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Biogas Production from Oil Palm Empty Fruit Bunches and Palm Oil Decanter Cake using Solid-State Anaerobic co-Digestion

Muthita Tepsour [1,2], Nikannapas Usmanbaha [2,3], Thiwa Rattanaya [2,3], Rattana Jariyaboon [1,2], Sompong O-Thong [4], Poonsuk Prasertsan [5] and Prawit Kongjan [1,2,*]

1 Department of Science, Faculty of Science and Technology, Prince of Songkla University, Pattani Campus, Meung, Pattani 94000, Thailand; muthita.tepsour@gmail.com (M.T.); rattana.sa@psu.ac.th (R.J.)
2 Bio-Mass Conversion to Energy and Chemicals (Bio-MEC) Research Unit, Faculty of Science and Technology, Prince of Songkla University, Pattani 94000, Thailand; nikannapas555@gmail.com (N.U.); phattew@gmail.com (T.R.)
3 Energy Technology Program, Faculty of Engineering, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand
4 Department of Biology, Faculty of Science, Thaksin University, Phatthalung 93110, Thailand; sompong.o@gmail.com
5 Research and Development Office, Prince of Songkla University, Hatyai, Songkhla 90110, Thailand; poonsuk.p@psu.ac.th
* Correspondence: kprawit.kongjan@gmail.com; Tel.: +6-89-728-7337

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Abstract: Oil palm empty fruit bunches (EFB) and palm oil decanter cake (DC) were used to investigate biogas production by using solid-state anaerobic co-digestion (SS-AcoD) with 15% total solid (TS) content. Solid state anaerobic digestion (SS-AD) using substrate to inoculum (S:I) ratio of 3:1, methane yields of 353.0 mL-CH_4/g-VS and 101.5 mL-CH_4/g-VS were respectively achieved from mono-digestion of EFB without oil palm ash (OPA) addition and of DC with 10% OPA addition under mesophilic conditions 35 °C. By adding 5% OPA to SS-AD using 3:1 S:I ratio under thermophilic conditions (55 °C), mono-digestion of EFB and DC provided methane yields of 365.0 and 160.3 mL-CH_4/g-VS, respectively. Furthermore, SS-AcoD of EFB:DC at 1:1 mixing ratio (volatile solid, VS basis), corresponding to carbon to nitrogen (C:N) ratio of 32, gathering with S:I ratio of 3:1 and 5% ash addition, synergistic effect is observed together with similar methane yields of 414.4 and 399.3 mL-CH_4/g-VS, achieved under 35 °C and 55 °C, respectively. According to first order kinetic analysis under synergistic condition, methane production rate from thermophilic operation is 5 times higher than that from mesophilic operation. Therefore, SS-AcoD could be potentially beneficial to generate biogas from EFB and DC.

Keywords: Gaseous bio-fuel; Oil palm biomass; Oil palm ash; Liquid anaerobic digestate; SS-AcoD

1. Introduction

Production of crude palm oil (CPO) by wet extraction from pulp of oil palm fruits is one of the major agricultural industries in southern Thailand. During CPO processing, one ton of fresh fruit bunches (FFB) could approximately generate residue/waste of Palm oil mill effluent (POME) 585 L, fiber 140 kg, palm shell 60 kg, palm oil decanter cake (DC) 42 kg and oil palm empty fruit bunches (EFB) 240 kg [1]. POME could be potentially used for biogas production by liquid state anaerobic digestion (L-AD) with satisfactory yield of 20–30 m^3-biogas/m^3-POME in a full scale plant [2]. Fiber and shell are burned effectively as solid fuels in a boiler to producing steam to preheat FFB prior mechanical pressing. As contained high nutrients content, DC could be practically converted to commercial grade pellet

animal feed. Partial EFB generated is mixed with DC and urea to producing fertilizer and composting material [1]. Directly massive using of EFB as solid fuel for boiler is still limited since high content of potassium that could potentially lead slagging and fouling problems during EFB combustion. High slagging and fouling indexes indicate obstruct effective heat transfer in boiler [3]. In order to create sustainable and cleaner society and to avoid consequently environmental problems, abundant EFB leftover could be potentially minimized by converting it into other useful products, such as biogas, bio-syngas, bio-oil, and bio-chemicals. These products would be used replacing products produced from non-renewable petroleum resources.

EFB classified as lignocellulosic biomass is comprised on dry weight basis with 41.3% cellulose, 24.2% hemicellulose, 20.5% lignin, and 8.9% extractives [4]. Typical extractive substances generally are consisted of proteins, fats, fatty acids, sugars, phenol, terpenes, resin acids, and resin [5]. As contained high organic content, EFB provided ultimate methane yield (Bio-methane potential, BMP) around 370 ml/g-VS_{added} under L-AD conditions [6]. Meanwhile, EFB was used as substrate under batch solid state anaerobic digestion (SS-AD) conditions and subsequently converted to around 358 mL-CH_4/g-VS_{added}. [7]. Since feasible to be obtained satisfactory methane yield from EFB SS-AD, which is not much lower than that from EFB L-AD, EFB general having high moisture content around 65–75% is thus more suitable to be anaerobically digested by deploying SS-AD. Furthermore, solid digestate from SS-AD still contained some carbon can be potentially further used as composting for soil fertility or solid fuel pellet more conveniently than effluent or liquid digestate discharged from L-AD system [8,9].

As reported by Suksong et al. [4], satisfactory methane yield obtained by SS-AD. EFB having high carbon to nitrogen (C:N) ratio around 72 is however to be added with urea to get favorable C:N ratio range of 30-40 for SS-AD system. To make SS-AD of EFB better economic feasibility, replacing urea with another biomass having low C:N as a co-substrate in anaerobic co-digestion (AcoD) system could be potentially viable option. Among biomass generated from crude palm oil mill, DC with low C:N ratio of around 20 has been used to mixed with EFB to get initial C:N ratio of 39 for starting-up composting process [1]. Furthermore, BMP yield of 370 mL-CH_4/g-VS_{added} was also achieved under L-AD of DC [6]. Thus DC could be alternatively used as co-substrate to adjust C:N ratio at desirable level for solid-state anaerobic co-digestion (SS-AcoD) system. AcoD could potentially enhance biogas production due to adaptation of nutrients ratios of C:N), and carbon to phosphorus (C:P) for positive synergisms on microbial systems, diluted toxic compound and decreasing free fatty acid [10]. SS-AcoD of EFB and DC is therefore hypothesized to enhancing biogas production, due to containing considerably different of carbon and nitrogen and other organic matters in both EFB and DC.

SS-AD system is rather risk on acidification caused by accumulation of VFA during the start-up phase, due to containing high substrate loading. Therefore, sufficient inoculum size in term of substrate to inoculum (S:I) ratio is required to assess in order to avoid this aforementioned risk of acidification [8,9]. For lignocellulose based SS-AD system contained insufficient buffer capacity, acidification could cause SS-AD system's pH decreasing to lower than pH 7, which is not favorable for methanogenic microorganisms. Bicarbonate buffer and/or caustic soda are normally added to prevent pH drop in lab scale AD system. However, using these chemicals might not be economical for industrial SS-AD system. Alternatively, highly alkali oil palm ash (OPA) generated from using oil palm fruit's fiber and shell as fuel in boiler is abundantly available in palm oil mill. pH above 10 could be possibly achieved when OPA is dissolved in water [11]. Thus OPA could be attracting for economical and environmentally friendly use to adjust pH in SS-AD system of EFB-DC.

SS-AcoD of different substrates has been reported with rising synergistic effect, causing enhanced methane yield. However, extra logistic cost for transportation of outside substrates would be economical limitation to up-scale SS-AcoD system [12]. In the context of economically feasibility for SS-AcoD, the scenario of whole substrates and additives (EFB, DC, and OPA), only discharged from a crude palm oil mill, was therefore focused in this research work to improve methane yield by mixing EFB or DC and/or adding OPA to change the raw material characteristics. The effect of using EFB or DC as

a substrate on biogas production by single (SS-AD) under mesophilic or thermophilic temperature (35 °C or 55 °C) was firstly conducted at S:I ratios of 2:1 and 3:1 and with various addition of OPA 0, 5, 7, and 10 (% of TS used). The effect of various EFB to DC mixing ratios of 1:1, 3:1, 9:1, and 19:1 (VS basis) on biogas production from SS-AcoD at selected S:I ratio and percent addition of palm ash under mesophilic or thermophilic temperature (35 °C or 55 °C) as well. Microbial community from SS-AcoD of EFB and DC at selected mixing ratios of EFB to DC was later investigated to describe relationship with SS-AcoD performance on biogas production.

2. Materials and Methods

2.1. Substrate and Inoculum Preparation

DC, EFB, OPA were collected from palm oil mill of Palm Pattana Southern Border Company Limited, Pattani, Thailand (6° 48′ 56.6568″ N, 101° 9′ 12.3732″ E). Loosen EFB fiber [3] discarded from consecutive mechanical pressing and shredding was collected for further use. The EFB used in this investigation had already pretreated by a kind of steam explosion during the FFB sterilization by holding 3 bar gauge steam correlated to around 145 °C for 90 minutes then sudden depressurization. This wetting steam pretreatment could decrease recalcitrance of lignocellulose by increasing internal surface area due to expansion [13]. Meanwhile mechanical pressing and shredding of EFB during additional oil recovery could enhance external surface area by changing in size and shape of EFB to be loosen EFB fiber. The loosen EFB fiber was grinded to increase further accessible surface area for biodegradation. EFB having size less than 2 mm was then used for an evaluation of biogas production by SS-AD. It was later dried by oven at 105 °C to have moisture content less than 10% to avoid biodegradation by microorganisms. The loosen EFB was later sequentially crushed and milled to have small particle passing through 2 mm sieve, after that storing in air tight container prior to be used. DC was sealed in plastic bag and kept in a refrigerator at 4 °C to prevent further biodegradation by microorganisms as well. OPA generated from combustion of oil palm fruit's fiber and shell at boiler's furnace was sieved through 2 mm sieve. It was stored in a closed container until further using as well.

Anaerobic sludge taken from bottom sediment of mesophilic anaerobic digester (Palm Pattana Biogas Company Limited, Pattani, Thailand) at hydraulic retention time 27 day was used as original inoculum. For using as inoculum at mesophilic temperature, anaerobic sludge was degassed for 1 week in the 35 °C incubator. Meanwhile anaerobic sludge was also used to acclimatize as an inoculum at thermophilic condition (55 °C) by adding with POME and incubated for 2 months in the 55 °C incubator. DC, EFB, and original anaerobic sludge were characterized for total solid (TS), volatile solid (VS), ash, lipid, and elemental composition. Meanwhile, OPA was analyzed for oxide components. Inoculums enriched for 35 °C and 55 °C of SS-AD were collected for further microbial community structures analysis.

2.2. Solid-State Mono-Anaerobic Digestion

This experiment was carried out in triplicate batch fermentation by using 500 mL serum bottle to evaluate biogas production from SS-AD of EFB and DC at different environmental conditions. S:I ratios were studied at 2:1 and 3:1 by adding OPA at various portions of 0%, 5%, 7%, and 10% (weight basis). Desire mixture with 15% initial TS was loaded into a serum bottle and purging with N_2 gas for 5 min. and kept in an incubator for 25–60 days in 35 °C or 55 °C incubator. A control bottle was also prepared by using sterilized sand, sieved through 35 mesh screener instead of EFB and DC in order to subtract background biogas possibly produced from inoculum.

2.3. Solid-State Anaerobic co-Digestion

Batch experimental assay for SS-co-AD was carried out by using 500 mL serum bottle at the S:I ratio of 3:1. EFB:DC ratios were varied at 1:1, 3:1, 9:1, and 19:1 (VS basis) gathering with 5% of palm ash addition. All capped bottles were flushed with N_2 gas for 5 min and later incubated in 35 °C or 55 °C

incubator for 25 days and 60 days, respectively. Batch assay was conducted in triplicates. Sterilized sands, sieved through 35 mesh screener were replaced to mixed EFB and DC substrate for a control bottle Anaerobic sludge from SS-AcoD of EFB:DC batch providing highest methane yield was taken microbial community analysis. Cumulative methane production achieved from this batch SS-AcoD could be further used to evaluate the hydrolysis constant (k_h) by using the first-order kinetic reaction as shown in Equation (1).

$$\ln \frac{B_\infty - B}{B_\infty} = -k_h t \quad (1)$$

The kinetics of methane formation under SS-AcoD of EFB with DC were estimated by fitting with a modified Gompertz model as shown in Equation (2)

$$B_t = B_\infty \times \exp\left\{-\exp\left[\frac{R_{\max} \times e}{B_\infty}(\lambda - t) + 1\right]\right\} \quad (2)$$

where B_t is methane cumulated at time t, B_∞ is the ultimate methane cumulating at the end of an experimental period and t is time (day). $R_{\max}$ is the maximum methane production rate (mL-CH_4/g VS-day); e = exp (1) = 2.7183; and is the lag phase period (day) [14].

2.4. Analytical Methods

The volume of produced gas was recorded by water displacement gas meter. Compositions in biogas were periodically analyzed by gas chromatography with thermal conductivity detector equipped with a 2 m stainless steel column, shin-carbon (80/100 mesh). Argon was used as a carrier gas at a flow rate 35 mL/min. The temperature values of the injection port, oven and detector were 100 °C, 100 °C, and 100 °C, respectively. The gas sample (0.5 mL) was injected in duplicates. pH, TS, volatile solid (VS), and oil and grease were determined in accordance with the procedures described in the APHA standard methods [15] for sample taken from the experiments. X-ray fluorescence spectrometry (XRF) was deployed to analyze major chemical composition of palm ash.

Volumetric gas production was report at standard temperature and pressure (STP) condition. All the measurements were done in triplicates and the results were plotted and reported in an average value with standard deviation.

2.5. Microbial Community Analysis

Inoculums for 35 °C and 55 °C of SS-AcoD, and anaerobic sludge from 35 °C and 55 °C of SS-AcoD of EFB:DC at 1:1 mixing ratio were taken for microbial community structure by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis. Genomic DNA was extracted from anaerobic sludge samples and purified by using QIAamp DNA Stool Mini Kit, according to its manual instruction (Qiagen Inc, Hilden, Germany). Genomic DNA was used as a template for first PCR reactions with the Arch2 1f-Arch958r primer pair and followed by second PCR with 340f-GC and 519r for the archaea population. The PCR reactions of the bacteria population were conducted with the 1525r -27f primer pair and followed by second PCR with 357f-GC and 518r [16]. The second PCR products with GC clamp were analyzed by electrolysis on 8% polyacrylamide gel containing a denaturant gradient ranging between 40% and 60% in the D-Code system (Bio-Rad Laboratories, CA, USA) [17]. Most of the bands were excised from the gel and re-amplified. After re-amplification, the PCR products were purified using E.Z.N.A cycle pure kit (Omega Bio-tek, USA) and sequenced (Macrogen Inc, Seoul, Korea). Closest matches for partial 16S rRNA gene sequences were identified by database searches in GenBank using BLAST [18].

3. Results and Discussion

3.1. Characteristics of EFB, DC, Anaerobic Sludge, Oxide Component of Oil Palm Ash

Table 1 demonstrates main characteristics of EFB, DC, and inoculum used for evaluation the potential of biogas production by using SS-AD system. Previously, biogas production from 2 mm particle size of sisal fiber was considerably higher than that from bigger particle size (5, 10, and 30 mm etc.) [19]. EFB is obviously rich in solid content and could be defined as carbon rich substrate with C:N ratio of 88.8, whilst, DC is contained with high moisture and rich in lipid and also is defined as nitrogen rich substrate with a C:N ratio of 19.23. C:N ratio is one of major parameters impact on biogas production. C:N ratio in a range of 20–30 is the most suitable for anaerobic digestion system [20]. For such high C:N ratio in EFB could lead to have lack of nitrogen for methanogens growth, consequently less biogas generated. Therefore, co-digestion of EFB with another substrate having low C:N ratio of DC is required to balance C:N ratio in the AD system. Anaerobic sludge used as inoculum in this investigation having C:N ratio of 8.0 is virtually similar to that of 8.2 reported by [12] but rather high than that of 3.0 and 2.5 reported by [21] and [22] respectively.

Table 1. Characteristics of empty fruit bunches (EFB), decanter cake (DC), anaerobic sludge and oil palm ash (OPA).

Parameter (%w/w)	EFB	DC	Anaerobic Sludge	Oxide Component (%w/w)	OPA
Total solid (TS)	96.38 ± 1.20	17.86 ± 0.25	5.14 ± 1.0	Silicon oxide (SiO_2)	50.30 ± 1.2
Volatile solid (VS)	90.22 ± 1.52	15.51 ± 0.32	3.14 ± 0.88	Calcium oxide (CaO)	14.90 ± 0.07
Lipid	1.08 ± 0.052	2.56 ± 0.10	0.11 ± 0.01	Potassium oxide (K_2O)	13.10 ± 0.013
Ash	6.16 ± 0.152	2.35 ± 0.11	2.0 ± 0.10	Magnesium oxide (MgO)	6.80 ± 0.18
Nitrogen	0.50 ± 0.002	2.22 ± 0.02	0.14 ± 0.001	Phosphorus oxide (P_2O_5)	4.80 ± 0.03
Carbon	44.29 ± 0.02	42.70 ± 0.14	1.12 ± 0.02	Ferric oxide (Fe_2O_3)	1.80 ± 0.01
Hydrogen	5.58 ± 0.02	5.77 ± 0.10	6.65 ± 0.02	Aluminum oxide (Al_2O_3)	0.87 ± 0.004
Sulfur	0.046 ± 0.002	0.260 ± 0.003	N.D.	Sulfur oxide (SO_3)	0.81 ± 0.002
Oxygen	38.27 ± 0.23	32.89 ± 0.12	63.98 ± 0.02	Manganese oxide (MnO_2)	0.31 ± 0.001
C:N ratio	88.76	19.23	8.0	Sodium oxide (Na_2O)	-

Anaerobic sludge from L-AD is suggested for being used as better inoculum for lignocellulose SS-AD system than other inoculum sources of activated sludge, manure, and rumen fluid due to higher content of microorganism, nutrients, and buffers [13]. OPA was characterized for oxide components by using x-ray fluorescence (XRF) as also shown in Table 1. The main inorganic component containing in OPA is Si, followed by Ca, K, Mg, and P. Ash alkaline solution is attributed mainly from K and Mg, while Si, Ca, and P is dissolved in smaller amount. Due to high alkali properties of OPA [11], ash is indeed expected to be helpful for simultaneous pH adjustment and alkali pretreatment during SS-AD process. Lignin from recalcitrant lignocellulose could be effectively solubilized to alkali solution during alkaline pretreatment, increasing accessible surface area of lignocellulosic substrate [23].

3.2. Solid-state Mono-anaerobic Digestion of EFB and DC

Methane production yields and pH-initial and -final obtained from single stage digestion of EFB and DC at different S:I ratios of 2:1 and 3:1 and various addition of % OPA portions (0, 5, 7, and 10) are shown in Figure 1. Under mesophilic condition (35 °C) at both S:I ratio 2:1 and 3:1, adding more portion of OPA for EFB SS-AD resulted in lower methane production yield. Highest yields of 307.5 mL-CH_4/g-VS and 353.0 ml-CH_4/g-VS was satisfactory achieved at S:I ratio 2:1 and 3:1, respectively by without OPA addition (Figure 1a,c). This suggests mesophilic inoculum itself provided sufficient buffering capacity to properly regulate relevant biochemical reactions for biogas production. Figure 1b,d, final pH of 7.86 and 7.55, which are in an appropriate pH range for AD system (6-8) [24], were approached by using S:I ratio at 2:1 and 3:1, respectively without adding OPA. However considerably low hydrolysis and acidogenesis could delay methane production. On contrary, SS-AD

of DC under mesophilic condition (35 °C) and also 2:1 and 3:1 S:I ratio, biogas yield was enhanced by adding OPA and maximized by adding 7% and 10% OPA for S:I ratio 2:1 and 3:1, respectively. However, methane yield obtained from suitable SS-AD of DC was significant lower compared to that from SS-AD of EFB, obviously due to high easily biodegradable organic contents in DC, especially lipids, which could contribute to rapid VFAs and long chain fatty acids accumulation and subsequent methanogens inhibition.

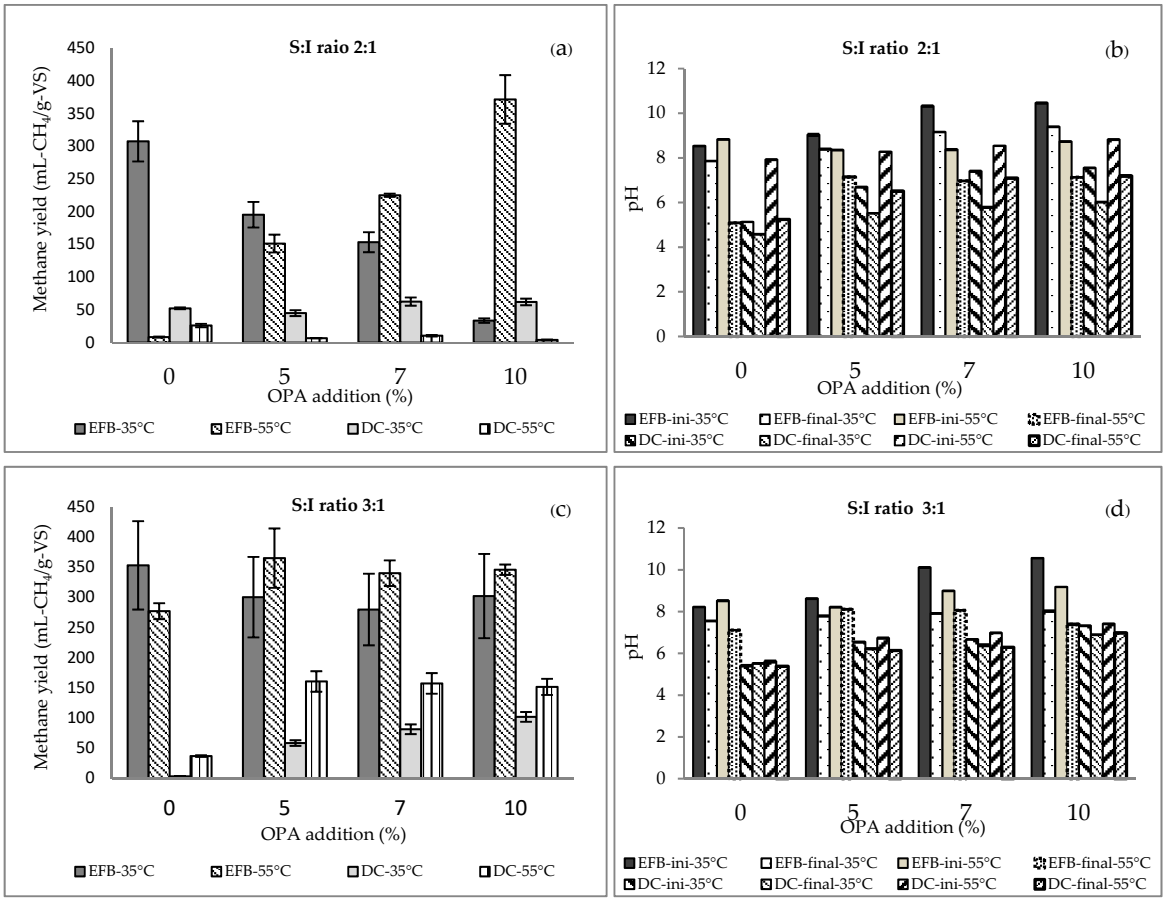

Figure 1. Methane production yield and pH from (SS-AD) of empty fruit bunches (EFB) and decanter cake (DC) at S:I ratio 2:1 and 3:1 with various % Oil palm ash (OPA) addition. Methane yield (**a**) and pH (**b**) at S:I ratio 2:1; Methane yield (**c**) and pH (**d**) at S:I ratio 3:1.

Under thermophilic conditions (55 °C), highest yields of 371.6 and 364.9 ml-CH_4/g-VS were generated from SS-AD of EFB by adding 10% and 5% OPA to EFB at S:I ratios 2:1 and 3:1, respectively (Figure 1a,c). These methane yield obtained from EFB of SS-AD is considerably higher than those methane yield obtained from EFB of SS-AD under mesophilic conditions. Thermophic conditions is more thermodynamically favorable for hydrolysis and acidogenesis [25]. Rapid hydrolysis and acidogenesis induce to have pH decreasing in the SS-AD, thus buffer agent is required to maintain pH in a suitable range for methanogenesis. Adding OPA as major buffer source appears to be helpful to enhancing methane yield by mean of pH regulation in the system for SS-AD of both EFB and DC at S:I ratio 3:1. Methane yields obtained from SS-AD of DC are however, comparably lower than those obtained from SS-AD of EFB. Furthermore, under high temperature of thermophilic conditions, organic acids other than acetic acid generated are easier to be degraded acetogenic step [26], faster methane production is thus accomplished. Operating under thermophilic temperature, S:I ratio 3:1 and adding higher portion of palm ash could be complementary factors to enhancing methane yield for single SS-AD of both EFB and DC.

As shown in Figure 1, at S:I ratio 3:1, methane yields obtained from SS-AD of both EFB and DC at S:I ratio 3:1 are considerably higher than that at S:I ratio 2:1, whether operating under thermophilic or mesophilic condition. Liew et al. [27] and Rouches et al. [9] reported previously an S:I ratio between 2:1 and 4:1 is capable to stabilize SS-AD process by regulating total VFA_S to alkalinity ($TVFA_S$:alkalinity) ratio not higher than 2.0, corresponding to a pH range between 6.5 and 7.8. Consequently, four distinct anaerobes including hydrolytic, acidogenic, acetogenic and methanogenic microorganisms allow to function effectively for methane production. Although the $TVFA_S$:alkalinity ratio was not analyzed during single solid-state anaerobic digestion of both EFB and DC, final pH achieved from each SS-AD batch producing highest methane shown in Figure 1 lies between 7.13 and 8.11, implying proper $TVFA_S$:alkalinity ratio for single SS-AD system achieved. A part from having sufficient inoculum and proper $TVFA_S$:alkalinity ratio, satisfactory methane yields of 353.0 mL-CH_4/g-VS and 364.9 mL-CH_4/g-VS obtained from SS-AD of EFB is to be contributed from size reduction by mechanical grinding to get EFB size less than 2 mm which in an optimal range between 0.5 and 2 mm for decreasing limitations of heat and mass transfer and for well digestibility. Mechanical grinding is able to disrupt matrix of poorly biodegradable lignin consisting in lignocellulosic substrate effectively. Consequently, hydrolytic bacteria and enzyme could easily access fermentable matters, resulting in enhancement of methane production [9].

3.3. Solid-state anaerobic co-digestion of EFB and DC

As previously stated, using S:I ratio 3:1, methane yields obtained from SS-AD of DC are drastically less than that obtained from SS-AD of EFB using S:I ratio 3:1, enhance methane production from DC is apparently required. AcoD is the practical strategy to increase methane yield from individual substrate for creating synergistic effect in the AD system by mean of mixing at least 2 different substrates [24]. Under mesophilic or thermophilic temperatures and at S:I ratio 3:1 gathering with adding 5% palm ash, ultimate methane production from batch SS-AcoD of EFB with DC was conducted by using trial EFB:DC ratios of 1:1, 3:1, 9:1, and 19:1 (VS basis). Cumulative methane yields obtained are demonstrated in a,b for mesophilic and thermophilic temperature, respectively. The highest methane yields obtained by using 1:1 EFB:DC mixing ratio, corresponding to substrate C:N ratio 32, are 414.40 mL-CH_4/g-VS and 399.3 mL-CH_4/g-VS at mesophilic and thermophilic temperatures, respectively. Nonetheless, other 3:1, 9:1, and 19:1 EFB:DC mixing ratios, corresponding to C:N ratios of 32, 47.3, 65.8, and 75.7, respectively, provide lower methane yields than 1:1 EFB:DC mixing ratio provided.

Either synergism or antagonism could be generated during AcoD of certain substrates. Synergism or synergistic effect is existed when methane yield experimentally obtained from mixed substrates co-digestion is considerably higher than weigh methane yield, which is calculated from individual substrate digestion. Thus, considerably lower methane generated from mixed substrates co-digestion is to be antagonistic effect or antagonism [28]. As demonstrated in Table 2, EFB:DC mixing ratio 1:1 having substrate C:N ratio 32, which is still in the suitable range for methane production suggested by [20], has clearly synergistic effect for operation at both mesophilic and thermophilic temperature. It is interestingly noticeable under mesophilic operation that synergisms is likely to be appeared even at EFB:DC mixing ratio 19:1 (with substrate C:N ratio 75.7). Nonetheless, under thermophilic operation, other mixing EFB:DC ratios than 1:1 (with C:N ratio 47.3–75.7) does not create synergisms. Table 2 demonstrates estimated hydrolysis constants (k_h) for thermophilic SS-AcoD are considerably higher than that in mesophilic SS-AcoD, confirming thermophilic temperature could be helpful to accelerate hydrolysis and acidogenesis. However, rapid VFAs generation from fast hydrolysis in thermophilic AD process at rather high C:N ratio could cause imbalance between acetogenesis and methanogenesis, leading to have antagonism. Concerning to synergism that lead to have higher methane yield, mesophilic SS-AcoD operation is therefore more flexible to adjust mixing substrates ratio. Previously, [29] reported previously SS-AcoD of spent mushroom with yard trimmings and wheat straw is able to provide synergistic effect at even rather high C:N ratio of 74.6 and 71.9, respectively.

Table 2. Methane generated from solid stage anaerobic co-digestion of EFB and DC at S:I ratio 3:1.

EFB:DC	SubstrateC:N Ratio	Temp	MY	WMY	MY-WMY	K_h	Modified Gompertz Model		
							B_∞	Rmax	l
VS basis		°C	mL-CH_4/g-VS			d^{-1}	mL-CH_4/g-VS	mL/d	d
1:0	89	35	300.2	300.2	0	N.D	N.D	N.D	N.D
		55	364.9	364.9	0	N.D	N.D	N.D	N.D
0:1	19.2	35	58.1	58.1	0	N.D	N.D	N.D	N.D
		55	160.3	160.3	0	N.D	N.D	N.D	N.D
1:1	32.0	35	414.4	179.2	+235.2	0.042	454.4	13.5	16.0
		55	399.3	262.6	+136.7	0.213	397.9	44.9	0.9
3:1	47.3	35	367.1	239.5	+127.6	0.062	371.8	17.8	12.7
		55	274.7	313.8	−39.1	0.150	280.4	27.9	1.3
9:1	65.8	35	324.8	276.0	+48.8	0.053	333.4	14.2	6.4
		55	222.0	344.4	−122.4	0.182	222.2	24.7	0.81
19:1	75.7	35	321.8	288.1	+33.7	0.028	327.7	14.2	6.7
		55	221.0	353.8	−132.8	0.195	224.3	25.9	1.1

Remark: MY: Methane Yield; WMY: Weigh methane Yield; MY-WMY: Yield difference; N.D: Not determined

Despite showing better synergism and methane yield for all mixing EFB:DC ratios used in this investigation, mesophilic SS-AcoD operation has much longer lag time and lower hydrolysis rate than thermophilic SS-AcoD. Consequently, as can be seen from Figure 2, operating time for ultimate methane production is around 25 days for thermophilic operation, 2 times less than that of mesophilic operation. Indeed, under synergistic condition of EFB:DC mixing ratio 1:1, methane production rate under thermophilic condition is 5 times higher than that under mesophilic condition, according to first order kinetic constant (K_h) as shown in Table 2. Furthermore, lag time estimated by using modified Gompertz model for thermophilic SS-AcoD of EFB with DC is obviously higher than that for mesophilic SS-AcoD of EFB with DC. Reactor size to be used for thermophilic operation is then projected to have 2 times smaller than that at mesophilic operation, implying that capital cost for reactor and land used is potentially reduced. Furthermore, operating at thermophilic temperature (55 °C) for 6 hours is capable of pathogen destruction in higher degree [30], digested solid effluent, which can be used further for organic fertilizer preparation is pathogen-free. Co-digestion is enable to have higher organic loading rate than mono-digestion, leading to further reduce reactor size and minimize capital cost [31]. Therefore thermophilic operation for solid state co-digesting EFB with DC at EFB:DC mixing ratio 1:1 on VS basis is the most preferable, when considering in context of both technical efficiency based on methane yield and economic efficiency based on methane productivity [32]. Since whole biomass and waste used for SS-AcoD in this investigation are generated in crude palm oil, high logistic cost for transportation of different feedstock for commercial scale, as one of the major disadvantages for SS-AcoD could potentially be reduced.

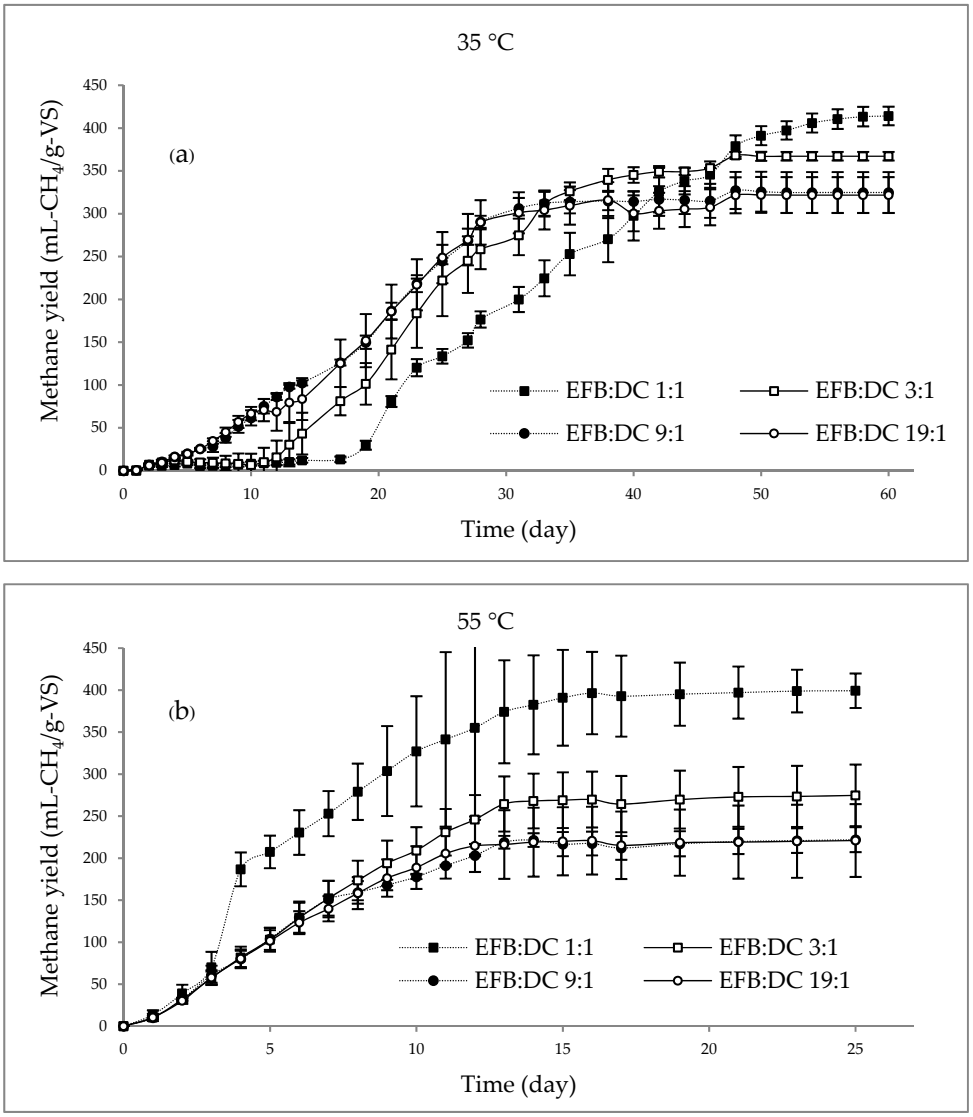

Figure 2. Methane yield achieved from different EFB:DC ratios for solid stage co-digestion with S:I ratio 3:1 at mesophilic temperature 35 °C (**a**) and thermophilic temperature 55 °C (**b**).

3.4. Microbial Community

Mesophilic (35 °C) and thermophilic (55 °C) inoculums collected prior using for SS-AcoD of EFB with DC and anaerobic sludge samples collected from batch SS-AcoD of EFB with DC using mixing ratio of 1:1 at day 60 for mesophilic (35 °C) and day 25 for thermophilic (55 °C) conditions were analyzed for predominant bacteria and archaea microbial community PCR-DGGE method as illustrated in Figure 3a,b, respectively. DGGE profiles of 16S rDNA gene fragment of microbial structure exhibit 74–96% sequence similarity. Both 35 °C and 55 °C of inoculum show distinct bacterial population, indicating effect of temperature on modulation of bacterial community structure [33]. *Prevotella* sp., *Ralstonia* sp., *Advenella* sp., *Clostridium* sp., *Alcaligenes* sp., and *Desulfatiglans* sp. *predominated* in mesophilic inoculum. Meanwhile, the main bacteria of *Ethanoligenens* sp., *Clostridium* sp., *Faecalibacterium* sp., and *Anaerofilum* sp. were majorly detected in thermophilic inoculum. Interestingly, *Clostridium* sp., majorly active in both 35 °C and 55 °C of inoculums, can normally decompose carbohydrates rich biomass to producing acetic acid, butyric acid, and hydrogen under the acidogenesis stage [34]. However, *Clostridium* sp., became less predominant in 35 °C anaerobic sludge collected from batch SS-AcoD of EFB with DC at 1:1 mixing ratio. This could be due to substrate change.

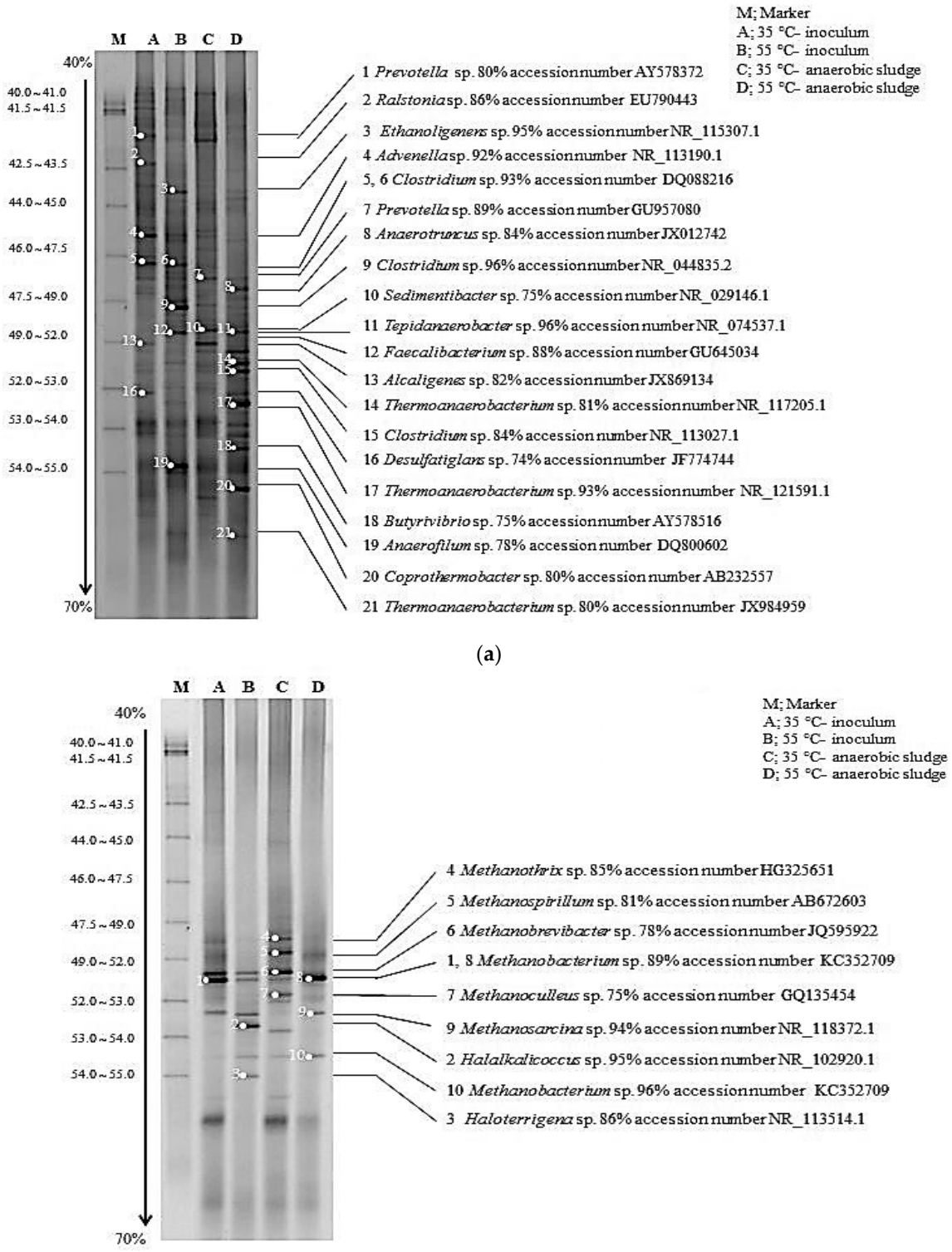

Figure 3. DGGE profiles of 16S rDNA gene fragment for bacteria (**a**) and archaea (**b**) from SS-AcoD of EFB: DC at 1:1 mixing ratio of 35 °C- inoculum (A), 55 °C-inoculum (B), 35 °C- anaerobic sludge (C), and 55 °C- anaerobic sludge (D).

Prevotella sp., *Sedimentibacter* sp., and *Alcaligenes* sp., predominant species found 35 °C of anaerobic sludge can produce acids from carbohydrate digestion [35,36] and can grow specifically at a temperature range between 30 °C and 37 °C [37]. In addition, as clearly demonstrated in Figure 3a, diversity of

the predominantly bacterial community under thermophilic conditions was higher than that under mesophilic conditions. High bacterial diversity enhance digestibility of complex substrates [38]. Therefore as mentioned previously mentioned in Table 2, considerably high hydrolysis constant and short lag time obtained from SS-AcoD of EFB with DC under thermophilic condition could be also contributed from high microbial diversity. Predominant *Thermoanaerobacterium* sp. in 55 °C of SS-AcoD, having specific growth temperature in thermophilic range between 50 °C and 60 °C can anaerobically converted starch, cellulose and sugar to VFAs [39].

The structure of archaea population in 35 °C of inoculum is majorly consisted with *Methanobacterium* sp., while *Halalkalicoccus* sp. was predominant in 55 °C of inoculum. However, both *Methanobacterium* sp., and *Halalkalicoccus* sp. become less predominant in 35 °C and 55 °C of anaerobic sludge, respectively. Hydrogenotrophic methanogens of *Methanospirillum* sp., *Methanobrevibacter* sp., and *Methanoculleus* sp. [40–42] and aceticlastic methanogen of *Methanothrix* sp. [43] were predominant archaea in 35 °C anaerobic sludge. While, hydrogenotrophic methanogen of *Methanobacterium* sp. [44], and aceticlastic methanogen of *Methanosarcina* sp. [45]. Mainly comprise in anaerobic sludge collected from 55 °C of SS-AcoD. In addition, hydrogenotrophic methanogens are capable of using hydrogen gas and carbon dioxide to produce methane, while aceticlastic methanogens are enable to convert acetate to methane and carbon dioxide [46].

The experimental results stated above could confirm that liquid digestate discharged from commercial biogas digester fed with POME potentially serve as an effective source of inoculum to start-up SS-AD system for co-digesting EFB with DC. Liquid anaerobic digestion process to producing biogas from POME is a mature technology, indicating abundant availability of liquid digestate to be further used as inoculum for industrial scale SS-AcoD of EFB with DC. Operating SS-AcoD of EFB with DC parallel with L-AD of POME could be highly potential approach for zero waste discharged from palm oil mill industries.

4. Conclusions

Liquid anaerobic digestate from commercial biogas production from POME is an effective inoculum source for both SS-AD of mono- and co-digestion of EFB and DC. Mono-digestion of EFB under mesophilic temperature is likely to be independent to palm ash addition. Meanwhile, under thermophilic temperature, addition of palm ash is required to have satisfactory methane yield from mono-digestion of EFB. Furthermore, mono-digestion of EFB provides methane yield much higher than that of DC. SS-AcoD of EFB with DC at 1:1 VS based mixing ratio, corresponding to C:N ratio 32, and at S:I ratio 3:1 under both mesophilic and thermophilic temperature is synergism with similar methane yields of 414.40 mL-CH_4/g-VS and 399.3 mL-CH_4/g-VS, respectively. According to first order kinetic analysis under synergistic condition, methane production rate from SS-AcoD of under thermophilic conditions is much higher than that of under mesophilic conditions.

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Abbreviations

AcoD	Anaerobic co-digestion
BMP	Bio-methane potential
C:N	Carbon to nitrogen ratio
CPO	Crude palm oil
DC	Decanter cake
EFB	Empty fruit bunches
FFB	Fresh fruit bunches
L-AD	Liquid state anaerobic digestion
OPA	Oil palm ash
PCR-DGGE	Polymerase chain reaction-denaturing gradient gel electrophoresis analysis
POME	Palm oil mill effluent
S:I	Substrate to inoculum
SS-AcoD	Solid-state anaerobic co-digestion
SS-AD	Solid state anaerobic digestion
TS	Total solid content
VFAs	Volatile fatty acids

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