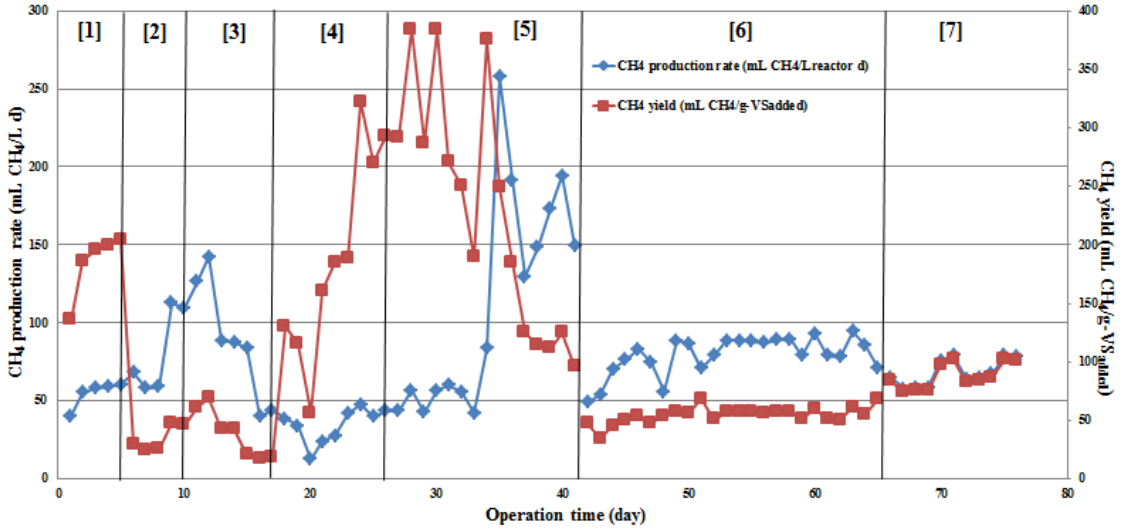

Fig. 4.8 Variation of methane production rate and methane production yield achieved from CH_4-UASB reactor at different HRTs under thermophilic temperatures; [1, 4] BA medium + sucrose 2 g/L, [2] (BA medium + sucrose 2 g/L) + effluent H_2 at 1:1 (%v/v), [3, 5, 7] BA medium + Effluent H_2 and [6] Effluent H_2 + $NaHCO_3$ 2 g/L

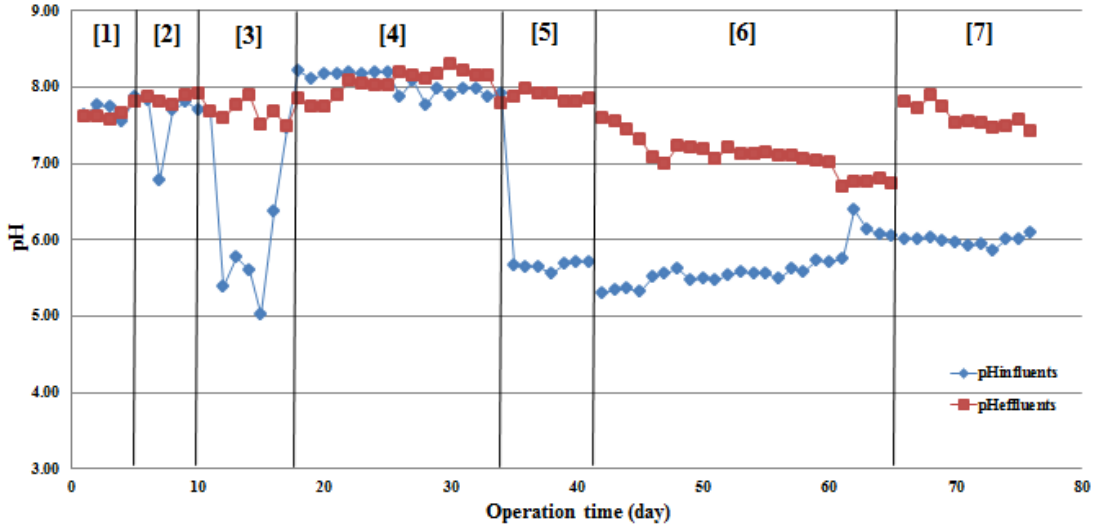

Fig. 4.9 Variation of pH in CH_4-UASB reactor which was operated at different HRTs under thermophilic temperatures; [1, 4] BA medium + sucrose 2 g/L, [2] (BA medium + sucrose 2 g/L) + effluent H_2 at 1:1 (%v/v), [3, 5, 7] BA medium + Effluent H_2 and [6] Effluent H_2 + $NaHCO_3$ 2 g/L

4.4.3 Energy achieved from two-stage co-digestion of SLS with POME

Two stages process for sequentially hydrogen and methane production from co-fermentation of SLS and POME was still lower in energy production yield in both hydrogen and methane phases. Under the optimal conditions, the hydrogen production yield was 34±2 mL H_2/g-VS_{added} and methane production yield 87±11 mL CH_4/g-VS_{added}, respectively. Corresponds to the energy yield was 434.52 kJ/kg-VS and 3482.78 kJ/kg-VS achieved from the first phase and the second phase, respectively. The total energy production yield achieved was 3917.30 kJ/kg-VS were obtained. Meanwhile, the total energy production yield achieved from sole fermentation of SLS and POME was 7505.98 kJ/kg-VS (Kongjan *et al*., 2014) and 14079 kJ/kg-COD (Mamimin *et al*., 2012), respectively. Thus, the optimization is still needed to further investigation to define the optimum conditions for methanogenic stage operation.

4.5 Conclusions

In this research, the hydrogen generated in H_2-CSTR reactor is still low with the hydrogen production yield was 34±2 mL H_2/g-VS_{added} because of there is several problems has occurred are: (i) overload the process; (ii) high hydrogen concentration in the liquid phase; (iii) the growing of sulfate reducing bacteria in the H_2-CSTR reactor. At the same time, relatively high acetate concentration produced and/or accumulated in CH_4-UASB reactor, resulting in low methane production yield was 87±11 mL CH_4/g-VS_{added}. Moreover, the other possible reasons for having low methane formation are: (i) low CH_4-UASB seed; (ii) high hydrogen partial pressure; (iii) the competition of sulfate reducing bacteria with methanogenic archaea has occurred. Although, the energy production yield achieved in this process was still had low of 3917.30 kJ/kg-VS, this study provides valuable information in order to further define the optimal conditions for acidogenic and methanogenic stages operation.