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ABSTRACT

High sulfate contents in skim latex serum (SLS) can be reduced by rubber wood ash (RWA). Subsequently, the desulfated skim latex serum (DSLS) can be further anaerobically treated more effectively with the accompanying generated biomethane. In this study, DSLS was treated using an up-flow anaerobic sludge blanket (UASB) reactor operated at 10-day HRT and under mesophilic (37°C) conditions. The effect of organic loading rates (OLR) at 0.89, 1.79 and 3.57 g-COD/L-reactor·d on DSLS biodegradability was investigated in Phase I-IV using $NaHCO_3$ as an external buffering agent. Maximum methane production yield of 226.35 mL-CH_4/g-COD_{added} corresponding to 403.25 mL-CH_4/L reactor·d was achieved at the suitable OLR of 1.79 g-COD/L-reactor·d. UASB effluent recirculation which was then applied to replace the $NaHCO_3$. It was found that with 53% effluent recirculation similar to an OLR of 2.01 g-COD/L-reactor·d, an average of 185.70 mL-CH_4/g-COD_{added} corresponding to 371.40 mL/L reactor·d of methane production was reached. The dominant bacteria in UASB reactor were members of *Proteobacteria*, *Bacteroidota*, *Firmicutes*, and *Desulfobacterota phyla*. Meanwhile, the archaeal community was majorly dominated by the genera *Methanosaeta sp.* and *Methanomethylovorans sp.* The study clearly indicates the capabilities of UASB reactor with effluent recirculation to treat DSLS anaerobically.

Keywords: Rubber latex wastewater, Sulfate removal, UASB reactor, Anaerobic Digestion, Methane production

1. Introduction

Skim latex serum (SLS) is the wastewater generated from the concentrated latex process after the sulfuric acid coagulation of skim latex which aimed at recovering the rubber. SLS with a high organic matter content of sulfate (3,580-7,500 mg/L), chemical oxygen demand (COD) (33.02-43.11 g/L), Volatile Solids (VS) (32.64-37.94 g/L) and low pH (5.22-5.78) (Jariyaboon et al., 2015; Raketh et al., 2021).

Anaerobic digestion (AD) is widely used for the treatment of high strength wastewaters in the rubber industry. The process is attractive for resource recovery and the production of sustainable energy carrier in the form of biogas. The AD is performed by a high variety of microorganisms in terms of biochemical reactions, i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Min et al., 2014) resulting in the production of a biogas containing 40-75% CH_4, 15-60% CO_2, 5-10% water, and 0.005-2% H_2S, and some amount of traces of other components such as siloxanes, halogenated hydrocarbons NH_3, O_2, CO, and N_2 (Rattanaya et al., 2021; Ryckebosch et al., 2011).

During AD process, sulfate ions contained in the substrate can be transformed to hydrogen sulfide (H_2S) by sulfate-reducing bacteria (SRB) (Mu et al., 2019). H_2S is the major problem for anaerobic treatment of sulfate-rich wastewater, as H_2S may lead to AD process failure. In previous research, SLS was used to produce biogas without reducing sulfate contents (Jariyaboon et al., 2015; Kongjan et al., 2014), resulting in the inhibition of high sulfate contents during the biogas production process.Thus, reducing sulfate contained in wastewaters before AD process is one of the strategies to achieve successful treatment of sulfate-containing wastewaters. In the previous experiments, rubber wood ash (RWA) was used to remove sulfate in the SLS. RWA can reduce sulfate, as high as 42% sulfate removal efficiency at a solubility equilibrium concentration of 10 g/L of added RWA (Raketh et al., 2021). Moreover, SLS with initial sulfate concentration of 5,417 and 1,625 mg/L was used as substrate to produce biogas in two stages and single stage AD, respectively. The results showed that the biogas production yields were lower with SLS than desulfated SLS (DSLS). Using DSLS, it had shown that 21% improvement of biogas production was achieved in the batch reactor compared to the raw SLS (Raketh et al., 2022).

Many reactor configurations have been reported for anaerobically treating concentrated latex wastewaters, mainly up-flow anaerobic sludge blanket (UASB), anaerobic baffled reactor (ABR), and continuous stir tank reactor (CSTR). Kongjan et al. (2014) reported that AD of SLS for hydrogen and methane production in separate process using a two-stage digestion in a series of UASB reactor.. A yield 178.70 mL-CH_4/g-COD_{added} was achieved under thermophilic conditions with 9-day HRT. Furthermore, single-stage AD under mesophilic conditions was studied for the treatment of concentrated latex wastewater (CLW) using ABR with organic loading rate (OLR) of 0.60 g-COD/L·d at 10-day HRT (Saritpongteeraka and Chaiprapat, 2008). A methane production yield of 242.31 mL-CH_4/g-COD_{added} was obtained. Moreover, based on the data collected from the concentrated latex factory in Songkhla Province, Thailand, whereby the factory uses covered lagoons to treat the concentrated latex wastewaters with the feed rate of 0.61 g-COD/L·d at HRT of 15.7 days. In this case the average methane yield was estimated at 219.97 mL-CH_4/g- COD_{added}. UASB reactor is a high-rate reactor, in which biological granules are formed as the anaerobic microorganism's community. Thus, the solid retention time (SRT) was found to be always much higher than HRT. However, the UASB reactor can productively digest organic matters in a low suspension solid (SS) containing wastewater (Angenent et al., 2004) .

Generally, AD performances depend on various parameters, such as the substrate composition, OLR, temperature, pH, C/N ratio, and HRT. Among these parameters, OLR is considered as a significant parameter because it is defined as the amount of COD or VS portions fed per day per unit digester's size. However, high OLR can reduce both the size of digester and consequently, the capital cost. The maximal OLR depends on the type of substrates fed into the digester as it imposes the level of biochemical activity of the digester (Babæe and Shayegan, 2011; Chandra et al., 2012; Cremonez et al., 2020).

In addition, AD reactors require sufficient alkalinity in order to maintain an optimal environment for methanogens whereby below the optimal pH range (6.7-8.0), it had resulted in the inhibition of methane-producing archaea (MPA) (Deublein and Steinhauser, 2011; Kongjan et al., 2014). KOH, NaOH, Na_2CO_3, and $NaHCO_3$ as alkali solutions is usually added to maintain the pH in methanogenic reactors. However, the cost of alkali chemicals is also an important element to be considered as well as the additional chemicals associated with the overloading of Na^+ and K^+ ions which can severely inhibit MPA at high concentration. One of the strategies which can be employed to overcome the above limitations is the recirculation of effluent/sludge from the AD process. This process can help to neutralize the pH through the dilution of influent fed into the reactor with subsequent improvement in the transformation. Previous studies reported that anaerobic digestion of vegetable market waste in a 4-chambered anaerobic baffled reactor (ABR) with effluent recirculation (25-100%) was regarded as feasible. The biogas and methane yields reached around 0.7–0.8 L biogas/gVS_{added}/d and 0.42–0.52 Lmethane/gVS $_{added}$/d, respectively, which were among the highest reported for anaerobic digestion of vegetable waste (Gulhane et al., 2016). More stable performances were also observed in the reactor with recirculation (Wikandari et al., 2018). Thereby, effluent recirculation is probably a good substitute for alkaline compounds to maintain appropriate pH and reach the optimum range of biogas production.

As mentioned earlier, the previous experiment has confirmed the enhancement of anaerobically treatment simultaneously biogas production of the DSLS using batch process. Then the process must be proved in a continuous mode before scaling up to the industrial application. Therefore, the objective of this study was to investigate the effect of OLR on the treatment performances of continuously treating DSLS wastewaters. The strategies to maintain a sufficient alkalinity by using $NaHCO_3$ buffering supplement and the UASB effluent recirculation were also compared. The treatment performances were assessed through methane production, COD and sulfate removal efficiencies, volatile fatty acids (VFA) accumulation, and microbial community.

2. Materials and methods

2.1. Substrate and Inoculum

Fresh raw SLS was collected from the skim latex serum coagulation baths in a concentrated latex factory located in Songkhla Province, Thailand. The collected SLS was stored at 4°C to minimize self-biodegradation and acidification (maximum storage was 1 month). Characteristics of SLS and Desulfated SLS are presented in Table 1. RWA was achieved from a high-pressure steam boiler of a glove factory situated in Songkhla Province, Thailand. The collected RWA was stored in covered container at room temperature.

DSLS was prepared following the method described in Raketh et al. (2021) (Raketh et al., 2021). A ratio of 10 g-RWA to 1 L SLS was used to remove sulfate from SLS. The mixer was continuously stirred at 150 rpm for 10 minutes at room temperature. Then, the ash residue was immediately separated from the mixed solutions and a desulfated solution, so-called DSLS was obtained.

Anaerobic granules used in this study were obtained from the UASB reactor of a frozen food factory in Songkla Province, Thailand. The mesophilic methane inoculum was sampled from a biogas plant using palm oil mill effluent as substrate in a palm oil mill factory located in Surat Thani Province, Thailand.

Table 1 Characteristics of skim latex serum (SLS) and Desulfated SLS

Parameters	**Unit**	**Value**	
		SLS	**DSLS**
pH		5.24 - 5.54	5.99 - 6.45
total Solids (TS)	g/L	38.89 - 41.01	39.82 - 41.99
Volatile Solids (VS)	g/L	32.45 - 34.42	33.23 - 35.25
Ash	g/L	6.44 - 6.59	6.59 - 6.75
Chemical Oxygen Demand (COD)	g/L	36.00 - 38.40	37.01 - 39.48
Total Organic Carbon (TOC)	g/L	14.25 - 15.12	NA
Sulfate	mg/L	4,452 - 4,728	2,793 – 2,979
Alkalinity	mg-$CaCO_3$/L	2,890 - 2,953	3,108 - 3, 267
Total Kjeldahl Nitrogen (TKN)	mg/L	1,548 - 1,588	NA

NA denoted not analyzed

2.2. Reactor set-up and operation

Firstly, to enrich the microorganisms, 100 mL basic anaerobic (BA) medium (supplemented with 3 g/L glucose) (Angelidaki and Sanders, 2004), 100 mL DSLS at a concentration of 33.23 g VS/L , and 1800 mL of anaerobic granules and methane inoculum mixture (70:30 by volume).were mixed in a batch reactor. The reactor was purged by 2 L/min nitrogen gas for 10 min to ensure anaerobically condition and incubated at ambient temperature (30-33 °C). The volume and composition of biogas were monitored daily. It was found the biogas production was steady within 14 days.

In this experiment, UASB reactor was operated with 1,200 mL working volume and maintained at 37 °C by circulating hot water inside a water jacket surrounding the reactor. The UASB began by adding 840 mL of the enriched microorganism's solution (70% of working volume) and 360 mL of 33.23 g VS/L DSLS. Nitrogen gas at 2 L/min was used to purge the reactor for 10 min to ensure anaerobically conditions. For the start-up phase , the reactor was operated at HRT of 20 days by feeding a mixture of DSLS and the 2.6 g/L final $NaHCO_3$ solution which corresponding to the OLR of 1.1 g-COD/L-reactor·d. The feed mixture of 30 mL was transferred to the UASB twice a day using a peristaltic pump. The methane production at start-up phase was continuously performed for 25 days.

According to the methane production profile of DSLS in batch mode reported by Raketh et al. (2022), it indicated that 90% of maximum methane production was obtained within 10 days. Thus, the HRT was decreased to 10 days in order to increase the methane production rate in a continuous process. The effect of OLR at 0.89, 1.79, and 3.57 g-COD/L-reactor·d on biogas production were carried out in phase I-IV, respectively. $NaHCO_3$ solution which was prepared from tap water was used to dilute DSLS and obtain the desired COD concentration for each OLR, with the 2.6 g/L final $NaHCO_3$ concentration in the feed. The feed mixture of 60 mL was transferred to the UASB twice a day using a peristaltic pump. In Phase V, the $NaHCO_3$ solution was substituted by adding 53% in volume of the UASB effluent, mixed with DSLS before feeding corresponding to OLR 2.1 g-COD/L-reactor·d was operated.

During UASB operation, biogas volume and composition were daily analyzed. pH and alkalinity of the effluent were also daily monitored. COD, sulfate content and VFAs in the effluent were analyzed at the steady-state of each phase. The steady-state was considered when the variation of biogas production was less than 10% as suggested in (Kongjan et al., 2014). Phase I-V conditions were operated for approximately three times of HRT.

Anaerobic granules samples were taken from the effluent at steady state. 10-15 whereby the granules were randomly separated from the effluent after 10 minutes of sedimentation, and their diameter were measured using a Vernier caliper. For microbial community analysis, the sediment granule samples were also taken from the effluent at steady state and stored at -20 ^{0}C before the analysis.

2.3. Analytical methods

The volume of produced biogas was recorded using a laboratory water displacement set. Biogas main composition of CH_4 and CO_2 were analyzed using gas chromatography equipped with a 2.5 m Porapak Q column and a thermal conductivity detector (Shimadzu GC 14A). A 30 mL/min. Helium was used as a carrier gas at a flow rate of 30 ml/min. The temperature of injection port, oven, and detector were set at 100, 60, and 110 ºC, respectively. A 0.5 mL sample of the gas was injected in triplicate. While, H_2S concentration in the biogas was measured using a gas chromatography fitted with a 2.5 m Porapak S column with Hayesep Q (80/100) and a flame photometric detector (Shimadzu GC 14A). Helium at a flow rate of 30 mL/min was used as the carrier gas. The injection port and detector were set at the same temperatures of 150 ºC. A 0.2 mL sample of the gas was injected in triplicate.

VFAs (acetic, propionic, and butyric acid) in the liquid sample were measured by using the gas chromatograph connected with a flame ionization detector (Shimadzu GC 8A). A 30 m capillary column packed with fused silica (Stabiwax® column) was used. The inlet temperature of 230°C and detector temperatures of 250°C were set. The running temperature of the column were set as 60 °C for 35 min, 2 °C/min to 110 °C, 10 °C/min to 200 °C, and hold for 1 min.

Total alkalinity, COD, Total Kjeldahl Nitrogen (TKN), TS, VS, ash, pH and sulfate content of the liquid sample were analyzed according to the standard methods (APHA, 2012). TOC-Liquid: multi N/C 3100 TOC analyzer (Analytik Jena) was used to determine the total Organic Carbon (TOC).

The microbial communities were analyzed by using the Next Generation Sequencing (NGS) technology. Total genome DNA from the samples was extracted using CTAB/SDS method. DNA concentration and purity was monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1ng/μL using sterile water. 16S rRNA/18SrRNA/ITS genes of distinct regions which were amplified using specific barcode. All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). PCR products quantification and qualification was carried out by mixing the same volume of 1X loading buffer (containing SYB green) with PCR products and with an operated electrophoresis on 2% agarose gel for detection. Samples with bright main strip between 400bp-450bp were selected for further experiments. PCR products were mixed at equal density ratios. The mixed PCR products were purified with Qiagen Gel Extraction Kit (Qiagen, Germany). The libraries generated with NEBNext® UltraTM DNA Library Prep Kit for Illumina and quantified via Qubit and Q-PCR, were analyzed by Illumina platform. Statistically significant differences in the results were determined using the one-way analysis of variance (ANOVA) of SPSS v26.0 software (IBM, USA).

3. Results and discussion

3.1. Performances of UASB reactor fed with $NaHCO_3$-supplemented DSLS

Daily methane production rates, methane yields and methane contents of the biogas in the UASB reactor fed with $NaHCO_3$-supplemented DSLS are presented in Fig. 1. A summary of the reactor performances at steady state are given in Table 2. For the start-up phase fed with DSLS at OLR 1.11 g-COD/L-reactor·d and HRT of 20 days, an average methane production rate of 174.52 mL/L-reactor·d was observed.

After the start-up phase, HRT was reduced to 10 days and the OLR was also reduced to 0.89 g-COD/L-reactor·d in Phase I. The higher feed flow rate with lower feed concentration has let the system to slowly acclimate to the higher shearing force. An average methane production rate at steady state of 148.98 mL/L-reactor·d slightly lower than the start-up phase was observed. The average methane yield was 166.40 mL-CH_4/g-COD_{added} and the average methane content in the biogas was 66.23%.

In Phase II, where the OLR was twice higher (1.79 g-COD/L-reactor·d), the average methane production rate reached 403.25 mL/L-reactor·d. This result indicates that a higher substrate density could enhance the activities of microorganisms present in the reactor, reaching also to a higher methane yield of 226.35 mL-CH_4/g-$COD_{added.}$ A slightly higher average methane content in biogas of 67.19% was also observed. In addition, the methane yield in this phase achieved 77.27% of the theoretical yield (350 mL-CH_4/g-COD) which was 270.75 mL-CH_4/g-$COD_{removed}$.

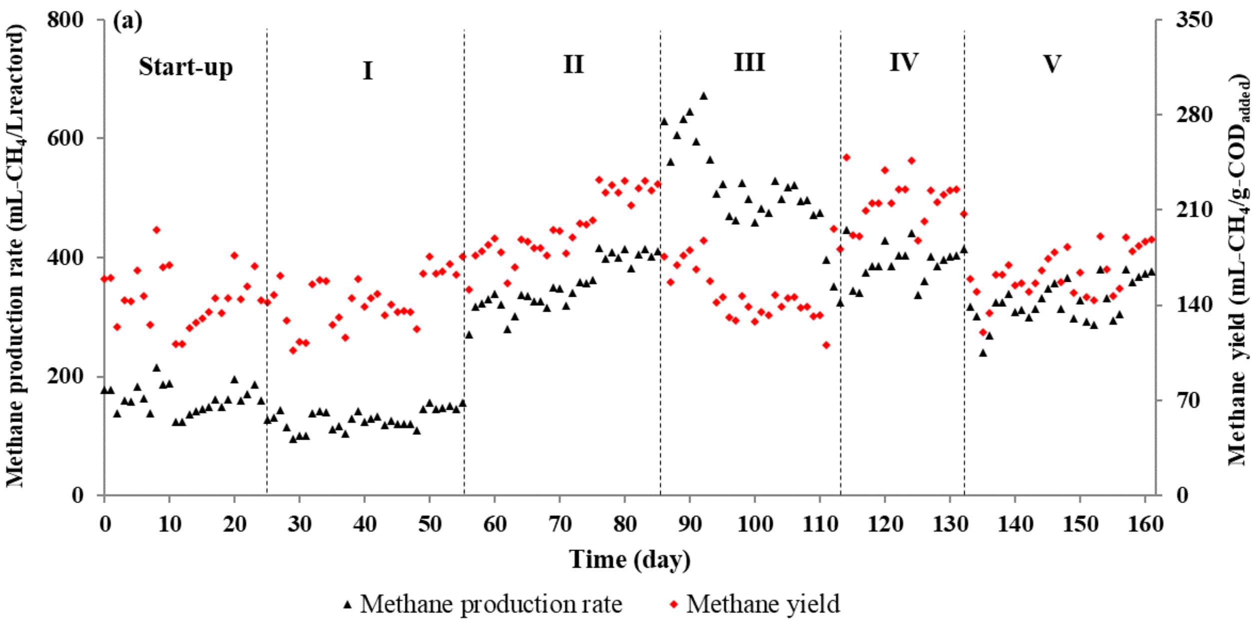
In phase III, the OLR was increased to 3.57 g-COD/L-reactor·d. In this phase, methane production rate achieved 467.61 mL/L-reactor·d which was 16% higher than phase II. However, the methane yield and average methane concentration has significantly decreased to 130.50 mL-CH_4/g-COD_{added} and 51.81%, respectively. The methane yield was 158.03 mL-CH_4/g-$COD_{removed}$ which was only 45.15 % of the theoretical yield. Meanwhile, an increase in VFA concentration was observed in Phase III. The remaining VFA concentrations in the effluent are presented in Fig.2. In all phases, butyric acid had the lowest concentration in the effluent. Acetic and propionic acids were detected in nearly amounts in the effluent. The VFA concentrations in phase III were higher than during the other phases operated at a lower OLR as shown in Fig.2 (1.54 g/L acetic acid, 1.48 g/L propionic acid, and 0.58 g/L butyric acid). Higher influent COD concentration had therefore led to higher VFAs concentration which possessed the potential to partly inhibit the methanogenic activity, hence lowering the methane yield.

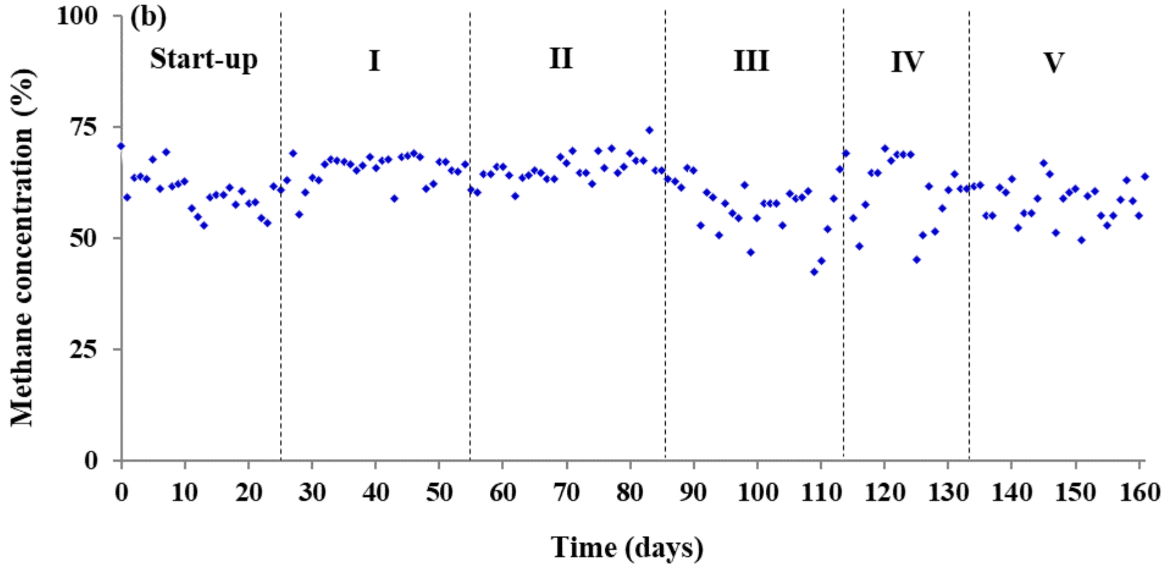
The optimum concentration of acetic acid, propionic acid, and butyric acid to achieve the maximum cumulative methane yield is 1.6, 0.3, and 1.8 g/L, respectively, as reported by Wang et al. (2009). A concentration level of 2.0 g/L of acetic, 0.9 g/L of propionic acid, and 4.5 g/L of butyric acid were reported as inhibition threshold levels of VFAs acid (Demirel and Yenigün, 2002).

Since the raw SLS has also exhibited a high sulfate concentration, thus the reduction of sulfate, to H_2S by sulfidogenesis is unavoidable and considered as a major concern for an effective anaerobic treatment. RWA was used to reduce sulfate in SLS, but since RWA also release some sulfate, thus a maximum of 10 g/L RWA loading was suggested (Raketh et al., 2021). Sulfate concentration in the high OLR phases are shown in Table 2. The DSLS in phase III contained the highest sulfate concentration.

This is also the reason that could have led to lower methane production yield. H_2S concentration during methane production in UASB reactor shown in Fig.1c. Trends of H_2S concentration in the biogas production fully correlated with the sulfate loading rate. Phase I with the lowest sulfate loading influent produced very low H_2S in the biogas. There was significant difference of sulfate removal efficiency between Phase I and III. In Phase III the highest H_2S generation in a range of 16,826-36,661 ppm was exhibited.

Then, the feed rate was reduced to OLR of 1.79 g-COD/L-reactor·d similarly to Phase II for the purpose of confirming the optimum feed rate to attain the highest methane yield. The methane production rate was however slightly lower (3% lower) than in Phase II, while the average methane content in biogas rebounded to 61.60 %. Furthermore, methane yield showed no significant difference between phases II and IV.





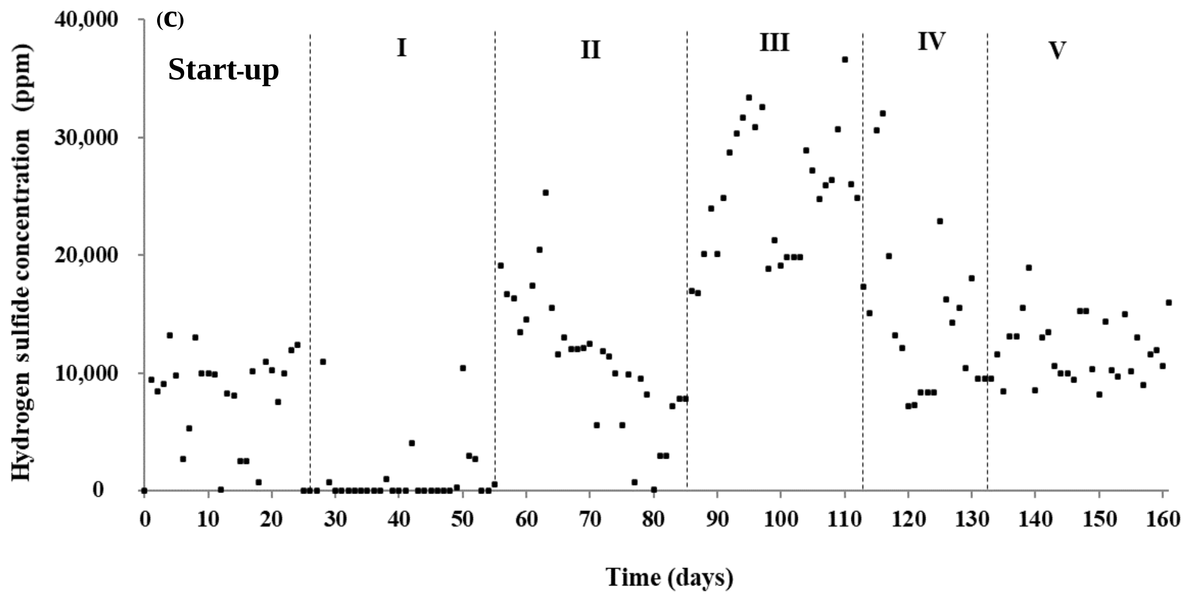


Fig. 1. UASB reactor performance of methane production: (a) methane production rate and methane yield, (b) methane concentration, and (c) hydrogen sulfide concentration, for various levels of OLR (Phase I=0.89, II=1.79, III=3.57, IV=1.79, and V= 2.01 g-COD/L-reactor·d) at 10-day HRT

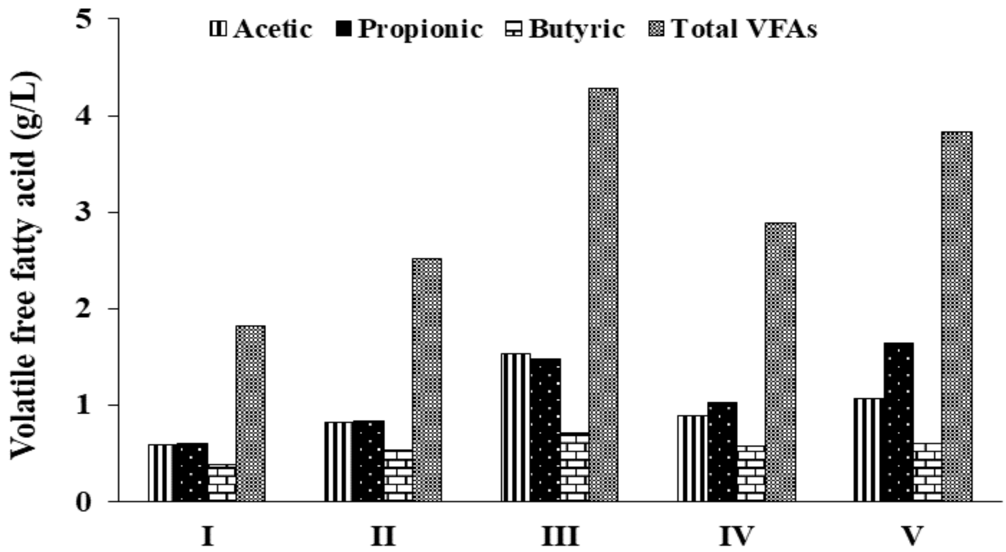


Fig. 2. Volatile fatty acids of effluent at steady-state in UASB reactor during methane production, for various levels of OLR (Phase I=0.89, II=1.79, III=3.57, IV=1.79, and V= 2.01 g-COD/L-reactor·d) at 10-day HRT.

Table 2 Summary of the steady-state methane production with DSLS at 10-day HRT.

Parameters	**Values**				
Phase	I	II	III	IV	V
OLR (g-COD/L-reactor·d)	0.89	1.79	3.57	1.79	2.01
Day at the condition (day)	26-55	56-86	87-112	113-132	133-162
Day at steady state (day)	50-55	77-86	108-112	127-132	158-162
CH_4 yield (mL/g-COD_{added})	166.40[a]	226.35[b]	130.50[c]	218.70[b]	185.70[d]
CH_4 yield (mL/g-$COD_{removed}$)*	210.45[a]	270.45[b]	158.03[c]	269.49[b]	224.83[a]
CH_4 production rate (mL/L-reactor·d)	148.98[a]	403.25[b]	467.61[c]	389.60[b]	371.40[d]
CH_4 composition (%)	66.23[a]	67.19[a]	51.81[b]	61.60[c]	59.79[c]

H_2S composition (%)	0.54[a]	0.65[a]	2.91[b]	1.24[c]	1.08[c]
Biogas yield (mL/g-COD_{added})	265.34	365.00	212.79	363.57	300.00
Biogas production rate (mL/L-reactor·d)	233.33	647.00	738.00	656.25	596.94
Influent pH	7.13	6.57	6.56	6.73	7.02
Effluent pH	7.48	7.70	7.63	7.62	7.71
Influent COD (mg/L)	8.87	17.85	35.50	17.85	20.05
Effluent COD (mg/L)	2.60	4.65	9.43	4.99	5.37
COD removal efficiency (%)	70.66[a]	73.95[a]	73.44[a]	72.04[a]	73.24[a]
Influent sulfate (mg/L)	695.10	1390.20	2780.40	1390.20	1488.45
Effluent sulfate (mg/L)	346.91	274.54	212.21	184.19	194.98
Sulfate removal efficiency (%)	50.09[a]	80.25[b]	92.37[c]	86.75[c]	86.90[c]
Energy recovery (kJ/g-COD_{added})	5.30	7.14	4.14	6.94	5.89

All the value is in average,

*The CH_4 yield was calculated at STP,

[a– d] are the statistically significant difference ($p \leq 0.05$).

3.2. Performances of UASB reactor fed with DSLS with effluent recirculation

To maintain the buffering capacity, the $NaHCO_3$ solution was replaced by a recirculation of the UASB effluent during Phase V. In this phase, OLR of 2.01 g-COD/L-reactor·d was applied, which was slightly higher than in Phase IV due to the remaining COD in the effluent. In Phase V, methane yield and methane production rate were lower than in the optimal feed rate (Phase II and IV) which was 185.70 mL-CH_4/g-COD_{added} and 371.40 mL/L-reactor·d. The average CH_4 composition was 59.79 % which is slightly lower than that in Phase IV. However, there was no significant difference of average CH_4 composition between phase IV and V.

Nonetheless, methane yield in this phase (185.70 mL-CH_4/g-COD_{added}) was higher than in Phase I and III as show in Table2.

3.3. Monitoring of alkalinity and pH

After removal of sulfate with RWA, the pH of DSLS increased from 5.24-5.54 to 5.99-6.45. The alkalinity of DSLS (3,108 - 3, 267 mg-$CaCO_3$/L) was also higher than in the raw SLS (2,890-2,953 mg-$CaCO_3$/L), most likely due to the the alkaline leachate from the metal oxide of RWA, released into desulfated SLS. The desulfated SLS used in AD process was diluted by the $NaHCO_3$ solution, hence the initial alkalinity of influent decreased during Phase I, II, III, and IV at 1,590, 2,110, 3,080, and 2,650 mg-$CaCO_3$/L, respectively. In Phase V, the DSLS was diluted by the rich alkalinity effluent, thus higher alkalinity of 4,930 mg-$CaCO_3$/L was obtained.

Alkalinity is the parameter referred to the buffer capacity of the AD system. The fact regarding this matter is that it should have high alkalinity enough to maintain the system pH. A digester should be kept higher than 2,000 mg-$CaCO_3$/L of alkalinity o to resist to the changes of pH in the system (Reungsang., 2019). Alkalinity of the effluent during all operation phases was higher than 2,000 mg-$CaCO_3$/L (Fig.3a), indicating that the digester was kept within the desired range of alkalinity by adjusting the buffering capacity in the feed. The start-up period showed a higher effluent alkalinity than in phases I and II due to a longer HRT. The effluent alkalinity increases when OLR increased from Phase I to Phase III because the influent alkalinity was also increased. The trend of effluent alkalinity in Phase III showed the highest trend due to higher COD loading in influent than the other phases. Moreover, a sharp increase in effluent alkalinity can also be used as a parameter to monitor and control the AD systems. In

Phase III, the trend of effluent alkalinity was increasing which can negatively affect the system if the value is too high, hence the operation in Phase III was switched back to lower OLR before the three times HRT operation time (at day 26 of Phase operation). The effluent alkalinity in Phase V was maintained at about 8,000 mg/L. This indicated that using the effluent recirculation strategy was successful for maintaining high alkalinity without external chemical addition to efficiently produce biogas from DSLS.

The pH of influent and effluent during methane production are presented in Fig.3b, which indicated that pH significantly affects the performance of AD system, and also considered as a crucial factor influencing the growth of diverse microorganisms. The optimal pH range for producing methane is 6.7-8.0 (Chandra et al., 2012; Cremonez et al., 2020; Kongjan et al., 2014). The result indicated that the pH of influent was maintained by using $NaHCO_3$ in Phase I to IV and by using effluent recirculation in Phase V. Influent pH fluctuated depending on the OLR of DSLS and the characteristics of the raw SLS which was collected from the factory once a month. During Phase I, the pH influent ranged 6.93-7 22. Then, change of OLR during Phase II had caused a slight decrease of influent pH (6.44-6.85) due to higher influent COD concentration. As expected, the lowest pH (6.24-6.62) was obtained at the highest OLR in Phase III. The other promising method to raise the pH of DSLS is mixing with the alkaline rich effluent. As shown in Phase V, the influent pH was increased to 7.20-7.43. It is worth to note that a rather stable pH of effluent in the range of 7.48-7.90 were obtained in Phase I – V. This indicated that all operating phases were run at the condition which provide sufficient buffering capacity to properly maintain the system pH.

In addition, monitoring the ratio of VFA and alkalinity (VFA/ALK) produced in the reactor is a more valuable tool in following the performances of the AD process. The VFA/ALK ratio is the specific bicarbonate alkalinity level that can help furnish insight into the reactor stability. In this study, the VFA/ALK ratio during operation varied between 0.02-0.31, which was lower than the 0.40 imposed in the literature as the inhibition thresholds. Most probably this was due to an excessive concentration of VFA which led to the process of acidification (El Gnaoui et al., 2020; Kim and Kim, 2020; Wilawan et al., 2014). Hence, the VFA/ALK ratio during operation remained in the optimum range and the reactor could maintain the stability of the buffering capacity for optimal methane production throughout the experiment.

During the AD, carbon to nitrogen (C/N ratio) has effects on methane production, and it is an essential factor for stable operation. The C/N ratio of the SLS in the current study is approximately 9.5, which is a low value when compared to the optimum C/N ratio (20-30) (Fu et al., 2012), due to the SLS contains a high concentration of nitrogenous compounds such as ammonia and protein. When C/N ratio of substrate is low, nitrogen will be rapidly consumed for growing most microbes, although this has a positive effect on methane production rate. However, the form of ammonium ions that increases the pH can adversely affects biogas production (Yen and Brune, 2007). This is one of the reasons which might probably described the product inhibition from the overloading in Phase III.

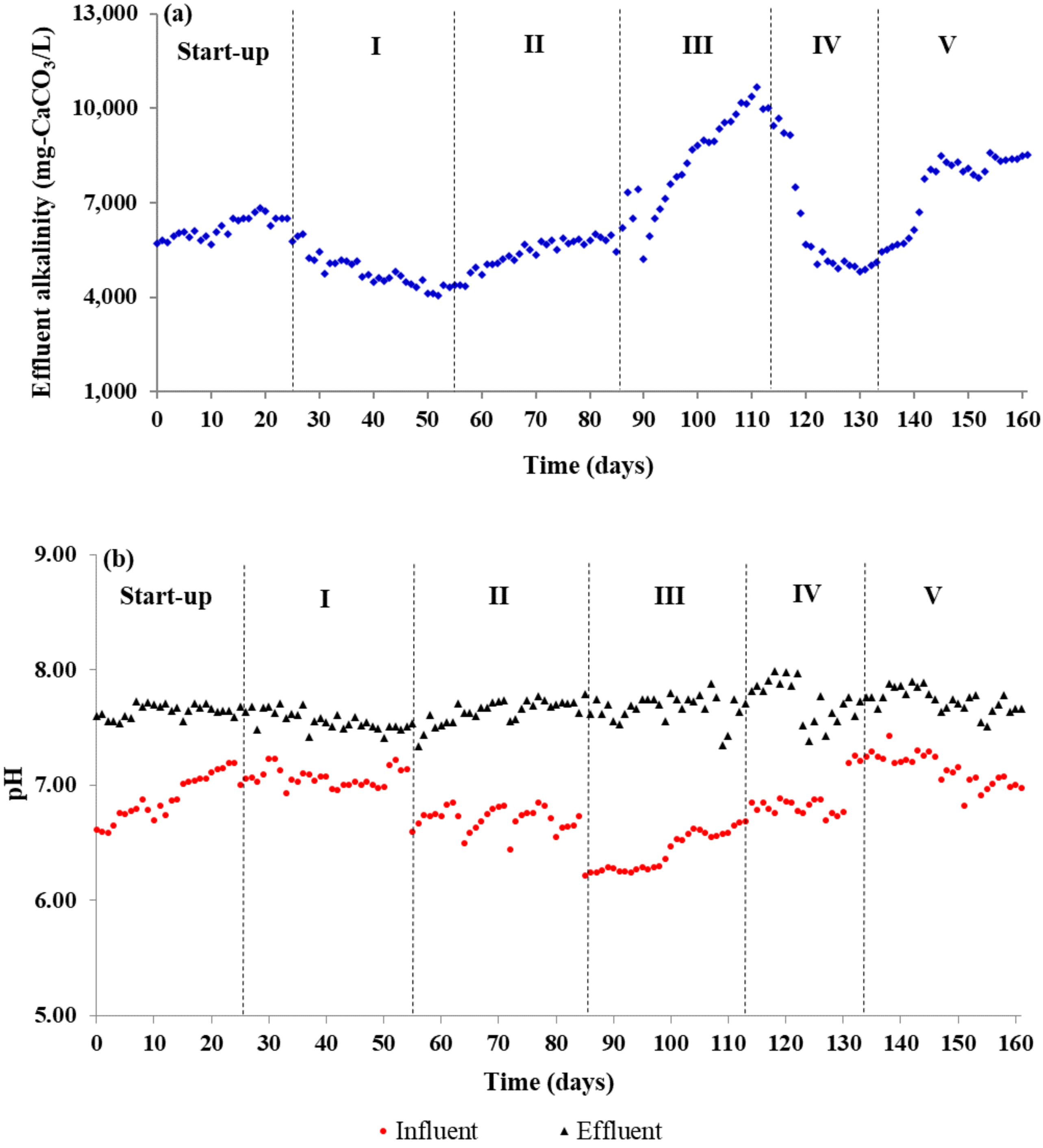


Fig. 3 UASB reactor performance of methane production: (a) effluent alkalinity, and (b) pH of influent and effluent, for various levels of OLR (Phase I=0.89, II=1.79, III=3.57, IV=1.79, and V= 2.01 g-COD/L-reactor·d) at 10-day HRT.

3.4. Anaerobic biodegradability

COD removal efficiencies in Phase I - V ranged between 70.66 and 73.95 %. The operation at Phase I obtained lower COD removal efficiency than other phases due to a lower COD concentration. When COD concentration was increased to 17.85 mg/L in Phase II, the COD removal efficiency slightly increased and was then constant when COD was increased to 35.50 mg/L in Phase III. For the result presented that there were no significant differences in COD removal efficiencies. Although the pathway of methanogenesis was changed due to different COD and sulfate concentration was feed during Phase I-V but COD removal efficiencies of all phases was not different.

In addition, The COD distribution in the effluent was also calculated to check the reliability which is presented in Table S1. The total of main VFAs were contributed to the effluent ranged from 54.80-88.24%. While the rest of the organic matter such as sugar, lactic acid, formic acid, and ammonium group were left in the effluent ranging in 11.76-45.20 %. Phase III had the highest total VFA concentration due to the highest COD concentration fed. However, when considering the COD contribution in the effluent, it was found that the other organic matter beside the main VFAs were also presented in higher portion compared to another phase with lower OLR loading. This observation confirms that the lower biodegradation causing by lower microorganism favorable at the overloading of OLR at Phase III.

Typically, during AD, organic substances are converted to biogas with the main composition of 40-75% CH_4, 15-60% CO_2, 5-10% water, and 0.005-2% H_2S. Higher CH_4 production yield is expected along with higher COD removal. However, in systems containing high sulfate concentrations, Sulfate-Reducing Bacteria (SRB) are also able to use the organic substances to generate H_2S, outcompeting MPA in using organic substances to produce methane. This resulted in a decrease of the methane production yield and higher H_2S production when sulfate concentration was increased. For instance, the VFAs can be converted to H_2S as illustrated in the following Equation (6)-(8) (Jariyaboon et al., 2015) .

$$C_2H_5COOH + 0.75H_2SO_4 \longrightarrow CH_3COOH + CO_2 + H_2O + 0.75H_2S \ \Delta^0 = -74.3\ kJ \quad (6)$$

$$CH_3COOH + H_2SO_4 \longrightarrow 2CO_2 + 2H_2O + H_2S \ \Delta^0 = -108.3\ kJ \quad (7)$$

$$4H_2 + H_2SO_4 \longrightarrow H_2S + 2H_2O \ \Delta^0 = -194.61\ kJ \quad (8)$$

In this study, the average sulfate removals ranged between 50.09 and 92.37 % and the highest sulfate removals were achieved at Phase III. The organic loading corresponding to initial sulfate loading in influent, the higher OLR was fed resulting in higher sulfate fed too. The result presented that higher sulfate removal efficiencies were obtained correspondingly with higher H_2S concentration observed in the biogas. Normally, when higher OLR was fed not only sulfate was higher but also the metal ions which was leached from RWA during sulfate removal process. The metal ion leached from RWA such as Ca, Mg, Fe, Ni, P, and K. Each ion had a limit value for the optimum condition for methane production in the AD process as mentioned in our previous research (Raketh et al., 2021). Therefore, metal ions leaching was also a one of the reasons which was affected to the AD process.

The energy production in this process was also assessed and the values are shown in Table 2. Only methane heating value was used to convert the produced biogas to energy. The energy yield from DSLS ranged 4.14-7.14 kJ/g-COD_{added}. Phases II and IV had high energy yield with 7.14 and 6.94 kJ/g-COD_{added}, respectively, while the highest OLR (Phase III) led to the lowest energy yield (4.14 kJ/g-COD_{added}) due to the production of minimum methane yield. Phases II and IV showed higher potential to recover energy from DSLS than Phases I and III. Energy recovered from Phase II was even 42.02% higher than the energy generated in Phase III. However, a higher COD concentration of DSLS was loaded causing the inhibition to possibly occur more significantly, and achieved a lower energy recovery yield. Phase V achieved higher energy recovery (5.89 kJ/g-COD_{added}) than Phase I and III while attaining 17.5 % of energy recovery, lower than Phase II.

3.5. Microbial community

Fig. 4 presents the relative abundance of the microbial community kingdom namely: (a), bacteria in phylum level (b), archaea in genus level (c) the microbial community in the UASB reactor for various levels of OLR.

The highest relative abundance of total bacteria was observed in Phase III, operated at the highest OLR, while Phases I and II showed similar quantities. The percentages of relative abundance present the indication of bacterial amounted for 77.86%, 78.25%, and 86.05% in Phase I, II, and III, respectively (Fig.4a). The microbial consortium exhibited the highest capability in Phase III due to the highest organic loading applied in this phase whereby the higher concentration of both organic compounds and nutrients in the system could drive the rate of the biochemistry reactions leading to more growth of bacteria community.

Sequences retrieved from Phase I showed dominant phyla within the bacterial community with *Bacteroidota* (23.46%), *Desulfobacterota* (17.73%), and *Chloroflexi* (15.45%). Simultaneously, the bacterial community in Phase II was dominated by *Bacteroidota* (25.69%), *Desulfobacterota* (18.04%), and *Synergistota* (14.83%) phyla. On the contrary, Phase III was illustrated among three main bacterial community phylum which was dominated by *Proteobacteria* (42.24%), *Firmicutes* (17.77%), and *Bacteroidota* (13.03%) as shown in Fig.4b. *Bacteroidetes* and *Firmicutes* represent important contributors for the degradation of saccharides and proteins. As well as enriching at an expeditious multiplication rate in a growth environment, it also indicated the high concentration of a soluble organic substance. Additionally, VFAs such as butyrate were reported to be biodegraded by *Firmicutes* as fermentative and syntrophic bacteria (Garcia-Peña et al., 2011; Kabisch et al., 2014) .

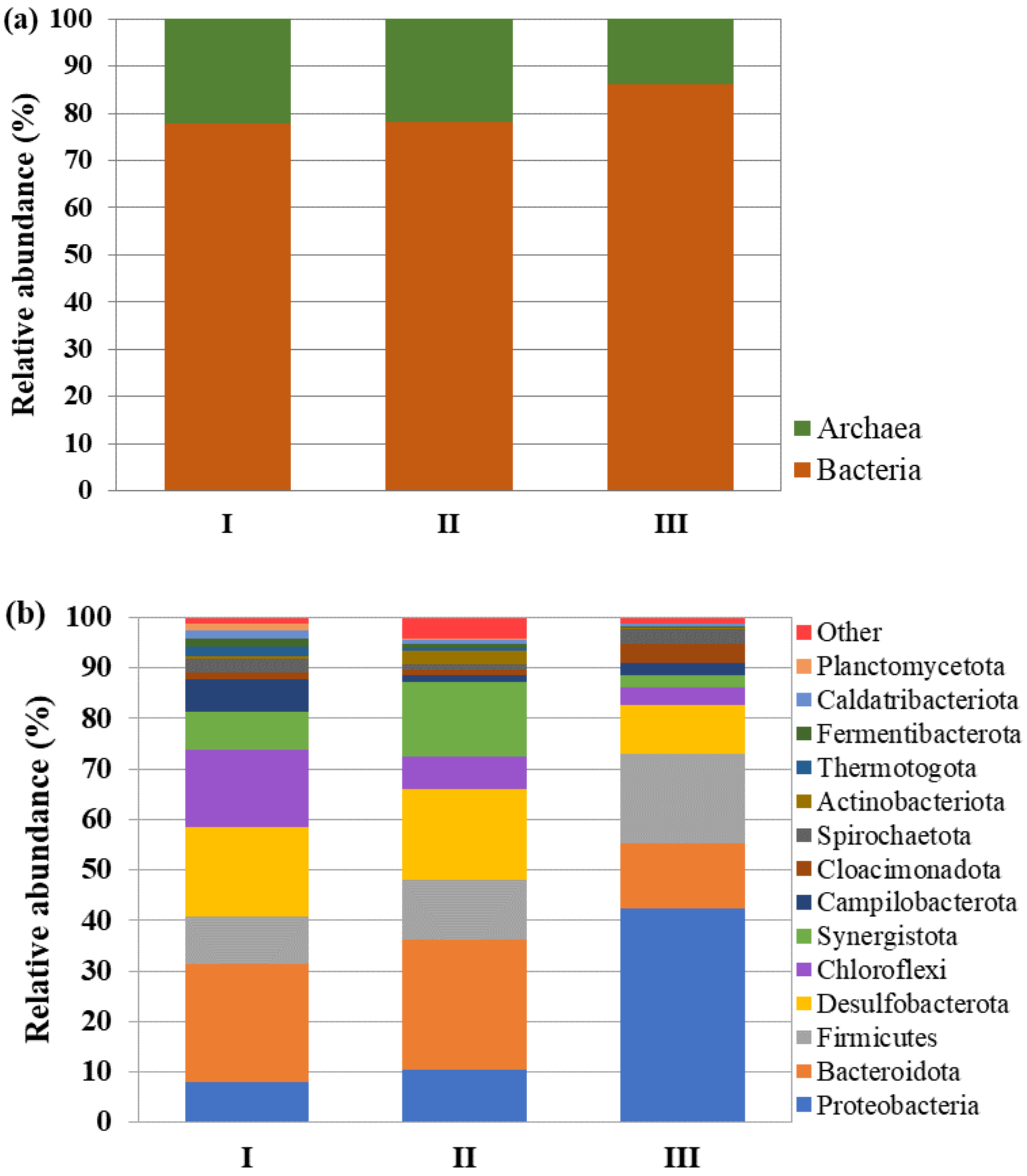
Similarly, *Proteobacteria* and *Chloroflexi* are also important bacteria involved in hydrolysis and acidification. These bacteria made the overall transformations that underpin the function of AD systems (Dai et al., 2016; Petriglieri et al., 2018). *Desulfobacterota* is a phylum known to harbor sulfur-cycling bacteria (Bell et al., 2022). The members affiliated with *Desulfobacterota* were the third most abundant phylum of Phase I and II. *Desulfobacterota* is mostly composed of a diversity of SRB (Yang et al., 2022). The presence of SRB in this study was found to be resulted from the use of sulfate-rich wastewater in AD process.

The abundance of archaea community is presented in Fig.4c. Archaea were more dominant in phases I and II than in Phase III. This corresponds to the decreasing in methane production yield in Phase III as mentioned previously. The third main genus of archaea in Phase I was affiliated to *Methanosaeta* (87.14%), *Candidatus_Methanofastidiosum* (3.40%), and *Methanomethylovorans* (3.15%). Furthermore, Phase II, and III were found to be majorly dominant by *Methanosaeta* and *Methanomethylovorans* which were 44.73%, 45.24% of Phase I, and 23.44%, 72.47% of Phase II, respectively. The results indicated that the *Methanomethylovorans* appeared to be relatively higher in Phase II and III. *Methanolinea* and

Methanobacterium species were also detected but at low level (<3%).

The *Methanosaeta* genus gathers acetoclastic methanogens utilizing acetate as a substrate for methane production (Dai et al., 2016). *Methanomethylovorans sp.* are methylotrophic methanogens and competent to grow and achieve methanogenesis from methanol, mono-, di-, and trimethylamine. Hydrogen and acetate are not utilized (Kim and Rhee, 2015; Whang et al., 2015). Methylotrophic methanogenesis is often presented to be responsible for methane production in sulfate-rich environments (Xiao et al., 2018). According to the SLS was used as a substrate which was sulfate-rich wastewater, resulting *Methanomethylovorans sp.* was found in all phases. Particularly in Phase III the highest *Methanomethylovorans sp*. appeared due to the highest OLR and sulfate were loaded. The results confirms that although the diverse genus-aerchare was found, it meant that the pathway to produce methane was different, but the COD removal efficiencies were still obtained in similar value which was mentioned in previous section.

The composition of the microbial community in the reactor is related to methane production performances, and seed organisms or inoculum type that could also have a large impact on reactor dynamics (Rajendran et al., 2020). A variety of anaerobic bacteria and methanogenic archaea were observed in this study with differences in their relative abundance. The relatively high abundance of *Proteobacteria, Bacteroidota, Firmicutes,* and *Desulfobacterota* were found in all phases in the AD process. Interestingly, it was also observed that a relative dominance of *Desulfobacterota* phylum was likely due to the use of sulfate-rich wastewater as substrate. An effective metabolism was achieved from archaeal community majorly dominated by the genera *Methanosaeta sp*. and *Methanomethylovorans sp.* Nonetheless, the microbial community analysis was performed to provide a better understanding of the granules functioning and support the macroscopical observations. Interestingly, a clear shift of the archaeal community was observed, providing new insight into the microbial community in granular systems. In practice, the efficiency and stability of the AD process could be monitored by the microbial community (Lim et al., 2020). A stable AD process desires an exquisite balance of microbial population dynamics and metabolic activities among the different guilds or trophic groups of the microorganism.



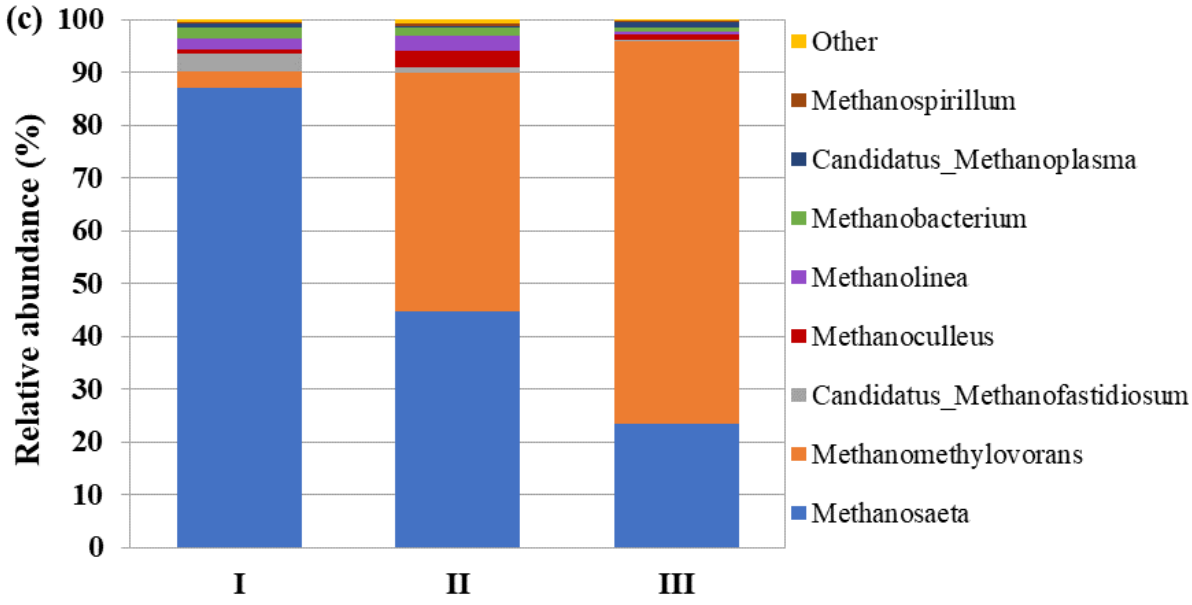


Fig.4 Relative abundance of microbial community in UASB reactor performance of methane production: (a) Kingdom, (b) phylum-bacteria, and (c) Genus-archaea, for various levels of OLR (Phase I=0.89, II=1.79, III=3.57 g-COD/L-reactor·d) at 10-day HRT.

Anaerobic granules are particulate biofilms, spontaneously formed by auto-immobilization of anaerobic bacteria without other additional support material (McHugh et al., 2003). These particles comprised of an intertwined mixture of the symbiotic anaerobic microorganisms that operate together in methane fermentation. The size of the anaerobic granules in the UASB reactor were examined as illustrated in Fig.5. The range of anaerobic granule size was 0.57-1.14 mm. The granule size also increases with higher CH_4 production yields. Consistently, the anaerobic granule size decreases following the declination of CH_4 yield at overload OLR in Phase III. This obviously indicates that the feed conditions favored the growth of anaerobic microorganisms and yielded higher auto-immobilization resulting in a bigger size of anaerobic granules and higher methane production.

After Phase I, the granule size increased from the original anaerobic granules, increasing from 0.57±0.08 mm to 1.01±0.10 mm of average anaerobic granules. Phases II and IV achieved the highest average anaerobic granule sizes of 1.14±0.08 and 1.11±0.15 mm, respectively. This result corresponded to the methane production yield which showed the highest production in both phases (II and IV), the bigger anaerobic granules size was also achieved producing higher methane yield. While the anaerobic granules size of Phase III decreased (0.96±0.11 mm) which was most probably due to the overload of organic substance, resulting in the disability of anaerobic granules to auto-mobilise on granules. Therefore, the size of the granules was significantly different in each phase.

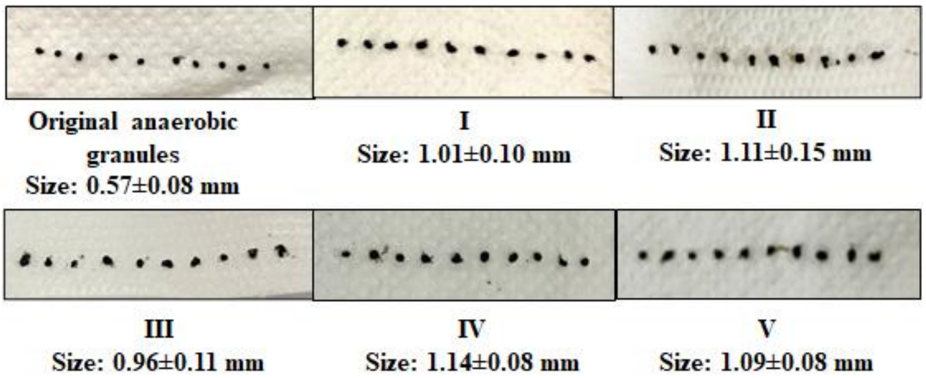


Fig. 5 Anaerobic granules in UASB reactor during methane production, for various levels of OLR (Phase I=0.89, II=1.79, III=3.57, IV=1.79, and V= 2.01 g-COD/L-reactor·d) at 10-day HRT.

3.6 Perspective for methane recovery from DSLS by using UASB reactor

Table 3 shows a comparison of single-stage AD and two-stage AD process performances on using wastewater of concentrated latex industry as a substrate. Methane production yield of single-stage AD in this study had higher significance compared to the Two-stage AD by Kongjan et al, 2014 which used the same type of wastewater as substrate (SLS) (Kongjan et al., 2014). This condition was likely due to a lower OLR and also reduced sulfate taken place before the AD process.

For other types of wastewaters issued from the concentrated latex industry, wastewaters at a latex mill were investigated with two-stage AD using acid tank and UASB reactor by Jawjit,2013. A methane yield of 95.12 mL-CH_4/g- COD_{added} was observed which is lower than the methane yield in this study. They used 3 days of HRT and 1.4 g-COD/L·d of OLR which is also lower than this study (Jawjit, 2013). This was possibly due to the fast feed flow rate that did not provide enough time for the completion of the biochemical reaction during AD process.

Furthermore, some previous studies reported a higher methane production than this study. Saritpongteeraka and Chaiprapat, 2008 represents that single-stage AD by using ABR demonstrated high performance for decomposing organic substances in concentrated latex wastewater (CLW) (Saritpongteeraka and Chaiprapat, 2008), which was 7.66% of methane production yield higher than this study. In addition, the data achieved from concentrated latex factory in Songkhla, Thailand found that production of methane from CLW by using anaerobic pond feed rate 0.61 g-COD/L·d for 15.7-day HRT achieved 219.97 mL-CH_4/g- COD_{added} of methane yield, which is lower than this study. This phenomenon might be due to a lower OLR and varying characteristics of substrate and reactor operation.

In practice, $NaHCO_3$ as an alkali solution is usually applied for pH control in AD process. The cost of alkali chemicals is relatively high. The effluent recirculation which has the potential of replacing the alkali solution is an attractive choice, although the yield is lower than using $NaHCO_3$ 17.5 %. For the industrial scale of concentrated latex factory, machine-washing wastewater was generated during the concentrated latex processing. This wastewater can use to dilute the effluent (replacing the tap water in the current study) before mixing with the substrate to be influent wastewater, then feeding into UASB reactor. Hence, this strategy in the experiment not only reduced the alkali chemicals cost but also reduced the volume of tap water for dilution. In addition, UASB technology is a good choice to replace the cover lagoon, the most popular low price-digester for concentrated latex wastewater. The UASB reactor is preferred for high organic loading rates application. Hence, it is possible to use compact UASB for treating large volumes or highly concentrated organic wastes.

Table 3 Comparison to previous reports on methane production in AD process from wastewater of concentrated latex industry.

Substrate	AD	Reactor type	Methane production						Reference
			Temperature (°C) of reactor	**OLR (g-COD/L·d)**	**HRT (days)**	**CH_4 Yield (mL-CH_4/g-COD_{added})**	**CH_4 production rate (mL/L-reactor·d)**	**% CH_4 Content**	
SLS	TSAD	UASB-UASB	55	4.47	9	178.70	712.00	57-65	(Kongjan et al., 2014)
Wastewater at a latex mill	TSAD	Acid tank-UASB	35	1.4	3	95.12	NS	60-70	(Jawjit, 2013)
CLW	SSAD	ABR	35	0.60	10	242.31	NS	65-75	(Saritpongteeraka and Chaiprapat, 2008)
CLW	SSAD	Anaerobic Pond	33	0.61	15.7	219.97	131.70	59.8	*
Desulfated SLS	SSAD	UASB	35	1.79	10	226.35	403.25	67.19	This study

SLS = Skim Latex Serum
CLW = Concentrated latex wastewater
NS = No Show
TSAD = Two stages anaerobic digestion
SSAD = Single stage anaerobic digestion
*Data achieve from Concentrated Latex Factory in Songkhla, Thailand
UASB-UASB = Up-flow anaerobic sludge blanket- Up-flow anaerobic sludge blanket
ABR = Anaerobic baffled reactor
UASB = Up-flow anaerobic sludge blanket

4. Conclusions

This study demonstrates that treatment of DSLS by using UASB reactor in single stage AD has the capability and efficiency for producing methane while using effluent recirculation method can replace the external buffering, $NaHCO_3$ solution. Due to the nature characteristic of DSLS which still contain some sulfate and has low C/N ratio, the investigation on suitable OLR is needed. The average maximal methane production yield of 226.35 mL/g COD_{added} was achieved and 7.14 kJ/g-COD_{added} of energy can be recovered in which OLR of 1.79 g-COD/L-reactor·d was fed (Phase II). Although the effluent recirculation is a practical and economical method to keep sufficient alkalinity for the stable system. However, with 53% of the effluent recirculation 18**%** decrease of methane yield than in Phase II was obtained.

For organic wastewater treatment, one of the main purposes is organic reduction. COD removal efficiency was within the range of 70**.**66**-**73**.**95**%** while the range of sulfate removal efficiency was within 50.09-92.37**%**. This indicated that post treatment of the effluent is still needed. The current study has demonstrated the capabilities of the UASB reactor in AD for the treatment of SLS**.** However, to enhance the methane productivity of SLS, other low cost method for reducing more sulfate content and co-digestion for increasing C/N ratio are suggested.

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Supplementary material for

Effect of Organic Loading Rate and Effluent Recirculation on Biogas Production of Desulfated Skim Latex Serum using Up-Flow Anaerobic Sludge Blanket Reactor

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Table S1 COD distribution in the effluent

	Phase I	Phase II	Phase III	Phase IV	Phase V
OLR (g-COD/L-reactor·d)	0.89	1.79	3.57	1.79	2.01
VFA in effluent (g/L)					
Acetic	0.60	0.82	1.54	0.90	1.07
Propionic	0.60	0.83	1.48	1.03	1.65
Butyric	0.39	0.53	0.71	0.58	0.61
VFA in effluent (g-COD/L)					
Acetic	0.64	0.88	1.64	0.96	1.14
Propionic	0.91	1.26	2.23	1.56	2.49
Butyric	0.70	0.96	1.29	1.06	1.10
Total VFA (g-COD/L)	2.25	3.10	5.17	3.58	4.73
Total COD in effluent (g-COD/L)	2.60	4.65	9.43	4.99	5.37
Other organic matter (g-COD/L)	0.35	1.55	4.26	1.42	0.63
% Contribution in Effluent					
Total VFA	**86.57**	**66.68**	**54.80**	**71.65**	**88.24**
Other organic matter	**13.43**	**33.32**	**45.20**	**28.35**	**11.76**