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Comparison of Methane Production Utilizing Raw and Acidogenic Effluent Coming from Sago Starch Processing in Anaerobic Sequencing Batch Reactor (ASBR)

(Perbandingan Penghasilan Metana Menggunakan Sisa Mentah dan Asidogenik Efluen Datang daripada Pemprosesan Kanji Sagu dalam Reaktor Sesekumpul Berjujukan Anaerobik (ASBR))

Rafiqqah Mohamad Sabri[a,c], Jamaliah Md Jahim [a,b*], Mohd Sobri Takriff [a,b]
[a]*Research Centre for Sustainable Process Technology (CESPRO),*
[b]*Chemical Engineering Programme*
Faculty of Engineering & Built Environment
Universiti Kebangsaan Malaysia

Nurleyna Yunus[c]
[c]*CRAUN Research Sdn. Bhd. Malaysia*

Bambang Trisakti[d]
[d]*Chemical Engineering Department, Faculty of Engineering,*
Universitas Sumatra Utara

ABSTRACT

In this research, a study of anaerobic digestion to generate methane in anaerobic sequencing batch reactor (ASBR) were conducted in a single stage system (using raw sago mill effluent (SME) as substrate) and two stage system (using acidogenic effluents from hydrogenic reactor treating sago mill effluent) operated under mesophilic condition. The experiment was carried out over a range of hydraulic retention times (HRT) of 12 to 1.5 days in both single and two stage system, respectively. The corresponding organic loading rate (OLR) was 1.21 to 9.90 kg COD/m^3.d and 1.41 to 11.17 kg COD/m^3.d, respectively. The performance of methane generation and organic matter degradation of these two systems were evaluated and compared. The highest methane production rate (MPR) and methane yield (MY) were obtained from the two-stage system under HRT 2 days at 1.7111 L $CH_4/L_{reactor}$.d and 0.2257 L CH_4/g $COD_{removed}$ respectively with a maximum percentage of COD reduction of 82%. Meanwhile, single stage system operated under the same HRT obtained lower methane production rate at 1.254 L $CH_4/L_{reactor}$.d and methane yield of 0.2217 L CH_4/g $COD_{removed}$. Overall, this study demonstrated a two-stage system is able to provide higher methane productivity as compared to a single stage system.

Keywords: Methane production; Metroxylon sago; Acidogenic effluent; Anaerobic digestion

ABSTRAK

Dalam kajian ini, kajian pencernaan anaerobik untuk menghasilkan metana dalam reaktor ASBR dilakukan dalam satu sistem peringkat tunggal (menggunakan sisa mentah efluen kilang sagu sebagai substrat) dan sistem dua peringkat (menggunakan efluen asidogenik daripada reaktor hidrogenik merawat efluen kilang sagu) dilakukan di bawah keadaan mesophilic. Eksperimen dijalankan sepanjang tempoh pengekalan hidraulik (HRTs) 12 hingga 1.5 hari masing-masing dalam sistem satu dan dua peringkat. Ini bersamaan dengan kadar pemuatan organik (OLR) sebanyak 1.21 hingga 9.90 kg COD / m^3.hari dan 1.41 kepada 11.17 kg COD / m^3.hari. Prestasi penghasilan metana dan degradasi bahan organik kedua-dua sistem ini dinilai dan dibandingkan. Kadar tertinggi pengeluaran metana dan hasil dicapai dari sistem dua peringkat yang mempunyai HRT 2 hari pada 1.711 L CH_4 / $L_{reaktor}$.hari dan 0.2257 L CH_4 / g $COD_{removed}$ masing-masing dengan 82% pengurangan COD . Sementara itu, sistem peringkat tunggal didapati memperoleh kadar pengeluaran metana yang lebih rendah di bawah HRT yang sama pada 1.254 L CH_4 / $L_{reactor}$.d. Secara keseluruhannya, hasil kajian ini menunjukkan sistem dua peringkat mampu memperolehi produktiviti metana yang tinggi berbanding sistem satu peringkat.

Kata kunci: Pengeluaran metana; Metroxylon sago; Efluen asidogenik; Penghadaman anaerobik

INTRODUCTION

Sago palm is one of the most essential species of palm that is useful for starch extraction. It has been utilized in South East Asia region for no less than 700 years (Oates & Hicks 2001). One palm of sago may produce between 150 to 300 kg of dry starch. At an average consumption of 1000 logs/day, a minimum of 400 tons of effluent is generated from a typical sago factory (Lo 2008). Massive amounts of waste water, bark of sago trunk, and fibrous residue commonly called *hampas* are produced during sago starch production (Apun et al. 1996). As reported by Chew and Shim (1993), about 50 to 110 tons of sago *hampas* are produced every day in Sarawak especially in Sibu and Mukah division. These waste likely to pollute water into which they are released which consequently will create environmental issues to the sago industry as a whole.

Thus, an alternative and effective solution to treat the wastewater from sago industry is via anaerobic digestion. It is a process by which bacteria break down organic waste such as industrial waste and food processing wastewater in oxygen-free condition. The process converts the input material into biogas which is mainly composed of methane, CH_4 (55-75 volume %) and carbon dioxide, CO_2 (25-45%) (Reith et al. 2003).

Two phase anaerobic fermentation process comprised of two steps production units of hydrogen and methane (Zhu et al. 2008). In the first step, organic matter in wastewater is hydrolyzed and transformed to hydrogen, carbon dioxide and volatile fatty acids (VFAs) specifically by acidogenic bacteria. The growth of acidogenic bacteria is favorable at acidic pH and shorter HRT. Meanwhile, the second unit of methane will be fed with hydrogenic effluent previously from the first unit to produce methane by methanogens; a type of archaea that grows slower with much longer HRT and requires neutral pH condition. (Cooney et al. 2007; Intanoo et al. 2014).

A two stage anaerobic digestion appears to be an increasingly popular method to deliver greater process stability: a controlled process in acidification stage would help in avoiding the overloading and/or inhibition of the methanogenic population in the second stage (Koutrouli et al. 2009), maximize biogas yield, enhance the efficiency of substrate degradation and thus improve the energy recovery (Xie et al. 2008, Hallenbeck 2009; Hallenbeck & Ghosh, 2009; Lee & Chung, 2010; Schievano et al. 2012, Liu et al. 2013, Wu et al. 2016).

Until now, the studies on sequential hydrogen and methane production in two stage system has been achieved and reported from various kinds of organic waste such as cassava (Intanoo et al. 2016; Cheng et al. 2015; Intanoo et al. 2014; Zhang et al. 2013), palm oil mill effluent (Krishnan et al. 2016a; Krishnan et al. 2016b; Mamimin et al. 2015) and food waste (Silva et al. 2018; Nathao, et al. 2013; Pisutpaisal et al. 2014; Liu et al. 2013; Lin et al. 2013). In addition, sago mill effluent has demonstrated its ability as substrate in cultivation of algae (Phang et al. 2000), biofuel generation through anaerobic fermentation such as hydrogen (Yunus et al. 2014) and methane (Yunus & AR, 2012). At present, there is no published research on two stage process of hydrogen and methane productions in the treatment of sago mill effluent.

An anaerobic sequencing batch reactor (ASBR) operates in a single reactor system through four steps in one cycle: feeding, reaction, settling and decanting. ASBR has the potential for its flexibility to control, very simple to be operated and no required any of primary and secondary settles. ASBR treatment has been widely developed and as well as been applied to treat various types of wastewater from low to high strength. Investigation on the feasibility of ASBR operation has been reported such as those from meat industry (Myra et al. 2015), olive mill (Ammary 2005), brewery industry (Zupancic et al. 2007), palm oil mill industry (Promtong et al. 2014), swine manure (Ndegwa et al. 2008) and syrup wastewater (Kwon & Nakasaki 2013).

This paper aims to study the performance of methane production in a single system and two-stage anaerobic digestions in an ASBR using raw sago mill effluent and acidogenic effluent coming from hydrogenic reactor treating sago mill effluent.

METHODOLOGY

SEED SLUDGE

The anaerobic sludge used as a digestion seed in the experiment were sampled from the drain of a sago starch processing factory in Mukah, Sarawak. The sludge was acclimatized in sago mill effluent using a laboratory scale (1 L) anaerobic sequencing batch reactor (ASBR) operated at 37°C in an incubator shaker.

PREPARATION OF SAGO MILL EFFLUENT

The sago mill effluent used in this study was prepared in the laboratory. Sago pith was homogenized using a laboratory blender with addition of tap water at ratio of 1:4. The resulting liquid mixture was filtered with muslin cloth to remove starch and large particles. The filtration process was repeated three times according to the conventional way of extraction that has been practiced by sago farmers in Mukah. After filtration, the supernatant was gathered, stored in reagent bottles at 4°C and to be used within 3 days.

Media for the reactor was made by adding to the SME solution comprised of 0.25 g/L potassium dihydrogen phosphate, KH_2PO_4, 0.25 g/L potassium hydrogen phosphate, K_2HPO_4, 0.5 g/L Cysteine, and 1.69 g/L ammonium hydrogen carbonate, NH_4HCO_3. To maintain the pH, 6.72 g/L of sodium hydrogen carbonate, $NaHCO_3$ was added into the media.

EXPERIMENTAL SETUP AND REACTOR OPERATION

Single stage (MR-1) and two stage (MR-2) experiments were performed in two identical 1L anaerobic sequencing batch reactors (ASBR) with effective volume of 800 mL and operated under mesophilic condition at 37 ± 1°C. The ASBR reactors were manually fed on a daily basis with a total cycle period of 24 h by using an external feeder pump. Each cycle consisted of 10 minutes feeding, 22.5 h reaction, 1 h settling and 10 minutes feed decanting. The rate of stirring was achieved at 100 RPM using incubator shaker during the react phase. Figure 1 illustrated the schematic diagram of ASBR for both MR-1 and MR-2.

Raw sago mill effluent was utilized as substrate in the single stage reactor (MR-1) whereas acidogenic effluent was used for the two stage reactor (MR-2). The acidogenic effluent were obtained from a hydrogenic reactor treating sago mill effluent in CRAUN laboratory. Conditions for the hydrogenic reactor were as described by Yunus et al. (2014).

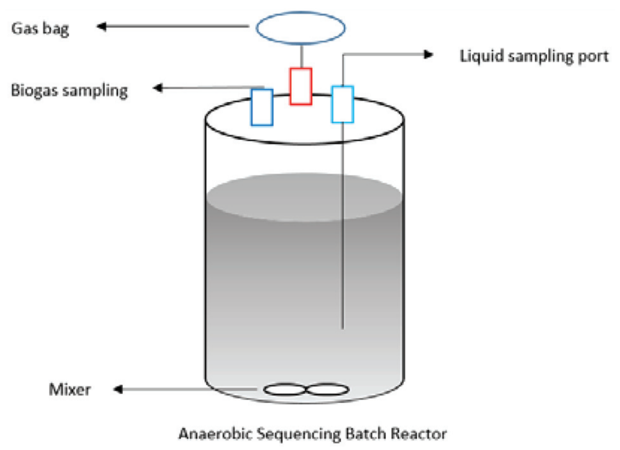

FIGURE 1. Schematic diagram of ASBR reactor MR-1 and MR-2

Seed sludge concentration was fixed at 20% (v/v) utilizing enriched methanogenic sludge. The seed sludge was added into methane reactors containing the substrates to designated working volume and purged with nitrogen gas for 10 minutes to create anaerobic condition.

Methane production in the single and two stage process were investigated at 12, 6, 4 2 and 1.5 days hydraulic retention times. The reactors were initially operated under batch mode for 20 days before shifted to semi continuous mode in accordance to tested HRTs as mentioned above. Steady state condition was defined when the daily biogas volume and methane volume obtained vary within ±15% for 6 days.

ANALYTICAL METHODS

Total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD), were carried out according to Standard Methods (APHA 1995). The biogas volume produced was measured using water displacement method. The composition of gas was measured using a gas chromatography (GC) (Hewlett Packard 5890) equipped with a thermal conductivity detector (TCD). The GC was fitted in series with a stainless steel molecular sieve column (10 ft 45/60) and Porapak Q packed column (9 ft 80/100). The injector, oven and detector temperatures were set at 90°C, 50°C and 200°C, respectively. Helium was used as the carrier gas with a flow rate of 18.7 mL/min.

Volatile fatty acids (VFA) and alcohol were analyzed using a gas chromatograph GC (Hewlett Packard 5890) equipped with flame ionization detector (FID). The column used was HP-FFAP (50 m length x 0.2 mm ID x 0.33 µm film thickness) with helium as carrier gas.

Injector and detector temperature were maintained at 270°C and 200°C respectively. Oven temperature was set to run at 60°C, held for 4 min, then ramped at 6°C/min to 92°C followed by a 20°C/min ramping to 150°C, then ramped again at a slower rate of 6°C/min to 175°C before finally increasing the temperature to 210°C at a rate of 30°C/min to completely remove remaining compound in the column. The separation of the primary volatile fatty acids and related solvents were obtained within 19.57 min.

RESULTS AND DISCUSSION

CHARACTERISTICS OF SAGO MILL EFFLUENT AND ACIDOGENIC EFFLUENT

Table 1 shows the average characteristics of both substrates (raw sago mill effluent and acidogenic effluent). Raw sago mill effluent was found to have a COD value that ranges between 7,000 to 10,000 mg/L and can be categorized as medium strength wastewater. In this study, acidogenic effluent was collected from a hydrogen reactor which generates hydrogen gas through anaerobic fermentation of sago mill effluent. The acidogenic effluent exhibited pH range between 5.04 - 5.96, which can be described by the presence of residual volatile fatty acids as a product of hydrogen fermentation. It is observed the COD concentration ranged between 16000-17000 mg/L.

TABLE 1. Characteristics of substrate of the ASBR in single and two-stage experiment

	Unit	Sago Mill Effluent (SME)	Acidogenic Effluent
pH		5.29-5.40	5.04-5.96
COD	mg/L	7000-10000	16000-17000
TSS	mg/L	980-3800	1700-3300
VSS	mg/L	780-3600	1000-2500
TS	mg/L	6600-9400	10000-17000
VS	mg/L	5800-8800	6000-10000

PERFORMANCE OF ASBR SINGLE STAGE EXPERIMENT, MR-1

METHANE AND BIOGAS VOLUME

Single stage process was performed by utilizing sago mill effluents as influent for methane production. Five experimental runs of different HRTs (12, 6, 4, 2 and 1.5 days) were tested. Table 2 summarizes the steady state results obtained during the course of the experiment. The biogas volume was found to increase from 325 to 1803 mL as HRT decreases from 12 to 2 days. As reported by Ndegwa et al. (2008), the increment in biogas was predicted as HRT is reduced due to the increase in loading rate and thus further supply of degradable substrate. When, HRT is progressed further to 1.5 days, a declining trend in biogas volume was observed. The fact that there was a declining trend below HRT 2 days was likely because of imbalance in microbial populations (excessive amount of acid

TABLE 2. Average steady state data in single stage experiment

HRT	Unit days	12	6	4	2	1.5
OLR	kg COD/m^3.d	1.21 ± 0.03	2.45 ± 0.10	3.66 ± 0.08	7.61 ± 0.42	9.90 ± 0.21
Effluent pH	-	7.84 ± 0.10	7.42 ± 0.12	7.00 ± 0.10	6.97 ± 0.02	7.26 ± 0.42
Biogas volume	mL/d	325 ± 27.57	423 ± 9.17	528 ± 45.87	1803 ± 56.10	1362 ± 123.68
Methane volume	mL	145.83 ± 18.13	216.97 ± 12.49	218.05 ± 22.31	1003.00 ± 18.97	735.44 ± 79.22
Methane content	%	35.80 ±1.70	41.10 ± 3.15	33.10 ± 2.55	44.60 ± 1.60	43.20 ± 1.84
Methane production rate	L CH_4/L_{ww}. d	0.182 ± 0.02	0.271 ± 0.03	0.273 ± 0.03	1.254 ± 0.02	0.919 ± 0.10
COD removal	%	70.27 ± 3.29	47.26 ± 2.19	43.98 ± 0.40	52.65 ± 2.23	31.05 ± 2.22
Methane yield	L CH_4/g $COD_{removed}$	0.1509 ± 0.01	0.1638 ± 0.02	0.1235 ± 0.03	0.2217 ± 0.02	0.2121 ± 0.03
VFA reduction	%	67	72	43	53	66

producing bacteria than methane producing microorganism) which in turn souring the reactor.

Methane production plays a vital part in anaerobic digestion that defines the efficiency of anaerobic digestion. The same trend as biogas volume was observed in methane volume as HRT was reduced down to 1.5 days. At the beginning of the experiment, methane volume was low because of low OLR and the evolved methane increase with the increment of OLR or short HRT. As indicated in Table 2, the volume of methane increased from 146 mL to 1003 mL as a result of shorter HRT applied. Meanwhile, methane content was found to be within the range of 33% to 45% in general.

METHANE PRODUCTION RATE AND METHANE YIELD

As Table 2 shows, the methane production rate (MPR) and methane yield (MY) was found to increase steadily from initial experiment start-up (at HRT 12 days) to a maximum result of 1.254 L CH_4/L_{ww}. d and 0.2217 L CH_4/g $COD_{removed}$, respectively, achieved under HRT 2 days. In spite of the gradual decrease in HRT of reactor, a higher MPR was successfully obtained. Meanwhile at HRT of 1.5 days, MPR and MY decreased to 0.919 L CH_4/L_{ww}. d and 0.2121 L CH_4/g $COD_{removed}$, respectively. Thus, it can be concluded that an HRT of 1.5 days was too short to allow substantial methane production in the system which was followed by high concentration of COD effluent (data not shown).

PH, VSS AND COD REMOVAL

In general, COD removal showed a decreasing trend from 70% to 31% as HRT is reduced from 12 to 1.5 days. This could be attributed to biomass loss as HRT is reduced thus contributing to the decreased in substrate removals (Banik & Daguet 1997). Although the biogas and methane volume were increased, it takes time for the reactor to stabilize as the COD removal started to decrease which then followed by the increase in COD concentration of the effluent which eventually would affect the performance of COD removal in the reactor. At the same time, when loading rate is too high, the time for microorganism to digest the organic matter is insufficient due to short HRT.

The results can be explained by the fact that single stage methane production contains complex carbohydrate in sago mill effluent which consequently would affect the degradation efficiency. This was also reflected in a study reported by Mamimin et al. (2015) where they were dealing with complex wastewater containing recalcitrant which could be inhibitive to the microorganism.

The pH range in the effluent was all above 7.0 which are optimal for production of methane. It was found to be stable within optimum range for methanogenesis (6.5-8.0) for the entire period of experiment. VSS is crucial criteria when loading anaerobic reactor as it represents solid material suspended and the population size of bacteria within the activated sludge process respectively. As illustrated in Figure 2, it can be seen that the concentration of solid biomass in the effluent was increasing. This behavior can be explained when HRT is declined, the organic loading increases accordingly and biomass synthesis is more significant (Banik & Daguet, 1997). It can be observed the ASBR was capable of holding up to 23000 mg/liter of biomass concentration. The high MLVSS of the reactors proves that the ASBR system has the ability to support high solids in the system and allowing efficient treatment of low-strength wastewater (Ndon & Dague 1997).

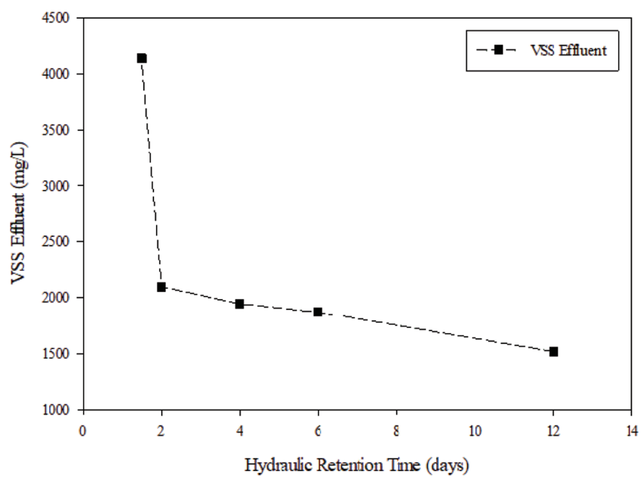

FIGURE 2. VSS concentration of effluent in single stage experiment

VOLATILE FATTY ACIDS AND ALCOHOLS ANALYSIS

At any given HRT, ethanol was the most abundant alcohols in the influent of ASBR. Unlike other studies, ethanol production was relatively high in this experiment, possibly due to the properties of sago mill effluent itself. Results achieved in Table 2 showed highest total VFA reduction at 72% for HRT 6 days, and the lowest was 43% at HRT 4 days. Based on the studies reported by Wang et al. (2009) most VFAs were degraded by a microorganism to acetic acid before converting to CH_4 according to sequence acetic acid > ethanol > butyric acid > propionic acid. Meanwhile, propionic acid was least detected in the reactor throughout the duration of the experiment.

PERFORMANCE OF ASBR TWO STAGE EXPERIMENT, MR-2

This study on methane production involves the utilization of acidogenic effluent from a reactor anaerobically treating sago mill effluents to generate hydrogen. The acidogenic effluent contains VFA and other residual organic matter produced during hydrogen fermentation. It was collected over a period for two months every day and stored at –4°C. The ASBR reactor MR-2 was operated for about 200 days. Throughout this period, various HRT (12, 6, 4, 2 and 1.5 days) were evaluated. The results suggested that HRT affects biogas volume, methane content, MPR, MY and COD removal.

METHANE AND BIOGAS VOLUME

The volume of biogas was found to increase as HRT is reduced. Maximum biogas volume achieved was 1535 mL at HRT 2 days (Table 3). Apart from that, the methane volume showed a general increment from 18 to 1369 ml as the HRT was decreased from 12 to 2 days. The methane content in the biogas was in the range between 14-71% and reached the maximum value of 71.40% at HRT 2 days. It is interesting to note that De Gioannis et al. (2017) also observed higher methane content in second stage than that observed in the one stage anaerobic digestion test of food waste.

TABLE 3. Average steady state data in two-stage experiment

HRT	Unit days	12	6	4	2	1.5
OLR	kg COD/m^3.d	1.41 ± 0.01	2.76 ± 0.05	4.15 ± 0.05	8.25 ± 0.09	11.17 ± 0.17
Effluent pH	-	7.95 ± 0.11	7.71 ± 0.07	7.72 ± 0.21	7.89 ± 0.06	8.00 ± 0.08
Biogas volume	mL/d	97 ± 11.29	412 ± 14.75	618 ± 6.89	1535 ± 43.20	757 ± 155.01
Methane volume	mL	18.02 ± 2.30	288.30 ± 8.31	546.24 ± 33.24	1368.89 ± 42.08	500.97 ± 101.53
Methane content	%	14.80 ± 0.33	56.01 ±3.37	70.80 ± 4.40	71.40 ± 1.20	53.00 ± 4.28
Methane production rate	L CH_4/L_{ww}. d	0.023 ± 0.00	0.360 ± 0.01	0.683 ± 0.04	1.711 ± 0.05	0.626 ± 0.13
COD removal	%	42.62 ± 2.65	49.41 ± 1.58	82.18 ± 1.86	65.04 ± 1.11	55.54 ± 2.33
Methane yield	L CH_4/g $COD_{removed}$	0.0264 ± 0.00	0.1863 ± 0.01	0.1414 ± 0.01	0.2257 ± 0.11	0.0715 ± 0.02
VFA reduction	%	79	72	48	67	77

METHANE PRODUCTION RATE AND METHANE YIELD

As depicted in Table 3, both methane production rate (MPR) and methane yield (MY) increased remarkably with reducing HRT from 12 to 2 days. The maximum MPR and MY were achieved at HRT of 2 days with values noted as 1.711 L CH_4/L_{ww}. d and 0.2257 L CH_4/g $COD_{removed}$, respectively. The results were comparable with a study done by Reungsang et al. (2016) where a MPR and MY of 1.27 ± 0.05 L-CH_4/L-culture day, and 348 ± 13 mL-CH_4/g-COD, respectively, was observed when using acidic effluent in the UASB as substrate. In other studies done with other types of substrate, the MPR and MY also correspond to decrease in HRT or increase in OLR (Göblös et al. 2008; Intanoo et al. 2014; Jung et al. 2012). When HRT was further shortened to 1.5 days, both the MPR and MY decreased as a result of overloaded feeding of the substrate. This trend was also reported by Kim et al. (2012) that states daily methane production decreased might be a result from the decrease substrate utilization efficiency of methanogens. In addition, Kim's study also found that the shorter the HRT, the lower the methane yield was due to the higher maintenance cost imposed by active cell synthesis to overcome the rapid HRT and in survival of washout environment.

PH, VSS AND COD REMOVAL

The trend achieved for COD removal showed an increasing pattern from 42% to 82% as the HRT is reduced from 12 to 4 days. As the HRT progressed beyond 2 days, the removal percentage decreased to 65%. The COD removal trend achieved in this study was found to concur with the study conducted by Intanoo et al. (2014) where it increased with increasing COD loading rate and reached a maximum value. Since longer HRT allowed a greater conversion of the substrate to biogas, more biomass washout could take place at the shorter HRT at any given temperature, which caused reduction of substrate removals. This pattern is also similar to observation reported by Dague (1998) that claims that with HRT reduced, the COD loading rate increased and removal rates deteriorated as well. At the same time, the COD in the effluent of methane reactor showed gradual increment (data not shown) as the OLR was increased, as indicated by the accumulation of COD in the reactor at high OLR. Interestingly, this results was similar to the findings by Jang et al. (2015) and Wu et al. (2016).

Besides this, the studies conducted by Mamimin et al. (2015) also addressed COD removal from a two stage experiment was found to achieve higher efficiency as compared to a single stage experiment which suggested the occurrence of incomplete degradation in the single stage at similar HRT thus causing lower production of gas. Anaerobic digestion is very sensitive to pH changes which has optimum pH of 6.5 to 8.0. As shown in the Table 3, pH for the experiment was found to be steadily maintained above 7.0 during methane fermentation, a sign of a good performance in the reactor. Apart from that, the trend of VSS in the effluent (data not shown) showed increment throughout HRT, a result similarly achieved for single stage experiment.

VOLATILE FATTY ACIDS AND ALCOHOLS ANALYSIS

The substrate of ASBR two stage was rich in VFAs as expected. According to VFA analysis, the main component of acids and alcohols that have been detected were ethanol, acetic acid, propionic acid and butyric acid. It was found that concentrations of ethanol was the highest, followed by acetic, butyric and propionic acid. In placing more emphasis, Yunus (2015) also claimed that the glycolysis of soluble carbohydrate present in SME is prone to the acetic acid/ethanol production fermentation pathway. The highest total VFA reduction was 79% at the highest HRT (12 days). The lowest total VFA reduction observed was 48% at HRT 4 days. From the results, it can be observed that the concentration of the acetic, propionic and butyric acid in the effluent were all constant over the time. On the other side, the ethanol was entirely consumed during digestion process as no ethanol was detected in the effluent.

COMPARISON OF SINGLE AND TWO STAGE

Referring to Table 4, it was observed that MPR obtained in two stage experiment was relatively higher compared to single stage experiment. The highest MPR in single stage was 1.254 L CH_4/L_{ww}.d, meanwhile 1.711 L CH_4/L_{ww}.d was recorded in two stage system both during HRT 2 d. This phenomenon was attributed to the characteristic of acidogenic effluent itself where most of the effluent from hydrogen reactor that was mainly made up of simple organic molecules which is highly degraded as a results from hydrolysis and acidogenesis process. This result concurred with the findings by Intanoo et al. (2016) who pointed out two stage anaerobic process are capable of producing very high methane content (greater than 80%) as compared to single stage anaerobic system. Additionally, it can also be observed that two stage process improves the COD removal efficiency at 82% compared to single stage at 70%. This findings is also in agreement with those obtained by Reungsang et al. (2016) who stated that two stage hydrogen and methane production increased the COD removal efficiency (75.6%) compared to the one stage methane production (59.52%). The same trend also could be seen in methane yield where there is an increment in two stage compared to single stage. It could be said that hydrogenic stage helps to increase methane yield in the second stage. Based on the study by Pakarinen et al. (2011) an increase in methane yield in two stage system is likely to happen due to the fact that hydrolysis and acidogenesis were improved hence producing the VFA which were then converted into methane in methane reactor. This is because, the complex organic molecules are already broken down into a soluble form that can be easily used by methanogenic bacteria during methane formation. This claim was also supported by similar research done by Liu et al. (2006).

TABLE 4. Comparison of single and two stage two stage anaerobic digestion

		Single stage	Two stage
Methane volume	mL	1003.00	1368.89
Methane content	%	44.60	71.40
COD removal	mg/L	70.27	82.18
Methane production rate	L CH_4/L_{ww}. d	1.254	1.711

On the aspect of methane content, the two stage process was found to produce higher methane content at 71.4% as compared to single stage process at 44.6%. The same can be said to methane volume where 1369 mL of methane can be obtained in two stage as compared to 1003 mL in a single stage system. This findings is consistent with Zhang et al. (2001) who mentioned the separation of two groups of bacteria gives the potential to prevent the failure in digester due to excessive acid accumulation, and reduction of the system HRT while maintaining high methane content in the biogas. This study showed that acidogenic effluent could be digested anaerobically in a two stage experiment at relatively short HRT values.

Mamimin et al. (2015) obtained similar observation where two stage hydrogen and methane production improved organic matter degradation and favored high yields and quality of biogas. Moreover, their results suggested that retention time of methanogenic process could be reduced and methane production rate could be increased through

COMPARISON WITH OTHER TWO STAGE FERMENTATION SYSTEMS USING DIFFERENT WASTES

Table 5 compares results obtained from this study with published findings utilizing various wastewater sources as substrate for methane production. Cogeneration of H_2 and CH_4 from cost effective molasses was tested in the continuous system by Park et al. (2010). Similarly, Laminaria japonica was used as substrate for H_2 and CH_4 production and fed continuously into UASBRr. Likewise, Zhang et al. (2013) and Intanoo et al. (2016) also had performed two stage anaerobicdigestion system using ASBR and UASB for treating cassava distillage and cassava wastewater, respectively. Meanwhile, there was also a research group of Reungsang et al. (2016) who utilized UASB for the sequential production of H_2 and CH_4 from sugarcane juice. In the current study, methane yield and methane production rate were comparable with that of revious research, only that slightly lower than that of two stage system which treated cassava distillage.

Apparently the possible point for this likely to happen was the different temperature condition in both reactor. This idea was supported by the work done by (Krishnan et al. (2016a)) where thermophilic condition favors the system thermodynamics, with enhanced biogas production. Meanwhile, the observed MPR 1.94 L CH_4/ $L_{reactor}$.d from the two stage mesophilic continuous treatment of cost effective molasses was greater than this study (1.711 L CH_4/L d). It could be due to the difference in the substrate characteristics whereby a high concentration of carbohydrates including sugars was detected in molasses as compared to in sago mill effluent. On top of that, considering the current and previous works, a higher and better methane yield, COD removal and process stability can be achieved in the two stage anaerobic digestion system.

TABLE 5. Comparison with other two stage fermentation systems using different wastes

Wastes	Reactor Type	Size	Mode	HRT	Organic Loading Rate (OLR)	Methane Production Rate (MPR)	Methane Yield (MY)	Methane Content	COD Removal	References
		(L)		(d)	(kg COD/m^3.d)	(L CH_4/L. d)	L CH_4/g $COD_{removed}$	(%)	(%)	
Cost Effective Molasses	PBR	2.5	Continuous	6	-	1.94	-	77-81	75.1	Park et al. (2010)
Laminaria Japonica	UASBr	3.5	Continuous	2	3.50	1.955	0.3298	-	98	Jung et al. (2012)
Cassava Distillage	ASBR	12	Semi Continuous	8	20.0	2.07	0.1470	-	80-64	Zhang et al. (2013)
Cassava Wastewater	UASB	24	-	-	8.00	0.91	0.1152	-	93.2	Intanoo et al. (2016)
Sugarcane Juice	UASB	30	Continuous	4	5.25	1.27	0.3480	57.94	69.46	(Reungsang et al. (2016))
Sago Mill Effluent	ASBR	1	Semi continuous	2	8.25	1.711	0.2257	71.4	65	This study

CONCLUSION

This study reveals that semi continuous anaerobic sequencing batch reactor methane production from sago mill effluent is achievable in mesophilic condition both in single and two stage system. The higher methane volume, methane content, MPR, MY, COD removal were recorded in two stage than single stage. However, the washout and poor performance of methane reactor was experienced at HRT 1.5 days for both cases as indicated by deterioration of gas volume and COD removal. Overall, this study successfully demonstrated better performance in two stage than a single stage Therefore, two stage system is recommended for more feasible way to treat sago mill effluents to extract H_2 and CH_4.

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REFERENCES

Ammary, Bashaar Y. 2005. Treatment of olive mill wastewater using an anaerobic sequencing batch reactor. *Desalination* 177(1): 157-165.

APHA. 1995. *Standard methods for the examination of water and wastewater* 19th ed. Washington D. C.: American Public Health Association.

Apun, K. K., Bujang, B.C. Jong & M.A. Salleh. 1996. Enzymatic activities of locally isolated bacteria on sago waste residue. *Proceeding of the 6th International Sago Symposium Sago: The Future Source of Food and Feed*, 9-12 December 1996, Indonesia.

Banik, G. C. & Dague, R. R. 1997. ASBR treatment of low strength industrial wastewater at psychrophilic temperatures. *Water Science and Technology* 36(2): 337-344.

Cheng, J., Lin, R., Ding, L., Song, W., Li, Y., Zhou, J. & Cen, K. 2015. Fermentative hydrogen and methane cogeneration from cassava residues: Effect of pretreatment on structural characterization and fermentation performance. *Bioresource Technology* 179: 407-413.

Chew, T.Y. & Shim, Y. L. 1993. Management of sago processing wastes. *Waste management in Malaysia– current status and prospects for bioremediation*. Ministry of Science, Technology and the Environment, Kuala Lumpur.

Cooney, M., Maynard, N., Cannizzaro, C. & Benemann, J. 2007. Two-phase anaerobic digestion for production of hydrogen–methane mixtures. *Bioresource Technology* 98(14): 2641-2651.

Dague, R. R., Banik, G. C. & Ellis, T. G. 1998. Anaerobic sequencing batch reactor treatment of dilute wastewater at psychrophilic temperatures. *Water Environment Research* 70(2): 155-160.

De Gioannis, G., Muntoni, A., Polettini, A., Pomi, R. & Spiga, D. 2017. Energy recovery from one-and two-stage anaerobic digestion of food waste. *Waste Management* 68: 595-602.

Göblös, S., Portörő, P., Bordás, D., Kálmán, M. & Kiss, I. 2008. Comparison of the effectivities of two-phase and single-phase anaerobic sequencing batch reactors during dairy wastewater treatment. *Renewable Energy* 33(5): 960-965.

Hallenbeck, P. C. 2009. Fermentative hydrogen production: principles, progress, and prognosis. *International Journal of Hydrogen Energy* 34(17): 7379-7389.

Hallenbeck, P. C. & Ghosh, D. 2009. Advances in fermentative biohydrogen production: the way forward?. *Trends in Biotechnology* 27(5): 287-297.

Intanoo, P., Chaimongkol, P. & Chavadej, S. (2016). Hydrogen and methane production from cassava wastewater using two-stage upflow anaerobic sludge blanket reactors (UASB) with an emphasis on maximum hydrogen production. *International Journal of Hydrogen Energy* 41(14): 6107-6114.

Intanoo, P., Rangsanvigit, P., Malakul, P. & Chavadej, S. 2014. Optimization of separate hydrogen and methane production from cassava wastewater using two-stage upflow anaerobic sludge blanket reactor (UASB) system under thermophilic operation. *Bioresource Technology* 173: 256-265.

Jang, H. M., Ha, J. H., Park, J. M., Kim, M. S. & Sommer, S. G. 2015. Comprehensive microbial analysis of combined mesophilic anaerobic–thermophilic aerobic process treating high-strength food wastewater. *Water Research* 73: 291-303.

Jung, K. W., Kim, D. H., Lee, M. Y. & Shin, H. S. 2012. Two-stage UASB reactor converting coffee drink manufacturing wastewater to hydrogen and methane. *International Journal of Hydrogen Energy* 37(9): 7473-7481.

Jung, K. W., Kim, D. H. & Shin, H. S. 2012. Continuous fermentative hydrogen and methane production from Laminaria japonica using a two-stage fermentation system with recycling of methane fermented effluent. *International Journal of Hydrogen Energy* 37(20): 15648-15657.

Kim, W., Shin, S. G., Cho, K., Lee, C. & Hwang, S. 2012. Performance of methanogenic reactors in temperature phased two-stage anaerobic digestion of swine wastewater. *Journal of Bioscience and Bioengineering* 114(6): 635-639.

Koutrouli, E. C., Kalfas, H., Gavala, H. N., Skiadas, I. V., Stamatelatou, K. & Lyberatos, G. 2009. Hydrogen and methane production through two-stage mesophilic anaerobic digestion of olive pulp. *Bioresource Technology* 100(15): 3718-3723.

Krishnan, S., Singh, L., Sakinah, M., Thakur, S., Wahid, Z. A. & Alkasrawi, M. 2016a. Process enhancement of hydrogen and methane production from palm oil mill effluent using two-stage thermophilic and mesophilic fermentation. *International Journal of Hydrogen Energy* 41(30): 12888-12898.

Krishnan, S., Singh, L., Sakinah, M., Thakur, S., Wahid, Z. A. & Sohaili, J. 2016b. Effect of organic loading rate on hydrogen (H2) and methane (CH4) production in two-stage fermentation under thermophilic conditions using palm oil mill effluent (POME). *Energy for Sustainable Development* 34: 130-138.

Kwon, S. H. & Nakasaki, K. 2013. Effects of operation conditions on the performance of the ASBR methane fermentation of syrup wastewater. *Procedia Environmental Sciences* 18: 427-432.

Lee, Y. W. & Chung, J. 2010. Bioproduction of hydrogen from food waste by pilot-scale combined hydrogen/methane fermentation. *International Journal of Hydrogen Energy* 35(21): 11746-11755.

Lin, Y., Wu, S. & Wang, D. 2013. Hydrogen-methane production from pulp & paper sludge and food waste by mesophilic–thermophilic anaerobic co-digestion. *International Journal of Hydrogen Energy* 38(35): 15055-15062.

Liu, D., Liu, D., Zeng, R. J. & Angelidaki, I. 2006. Hydrogen and methane production from household solid waste in the two-stage fermentation process. *Water Research* 40(11): 2230-2236.

Liu, X., Li, R., Ji, M. & Han, L. 2013. Hydrogen and methane production by co-digestion of waste activated sludge and food waste in the two-stage fermentation process: substrate conversion and energy yield. *Bioresource Technology* 146: 317-323.

Lo, May Chiun. 2017. Sago starch factory effluent: salvaging a potential pollutant, 22 April 2008 Available from http://www.researchsea.com/html/article.php/aid/3069/cid/2.

Mamimin, C., Singkhala, A., Kongjan, P., Suraraksa, B., Prasertsan, P., Imai, T. & Sompong, O. 2015. Two-stage thermophilic fermentation and mesophilic methanogen process for biohythane production from palm oil mill effluent. *International Journal of Hydrogen Energy* 40(19): 6319-6328.

Myra, T., David, H., Judith, T., Marina, Y., Ricky, B. J. & Reynaldo, E. 2015. Biological treatment of meat processing wastewater using anaerobic sequencing batch reactor (ASBR). *International Research Journal of Biological Sciences* 4(3): 66-75.

Nathao, C., Sirisukpoka, U. & Pisutpaisal, N. 2013. Production of hydrogen and methane by one and two stage fermentation of food waste. *International Journal of Hydrogen Energy* 38(35): 15764-15769.

Ndegwa, P. M., Hamilton, D. W., Lalman, J. A. & Cumba, H. J. 2008. Effects of cycle-frequency and temperature on the performance of anaerobic sequencing batch reactors (ASBRs) treating swine waste. *Bioresource Technology* 99(6): 1972-1980.

Ndon, U. J. & Dague, R. R. 1997. Effects of temperature and hydraulic retention time on anaerobic sequencing batch reactor treatment of low-strength wastewater. *Water Research* 31(10): 2455-2466.

Oates, Christopher & Alastair Hicks. 2001. Sago Starch Production in Asia and the Pacific – Problems and Prospects. *Proceedings of the International Symposium on Sago: New Frontiers of Sago Palm Studies, at Tsukuba, Japan.*

Pakarinen, O. M., Kaparaju, P. L. N. & Rintala, J. A. 2011. Hydrogen and methane yields of untreated, water-extracted and acid (HCl) treated maize in one-and two-stage batch assays. *International Journal of Hydrogen Energy* 36(22): 14401-14407.

Park, M. J., Jo, J. H., Park, D., Lee, D. S. & Park, J. M. 2010. Comprehensive study on a two-stage anaerobic digestion process for the sequential production of hydrogen and methane from cost-effective molasses. *International Journal of Hydrogen Energy* 35(12): 6194-6202.

Phang, S. M., Miah, M. S., Yeoh, B. G. & Hashim, M. A. 2000. Spirulina cultivation in digested sago starch factory wastewater. *Journal of Applied Phycology* 12(3-5): 395-400.

Pisutpaisal, N., Nathao, C. & Sirisukpoka, U. 2014. Biological hydrogen and methane production in from food waste in two-stage CSTR. *Energy Procedia* 50: 719-722.

Promtong, T., Dongbang, W. & Sittikijyothin, W. 2014. Biogas production using anaerobic sequencing batch reactor (ASBR) system for palm oil wastewater. *2nd AUN/SEED-Net Regional Conference on Energy Engineering, Bangkok.*

Reith, J. H., R. H. Wijffels & H. Barten. 2003. *Bio-Methane And Bio-Hydrogen: Status and Perspectives of Biological Methane and Hydrogen Production*. Petten: Dutch Biological Hydrogen Foundation.

Reungsang, A., Sittijunda, S. & Sreela-or, C. 2016. Methane production from acidic effluent discharged after the hydrogen fermentation of sugarcane juice using batch fermentation and UASB reactor. *Renewable Energy* 86: 1224-1231.

Schievano, A., A. Tenca, B. Scaglia, G. Merlino, A. Rizzi, D. Daffonchio, R. Oberti & F. Adani. 2012. Two-stage vs single-stage thermophilic anaerobic digestion: comparison of energy production and biodegradation efficiencies. *Environmental Science & Technology* 46(15): 8502-8510.

Silva, F. M., Mahler, C. F., Oliveira, L. B. & Bassin, J. P. 2018. Hydrogen and methane production in a two-stage anaerobic digestion system by co-digestion of food waste, sewage sludge and glycerol. *Waste Management* 76: 339-349.

Wang, Y., Zhang, Y., Wang, J. & Meng, L. 2009. Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. *Biomass and Bioenergy* 33(5): 848-853.

Wu, L. J., Higashimori, A., Qin, Y., Hojo, T., Kubota, K. & Li, Y. Y. 2016. Comparison of hyper-thermophilic–mesophilic two-stage with single-stage mesophilic anaerobic digestion of waste activated sludge: process performance and microbial community analysis. *Chemical Engineering Journal* 290: 290-301.

Wu, L. J., Kobayashi, T., Kuramochi, H., Li, Y. Y. & Xu, K. Q. 2016. Improved biogas production from food waste by co-digestion with de-oiled grease trap waste. *Bioresource Technology* 201: 237-244.

Xie, B., Cheng, J., Zhou, J., Song, W. & Cen, K. 2008. Cogeneration of hydrogen and methane from glucose to improve energy conversion efficiency. *International Journal of Hydrogen Energy* 33(19): 5006-5011.

Yunus, Nurleyna. 2015. *Batch and Continuous Fermentative Hydrogen Production from Sago Mill Effluent Seeded with Acclimatised Sago Sludge Consortia*. PhD Dissertation, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia Bangi.

Yunus, Nurleyna & Azhar AR. 2012. Biogas generation potential via anaerobic treatment of sago mill effluent. *2nd ASEAN Sago Symposium*, at Universiti Malaysia Sarawak, Kuching, Sarawak.

Yunus, N., Jahim, J. M., Anuar, N., Abdullah, S. R. S. & Kofli, N. T. 2014. Batch fermentative hydrogen production utilising sago (Metroxylon sp.) starch processing effluent by enriched sago sludge consortia. *International Journal of Hydrogen Energy* 39(35): 19937-19946.

Zhang, Q., Zhu, X., Kong, L., Yuan, G., Zhai, Z., Liu, H. & Guo, X. 2013. Comparative assessment of the methanogenic steps of single and two-stage processes without or with a previous hydrolysis of cassava distillage. *Bioresource Technology* 147: 1-6.

Zhang, R. H., Tao, J. & Dugba, P. N. 2000. Evaluation of two-stage anaerobic sequencing batch reactor systems for animal wastewater treatment. *Transactions of the ASAE* 43(6): 1795-1802.

Zhu, H., Stadnyk, A., Béland, M. & Seto, P. 2008. Co-production of hydrogen andmethane from potato waste using a two-stage anaerobic digestion process. *Bioresource Technology* 99(11): 5078-5084.

Zupančič, G. D., Stražiščar, M. & Roš, M. 2007. Treatment of brewery slurry in thermophilic anaerobic sequencing batch reactor. *Bioresource Technology* 98(14): 2714-2722.

Rafiqqah Mohamad Sabri
Research Centre for Sustainable Process Technology (CESPRO),
Faculty of Engineering & Built Environment,
Universiti Kebangsaan Malaysia, Bangi, Malaysia

Nurleyna bt Yunus
Downstream Technology Division,
CRAUN Research Sdn Bhd, Faculty of Lot 3147,
Block 14, Jalan Sultan Tengah,
93055, Kuching, Sarawak, Malaysia

*Jamaliah Md Jahim
Research Centre for Sustainable Process Technology (CESPRO),
Faculty of Engineering & Built Environment,
Universiti Kebangsaan Malaysia,
43600 UKM Bangi, Malaysia

Mohd Sobri Takriff
Research Centre for Sustainable Process Technology (CESPRO),
Faculty of Engineering & Built Environment,
Universiti Kebangsaan Malaysia,
43600 UKM Bangi, Malaysia

Bambang Trisakti
Chemical Engineering Department,
Faculty of Engineering, Universitas Sumatra Utara,
Jalan Almamater, Komplek USU, Medan,
20155 Indonesia

*Corresponding author; email:
jamal@ukm.edu.my

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