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Effects of size and thermophilic pre-hydrolysis of banana peel during anaerobic digestion, and biomethanation potential of key tropical fruit wastes

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ABSTRACT

Methane production potential of tropical fruit wastes, namely lady-finger banana peel, rambutan waste and longan waste were compared using BMP assay and stoichiometric modified Buswell and Mueller equation. Methane yields based on volatile solid (VS) were in the order of ground banana peel, chopped banana peel, chopped longan waste, and chopped rambutan waste (330.6, 268.3, 234.6 and 193.2 $mLCH_4$/gVS) that corresponded to their calculated biodegradability. In continuous operations of banana peel digestion at feed concentrations based on total solid (TS) 1–2%, mesophilic single stage digester run at 20-day hydraulic retention time (20-day HRT) failed at 2%TS, but successfully recovered at 1.5%TS. Pre-hydrolysis thermophilic reactor (4-d HRT) was placed as pre-treatment to mesophilic reactor (20-d HRT). Higher biogas (with an evolution of H_2) and energy yields were obtained and greater system stability was achieved over the single stage digestion, particularly at higher solid feedstock. The best performance of two stage digestion was 68.5% VS destruction and energy yield of 2510.9 kJ/kgVS added at a feed concentration of 2%TS.

1. Introduction

Banana is the general name for a number of species in genus *Musa*. It is an elliptically shaped fruit widely grown in tropical and subtropical countries mostly in Asia and Africa, and one of the most versatile and earliest crops cultivated in the history of human agriculture. The banana fruit is available throughout the year and it is harvested in its green unripe stage. As the banana fruit ripens in postharvest senescence, physical, chemical and biological changes take place and the color changes from green to yellow as the fruit softens (Yuan et al., 2017). The ripe banana is made up of a soft seedless edible pulp and a skin known as peel which is about 30–40% of the total weight of the fruit and could serve as a good source of easily biodegradable biomass (Bardiya et al., 1996; Happi Emaga et al., 2008).

Rambutan (*Nephelium lappaceum*) and longan (*Euphoria longan*) are also tropical fruits largely cultivated and produced in Thailand and other South East Asian counties, and have made their way to the canning industry. The wastes or fragments of these fruits could be used as feedstock with ample supply for anaerobic digester. Seed, peel, and pulp of fruits although possess good potential, their digestibility varies greatly, and most studies focused on only batch assays (Escalante et al., 2016; Sanjaya et al., 2016; Zhao et al., 2016). This research focuses on banana peel with some comparative potential data of rambutan and longan wastes (peel and seed).

Based on existing statistics, rambutan and longan production are stable in the past 3 years (2013–2015) for Thailand at around 0.32 and 0.91 million tons per annum on average, respectively. The averaged export during the same period is mostly as processed fruit at 13,011 and 561,595 tons per annum. Fresh banana production, subsequent conversion to various products and its total exportation of Thailand are growing in recent years. An amount of 22,570 tons of banana was exported with a value of 265 million Thai baths (35.0 Thai baht per US Dollar) in 2014 (DOA, 2014) from the total banana production of 1.51 million tons per annum. With recent interest from Chinese market, the banana production in Thailand is getting further expansion. For domestic use, about 80% of ripe banana is processed to different products or used as ingredient in several types of food (Pisutpaisal et al., 2014). The flesh of banana, rambutan and longan must be taken from the whole fruit before further processings. Peel and seed are generated

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in large quantity from such productions. There is a need for sustainable management of these organic wastes in an environment-friendly and economical way as opposed to the landfilling and land application that releases greenhouse gases from decomposition (Permpool et al., 2016). Burning of these wastes is not an economical option due to its high moisture content. Anaerobic digestion seems to be an appropriate technology for energy recovery and sustainable waste stabilization of the banana peel (Bardiya et al., 1996; Nagao et al., 2012; Pisutpaisal et al., 2014). The methane produced is a source of renewable energy, environmental pollution caused by open dumping can be reduced or eradicated, and the left over organic residue can be used as a stable soil amendment (Clarke et al., 2008; Ge et al., 2014).

In anaerobic digestion (AD), organic materials are broken down in the absence of oxygen producing biogas as a product. For solid substrate, hydrolysis is often a rate limiting step since the biomass usually has various degrees of structural integrity which needs to be solubilized by enzymes from the microorganisms in the reactor. Once it was solubilized to soluble monomers such as sugars and amino acids, volatile fatty acids (VFA), hydrogen, and carbon dioxide are produced in the acidogenesis step before further converted to methane. Different preparation of biomass can be employed to improve digestibility and rate of AD, either by or the combination of physical, chemical, or biological methods (Kumar et al., 2009; Zhu et al., 2008). However, size reduction is most simple and usually an economical option with soft biomasses.

Several designs of AD systems have been developed over time for treatment of organic wastes, such as wet process, dry process, single stage, or multiple stage system, just to name a few. Wet AD (feeding 1–10% total solid concentration, TS) is simple in design and construction and demonstrated a higher system stability due to good mass transfer through effective mixing, proper temperature control and dilution of inhibitors compared to semi dry (feed 10–20% TS) and dry AD (feed 20–40% TS) (Ge et al., 2014; Zhang et al., 2007). Nevertheless, AD is a delicate process and rates of acid formation and methane production should be balance or equal to prevent VFA accumulation and pH drop (Nagao et al., 2012). Digester acidification is often a cause of system failure at high organic loadings (Ahring et al., 1995; Boe et al., 2010; Nagao et al., 2012). Fast degradability substrates could also cause over acidification and banana peel could be one in this category judging from its soft and thin physical feature. In a single stage system, hydrolysis, acidogenesis, acetogenesis and methanogenesis all take place in one reactor. However, in a multiple stage system, anaerobic reactions are separated to more than one reactor mostly for the purpose of process efficiency and stable digestion. Since enzymatic hydrolysis is dependent on temperature (Luo et al., 2012), thermophilic as a pretreatment stage shall benefit particularly in the digestion of biomass. It has been proven that the optimum operational time in thermophilic first phase should be below 7 days with best conditions at 4 or 5 days for organic fraction of municipal solid waste (Fernández-Rodríguez et al., 2015). In addition, a part of intermediate hydrogen produced in the first stage could be of use while leaving a more degradable substrates to the ensuing stage.

The first part of this study focused on identifying biomethanation potential of key tropical fruit wastes from banana, rambutan and longan in order to find a suitable feedstock for anaerobic digestion. Effects of physical pre-treatment by size reduction of the selected substrate were also studied. The main objective in the second part of this work was to evaluate an appropriate digestion strategy of the selected substrate (banana peel) in continuous anaerobic digestion systems. Impacts of staging were assessed in long-term operations of a mesophilic single stage digester versus a two stage digester with a front-end thermophilic pre-hydrolysis stage which enable the co-production of hydrogen and methane.

2. Methodology

2.1. Inocula

Inoculum used in this experiment was a mixed sludge from two full scale anaerobic digesters; a concentrated rubber latex factory and a pig farm in Songkhla province, Thailand to ensure diversity in the microbial population. The concentrated rubber latex industry digester treats wastewater with high sulfate concentration from concentrated rubber latex processing, and the commercial pig farm digester treats pig waste slurry with high nitrogen and solids. The solid contents and pH of the digester sludges were determined prior to the experiments after screening and sedimentation. The inoculum was prepared by mixing the two sludges at 1:1 ratio based on total solid concentration (TS). Past studies on microbial population using denaturing gradient gel electrophoresis (DGGE) revealed that rubber latex digester sludge has high concentration of active acetogenic and methanogenic microorganisms while pig farm digester sludge has high concentration of hydrolytic microorganisms (Dechrugsa et al., 2013).

The two fresh anaerobic digester sludges, pH values within 7.5–8.5, were kept to settle overnight in separate containers to remove scum and thicken the sludges for use. Rigorous biogas production was observed indicating high microbial activity in the inocula. These sludges were mostly dispersive, but settled well without granulated sludge.

2.2. Substrate

Ripe lady-finger banana (*Lusa sapientum Linn.*) was collected from southern Thailand and the fresh yellow-colored peels were removed, prepared and analyzed for total solid (TS) and volatile solid (VS) contents. The fresh peel was prepared in two sizes to be used in biochemical methane potential (BMP) assay, including a sample of chopped peel of 5 mm, and a sample of ground peel of about <1 mm (using mortar and pestle). Ripe longan and rambutan fruits were also collected from the local fruit market in Songkhla province. Chopped longan and rambutan wastes, composed of peel and seed, of 5 mm size were prepared to be used in BMP assay. All samples were dried at 60 °C to a constant weight and ground using mortar and pestle to perform the analysis of the elemental compositions (CHNS-O), cellulose and hemicellulose contents.

2.3. Biochemical methane potential (BMP) assay

BMP assay was set up according to the procedure based on Angelidaki et al. (2009), in 120 mL glass bottles with effective volume of 60 mL. Each BMP glass reactor was provided with 1% (v/v) of nutrients and trace element solution, and a buffer solution of 50 g/L $NaHCO_3$ at 10% (v/v). Seed inoculum of 15 g TS/L was added to each bottle, and 2–5 g/L TS of the materials were used. The pH was adjusted to 7.0 using small drops of 0.1 M NaOH or 0.1 M HCl. Effective volume of 60 mL was attained by addition of distilled (DI) water. The BMP glass reactors were flushed with nitrogen gas for 60 s and sealed immediately to maintain anaerobic condition. The glass reactors were placed in an incubator shaker with continuous shaking at 150 rpm (rpm) and at a temperature of 35 ± 1 °C. The assay was run against a control of blank containing only 15 g TS/L of inoculum, nutrient, with no substrate and filled to the effective volume with DI water. All BMP assays were run in three replicates for substrate samples and blank. The stability of reaction from solid substrate to gaseous end products was investigated by measuring pH, total alkalinity and total volatile fatty acids (TVFA) of the digestate at the end of 30 days. BMP methane yields were derived from dividing the volume of methane by the weight of sample VS added to the reactor.

2.4. Calculations of theoretical methane production and energy yield

Theoretical methane potential (TMP) assay was used to determine the maximum theoretical methane production from the complete degradation of the samples. TMP was calculated from elemental compositions (C, H, O, N, and S) using the modified Buswell and Mueller equation which includes nitrogen and sulfur to obtain the fraction of ammonia and hydrogen sulfide in the produced biogas as shown in Eq. (1) and Eq. (2) (Buswell and Mueller, 1952).

$$C_cH_hO_oN_nS_s + yH_2O \rightarrow xCH_4 + zCO_2 + nNH_3 + sH_2S \tag{1}$$

$$\begin{aligned} x &= 0.125(4c + h - 2o - 3n - 2s), \\ y &= 0.250(4c - h - 2o + 3n + 2s), \\ \text{and } z &= 0.125(4c - h + 2o + 3n + 2s) \end{aligned} \tag{2}$$

where c, h, n, s, and o are the moles of carbon, hydrogen, nitrogen, sulfur and oxygen, respectively, assigning s = 1. The theoretical methane yield was reported in litres of methane per gram VS of substrate.

Energy contents (LHV and HHV in kJ/g) were computed using modified Dulong's equation according to Hosokai et al. (2016) from elemental compositions as shown in Eqs. (3) and (4). Higher heating value (HHV) can be calculated by adding the heat of water vaporization of the sample as shown in Eq. (4). This energy content is an estimate of the ultimate conversion of combustible biomass (volatile solid) to carbon dioxide, of which the energy released from its anaerobic digestion pathway represents only a fraction. This is later discussed in mass-energy balance in our results.

$$\text{Energy content (LHV, kJ/g)} = 38.2m_C + 84.9(m_H - m_O/8) - \Delta H_l \tag{3}$$

$$\text{Sample's heat of water vaporization} = (M_{H2O}/M_{H2}) \times \Delta H_{boil} \times m_H = (18/2) \times 2.44m_H = 22.0m_H \tag{4}$$

where m_C, m_H and m_O are the contents (value between 0 and 1) of carbon, hydrogen, and oxygen, respectively, on dry basis, ΔH_l is the latent heat of sample (kJ/g), M_{H2O} and M_{H2} are the molecular weight of water and hydrogen, and ΔH_{boil} is the heat of water vaporization (2.44 kJ/g). According to Hosokai et al. (2016), the modified Dulong's formula employs ΔH_l of 0.62 kJ/g for determining the heating value of solid fuels.

2.5. Continuous anaerobic digestion configuration and operational procedures

The continuous digestion study was carried out using two bioreactor systems; a single stage mesophilic digester (35 ± 1 °C), designated as MS reactor, and a two stage system comprising of a first stage thermophilic digester (55 ± 1 °C), designated as T1 reactor, serving as a pre-hydrolysis step followed by a second stage mesophilic digester (35 ± 1 °C), designated as M2 reactor. The bioreactors were made of glass and covered with gastight capseal. The reactors were connected to gas bags for biogas collection using low permeability tubing. Nitrogen gas was used to flush head space before sealing the reactors to ensure anaerobic condition. The operational temperatures were controlled using water baths and electrical heaters equipped with temperature sensors connected to automatic switch. The reactors were inoculated at 44,000 mg/L MLSS (Mixed Liquor Suspended Solid) of the anaerobic sludge described in Section 2.1. The inoculum was used within 3 days of collection from the full scale anaerobic digesters to ensure active microorganisms.

Ground lady-finger banana peel feedstock was prepared into slurry every 2–3 days to the desired TS concentrations, and stored in the refrigerator at 4 °C until use. MS reactor with 4 L effective volume was operated at HRT 20 days and solid feed concentrations of 1%TS, 2%TS, and 1.5%TS corresponding to organic loading rate (OLR) of 0.41, 0.83, and 0.62 kg/m^3.d, respectively. Feed concentration was reduced from 2% to 1.5% TS due to acidified system failure which is discussed in Sections 3.5.1 and 3.5.2. In two stage system, T1 reactor, 1.2 L effective volume, was operated at HRT 4 days at the same feed concentrations corresponding to 2.07, 4.14, and 3.11 kg/m^3.d, respectively, while M2 reactor, 2.8 L effective volume, was operated at HRT 20 days. A 140-mL homogeneous effluent from T1 was fed to the M2 reactor while the rest was used in sample analyses. T1 reactor was employed as a thermal hydrolytic pre-treatment of the substrate. pH adjustment to the MS reactor was carried out when significant pH drop occurred during 2% TS feeding. This was done to prevent system failure due to accidental or random pH drop incidents, in turn, assuring the system inability to handle such OLR.

2.6. Analytical methods

Total solids (TS) and volatile solids (VS) were determined using Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Samples of ripe lady-finger banana peels, longan wastes and rambutan wastes were dried at 60 °C to a constant weight and ground for the analysis of carbon, hydrogen, oxygen, nitrogen, and sulphur (CHONS) elemental compositions by dynamic flash combustion method. Cellulose and hemicellulose contents were determined using detergent fibre technique. Natural detergent fibre (NDF) and acid detergent fibre (ADF) were both measured using the Van Soest method from which the content of cellulose and hemicellulose were calculated (Van Soest et al., 1991).

Biogas volume was measured on daily basis using a high precision flow gas meter, multi-chamber rotor, drum-type wet gas meter Model TG0/5, Ritter (Germany). Biogas sampling was done through a septum gas sampling point connected in-line with the biogas tubing to the gas bag. Ten mL of biogas was injected to a gas chromatography (GC Agilent™ 7820 A Agilent Technologies), equipped with a thermal conductivity detector (TCD) and a stainless steel packed column SS Hayesep Q80/100 (6 m × 1/8 in.) using helium (He) as carrier gas to determine the methane composition. The standard calibration curve was made with pure CH_4, CO_2, and N_2 gases, and verified with a standard gas mixture of 5% N_2, 60% CH_4, and 35% CO_2. For hydrogen composition measurement, GC (Agilent™ 7820 A) with a Packed Column: Shin Carbon ST100/120 (2 m × 1 mm) was used with argon (Ar) as carrier gas. The standard calibration curve was made from the peak of pure nitrogen (100%), hydrogen (99.99%), methane (99.99%) and carbon dioxide (99.99%) gases.

Alkalinity and total volatile fatty acids (TVFA) analyses of effluents were done using direct titration methods (DiLallo and Albertson, 1961), and pH was measured following Standard Methods (APHA, 2005). Supernatant samples were filtered through a 0.22 μm Teflon filter and VFA species were analyzed by gas chromatography (GC 7820 A Agilent Technologies) with a flame ionization detector (FID) and a capillary column 30 m × 0.25 mm Internal Diameter.

2.7. Statistical methods

Mean and standard deviation values from data were computed at stable conditions. The SPSS software version 15.0 was used to compare the means using one-way analysis of variance (ANOVA) and independent sample T-tests. Significant differences were shown at $p < 0.05$, while at $p > 0.05$, data are statistically the same.

3. Results and discussion

3.1. Substrate characteristics

The characteristic weight distribution of the substrates including ripe lady finger (LF) banana peel, rambutan waste and longan waste is presented in Table 1. The combined elemental composition (CHONS) of substrates was reasonably close to the VS content at the difference to VS of only 2.3% in LF banana peel, 3.6% in rambutan waste and 0.8% in longan waste. This may be contributed to the minor minerals not measured in this study, such as potassium, manganese, sodium, calcium, iron, etc. The VS of LF banana peel in this study, 88.1% of dry matter, is comparable to the value obtained for different types of banana peels in past research; 87%, 86.9% and 87% of dry matter by Bardiya et al. (1996), Gunaseelan (2004) and Pisutpaisal et al. (2014), respectively. This shows the similarity of the organic content of ripe banana peel across different consumable banana species. However, the variation in fibre composition mainly cellulose, hemicellulose and lignin persists in different genotypes, traceable to the slight genetic makeup of each variety. For example banana genotype AAA, Grande Naine, in ripe yellow stage contains higher hemicellulose (8.4%) than cellulose (7.5%) as reported by Happi Emaga et al. (2008) while Bardiya et al. (1996) reported higher cellulose (11.1%) than hemicellulose (5.4%) but these are in a lower range. LF banana peel in this study contains about 3 times higher hemicellulose than cellulose content (Table 1). The ladyfinger banana peel has thin and softer texture than most of the other types of banana peel such as Cavendish banana, Red Java banana, etc.

Rambutan and logan wastes comprise of the fruit peel and seed. VS in rambutan and longan wastes are 96.8% and 92.0% dry matter, respectively. The basic components of a typical plant cell are cellulose, hemicellulose, lignin, pectin, wax and cutin. The biodegradable contents of the plant cell wall are, however, mainly cellulose, hemicellulose and pectin (Taherzadeh and Karimi, 2008) although other components will be released to the degradation products. Since the other components of the cell wall were not investigated in this study except cellulose and hemicellulose, therefore conclusion cannot be made on their digestibility by only considering biomass characteristics. The halocellulose content, a combination of cellulose and hemicellulose, could however be used to indicate the tendency of substrate biodegradability. The banana peel in this study possessed higher percentage of halocellulose than rambutan and longan wastes, which coincides with the order of biogas production potential from the experimental results in the following section of this paper. In addition, C/N ratio in LF banana peel (23.1) and longan waste (29.5) are within the optimal range for anaerobic digestion of 20–30 while rambutan waste possesses rather high C/N of 44.2. If the rambutan waste were to be used as substrate in continuous anaerobic digestion system, nitrogen deficiency may result leading to an incomplete metabolism whereby the carbon content in the substrate is not efficiently degraded. Co-digestion of rambutan waste with other types of biodegradable materials having low C/N ratio, such as swine or chicken manure, may serve as a way to improve the nutrient balance and methane production.

3.2. BMP of lady-finger banana peel, rambutan waste and longan waste

In digestability test, the three fruit wastes were subjected to anaerobic digestion assessment of Biochemical Methane Potential (BMP) assay. Data shown in Fig. 1 are the mean values from three replicate experiemnts as previously described. Methane production started quickly after the commencement of the assay in all substrates tested. This could be a result of the mixed active inocula from 2 sources that gave a more diverse microbial community (Dechrugsa et al., 2013). The cumulative methane yield at day 30 obtained for chopped LF banana peel, ground LF banana peel, chopped longan waste and chopped rambutan waste were 268.3, 330.6, 234.6 and 193.2 $mLCH_4/gVS$, respectively. Results clearly showed that LF banana peel has the highest methane yield and evolution rate, and size of the substrate played a significant role in anaerobic digestion, a 23.2% increase in methane yield during 30-d digestion. Banana waste showed a promising biogas output with its great volume and less seasonal variation although size reduction of rambutan and longan wastes could also increase their biogas yield. Seeds of rambutan and mangosteen have reportedly high methane potential due to high fat content (Sanjaya et al., 2016) but the weight contribution is small and require higher energy for grinding. Moreover, the peel or skin of fruits always has the lowest methane potential compared to their pulp and seed, owing to the complexity of the cellulose and hemicellulose structure in this protective layer, particularly the phenolic components within rambutan peel (Thitilertdecha et al., 2008). It is noticeable that higher VS percentage of rambutan and longan wastes compared to LF banana peel did not always translate to the higher biogas production. Direct measurement of biogas yield by BMP assay is proven to be a crucial prerequisite in AD project design and appraisal.

The methane yield obtained from ground LF banana peel in this study was higher than that for different varieties of ground ripe banana peels published by Gunaseelan (2004) and maximum methane yield for ground banana peel reported by Pisutpaisal et al. (2014) as shown in Table 2. The rate of methane and biogas production was also faster for LF banana peel in this study than

Table 1
Characteristics of substrates used in this study.

Parameters	Unit	Banana waste (peel)	Rambutan waste (peel + seed)	Longan waste (peel + seed)
Moisture	% fresh wt.	82.3	69.8	34.5
Volatile solids	% dry wt.	88.1	96.8	92.0
Fixed solids	% dry wt.	11.9	3.2	8.0
Carbon	% dry wt.	39.2	48.6	44.3
Hydrogen	% dry wt.	5.3	5.9	5.5
Nitrogen	% dry wt.	1.7	1.1	1.5
Sulphur	% dry wt.	0.1	0.1	0.2
Oxygen	% dry wt.	39.6	37.6	39.7
Sum CHNS-O	% dry wt.	85.8	93.2	91.2
Cellulose	% dry wt.	13.5	9.8	17.8
Hemicellulose	% dry wt.	39.7	10.0	24.2
C/N ratio	–	23.1	44.2	29.5

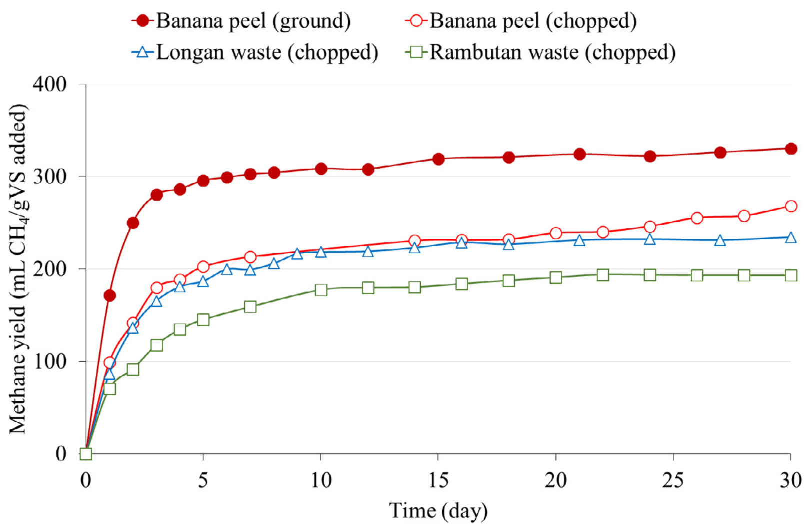

Fig. 1. Cumulative methane yields of chopped LF banana peel, ground LF banana peel, rambutan waste (peel + seed) and logan waste (peel + seed) in BMP assay.

Table 2
Comparison of past researches investigating the methane yield of agricultural wastes using batch fermentation.

	Materials	CH_4 yield (L CH_4/g VS added)	References
1	Stalk of grape bunch (ground)	0.180	Gunaseelan (2004)
2	Fresh orange peel (ground)	0.072 ± 0.033	Sanjaya et al. (2016)
3	Fresh banana peel (ground)	0.342 ± 0.055	Sanjaya et al. (2016)
4	Pine apple peels (ground)	0.360	Gunaseelan (2004)
5	Fresh rambutan (ground)	0.203 ± 0.041	Sanjaya et al. (2016)
6	Banana peels (chopped/ground)	0.190/ 0.200	Bardiya et al. (1996)
7	Banana peels varieties (ground)	0.243–0.322	Gunaseelan (2004)
8	Banana peels (chopped)	0.077	Pisutpaisal et al. (2014)
9	LF banana peel (chopped/ground)	0.268/0.331	This study
10	Rambutan waste (chopped)	0.193	This study
11	Longan waste (chopped)	0.235	This study

for the different varieties reported by Gunaseelan (2004). The variation in genotype and thus biochemical composition of the peel dictate their digestibilty and this finding supports the possible utilization of LF banana peel as feedstock to anaerobic digester in Thailand and other tropical countries. Size reduction by grinding LF banana peel increased the methane yield and the rate of methane production (slope of methane evolution) as shown in Fig. 1 and Table 3. This mechanical rupturing of biomass cell was capable of deforming β-glucosidic linkages in cellulose and hemicellulose (Gañán et al., 2004). Over 80% of the cumulative volume of methane production at day 30 was attained at only day 3 in ground banana peel in comparison with chopped banana peel at day 14 as shown in Table 3. At the end of a 30-day BMP assay, pH was in the range of 7.4–8.1, total alkalinity was in the range of 3500–4500 mg/L as $CaCO_3$, and the ratio of total VFA/total alkalinity was lower than 0.1 for all the substrates. These are within the optimum range for methane production (Pisutpaisal et al., 2014) and justify the balance in BMP assay performed.

3.3. Theoretical methane potential (TMP) and biodegradability of substrates

Generally, there will be a portion of plant cells that would not be converted biologically under the test condition, or in any condition. The degree of conversion in any particular pathway depends on various parameters and can be represented by the term "biodegradability" under a defined condition (i.e. pretreatment, temperature, etc.). In this work, to estimate the biodegradability of the fruit wastes, theoretical methane potential was calculated from the elemental composition CHNS-O using the modified equation of Buswell and Mueller (1952). The stoichiometric equations generated for each substrate using CHNS-O elemental composition are stated in Eqs. (5)–(7). This method assumes that all of the VS in the substrate is 100% converted. It should be noted that fixed solids content in the substrate was already accounted for in our calculations. Thus, the theoretical methane yield of LF banana peel, rambutan and longan wastes are 364.1, 477.5, and 417.5 L CH_4/kg

Table 3
Milestone of the ratio of cumulative methane yield attained to the ultimate methane yield in BMP at day 30.

CH_4 yield ratio against 30-day BMP	Duration (in days)			
	LF Banana peel (ground)	LF Banana peel (chopped)	Rambutan waste (chopped)	Longan waste (chopped)
>0.5	1	2	3	2
>0.6	2	3	3	3
>0.7	2	5	5	3
>0.8	3	14	7	6
>0.9	6	24	10	9

dry substrate, or the equivalence of 413.2, 493.1, and 453.7 L CH_4/kg VS, respectively.

LF banana peel:

$$C_{1055}H_{1704}O_{801}N_{38}S + 257.9H_2O \rightarrow 526.0CH_4 + 529.4CO_2 + 38.1NH_3 + H_2S \quad (5)$$

Rambutan waste:

$$C_{954}H_{1368}O_{553}N_{18}S + 349.7H_2O \rightarrow 502.7CH_4 + 451.6CO_2 + 18.3NH_3 + H_2S \quad (6)$$

Longan waste:

$$C_{619}H_{918}O_{417}N_{18}S + 194.8H_2O \rightarrow 312.9CH_4 + 305.8CO_2 + 17.9NH_3 + H_2S \quad (7)$$

Biodegradability was computed as the ratio of total cumulative methane yield (in L CH_4/g VS) of BMP assay at 30 days to the theoretical methane yield (in L CH_4/g VS) expressed in percentage as shown in Table 4. The biodegradability of LF banana peel increased from 65.4% for the chopped peel to 80.5% for the ground peel. Size reduction had an immense positive impact on the methane production rate and yield. Nevertheless, the uptrend of the biogas production of the chopped banana peel appeared toward the end of the 30 days, which should ultimately approached the same level as the ground peel's. Meanwhile, the biodegradability of longan waste and rambutan waste are only 52.0% and 39.4%, respectively. Higher halocellulose, particularly hemicellulose which are more biodegradable, was a unique property of LF banana peel beside its soft texture and more balance nutrients.

A big gap between the theoretical and BMP assay yields suggests that more biogas may be produced from the rambutan and longan wastes if pre-treatment techniques were employed. Rambutan waste has the highest theoretical methane yield of 0.49 L CH_4/g VS, followed by longan waste having 0.45 L CH_4/g VS and then lady-finger banana peel having 0.41 L CH_4/g VS. This is remarkably in reverse order to the BMP results. In terms of energy, it is prudent to estimate the energy yield from the wastes in this experiment. Table 4 also shows higher heating value (HHV) of the biogas derived from theoretical and BMP results in comparison with the heating content in the biomass by the modified Dulong's equation (Eq. (3)). It is noted that Dulong's Eq. is typically used to estimate the heating value through combustion of the solid fuels. Here, the Dulong's energy release estimation represents ultimate conversion of our fruit wastes to energy by complete oxidation.

It was obvious in Table 4 that HHV values from the modified Dulong's equation are in agreement with those from stoichiometric calculation (TMP). Energy derived through anaerobic biomethanation could accomplish only around the biodegradability level (%) and the rest of the energy to be released remained in the digestate (digested solids). Due to its wetness of the digestate, it becomes impractical to combust it directly. Drying methods, such as bio-drying, sun-drying, or other thermal drying techniques is required, which increase complexity or the process. Field application as solid amendment or fertilizer in agriculture is generally practiced (Al Seadi et al., 2013).

3.4. Cost implication and benefits of anaerobic digestion of fruit wastes

The value of selling the biogas produced from the LF banana peel can be estimated for liquid petroleum gas (LPG) and electricity as shown in Eqs. (8) and (9):

Case 1: Biogas produced replaces LPG

$$\text{Value} = (0.45\ \text{kg LPG/m}^3\text{biogas}) \times (24.16\ \text{Baht/kg LPG}) = 10.9\ \text{baht/m}^3\text{biogas} \quad (8)$$

Case 2: Biogas produced generates electricity

$$\text{Value} = (2.0\ \text{kWh/m}^3\text{biogas}) \times (4.2\ \text{baht/kW h}) = 8.4\ \text{baht/m}^3\text{biogas} \quad (9)$$

Assumptions: energy equivalent of biogas at 60% CH_4 is 0.45 kg LPG; current price of LPG in Thailand is 24.16 baht/kg; 1 m^3 of biogas at 60% CH_4 is required to produce 2 kWh of electricity through standard typical biogas engine; and the price of electricity in Thailand is 4.2 baht/kWh. Currency exchange rate is 35.0 Thai baht per US Dollar. Moreover we can also predict the cost of biogas production based on biogas yield from ground lady-finger banana peel in BMP assay as shown in Eq. (10):

$$\text{Cost} = (\text{A baht/kg substrate})/(0.051\ \text{m}^3\text{CH}_4\text{/kg fresh}) \quad (10)$$

where A is total cost (procurement + transportation + O&M) per kg substrate, and 0.051 is the CH_4 yield from BMP assay in m^3 of methane per kg fresh banana peel (0.330 L CH_4/gVS, VS/TS = 0.881, moisture = 82.3%). It should be noted that the CH_4 yield in Eq. (10) must be replaced with the value from the yield from continuous system experiment in order to more realistically appraise the cost of methane production.

$$\text{If}\quad \begin{aligned} &\text{Cost} < \text{Value Positive return for the investment of the biogas system}\\ &\text{Cost} = \text{Value Break-even}\\ &\text{Cost} > \text{Value Negative return or loss}\end{aligned}$$

Commercial biogas plant is typically run on the positive return and minimization of cost can be done through finding cheap substrate (smallest A) either procurement, transportation and O&M, or high biogas yield substrates or multi substrates. In depth analysis needs to be undertaken for seasonal fruit wastes as their supplies could vary over time in a year, where co-digestion system capable of accepting multiple wastes is probably required. Fortu-

Table 4
Methanation potential and energy derivation from the studied substrates.

Substrate	TMP yield	BMP yield	Biodegradability	Energy content (kJ/kg)		
	(LCH4/gVS)	(LCH4/gVS)	(%)	Dulong's Eq. (LHV/HHV)	TMP (HHV)	BMP (HHV)
LF banana peel (ground)	0.413	0.330	80.5	16,631/17,954	16,520	13,200
LF banana peel (chopped)	0.413	0.268	65.4	16,631/17,954	16,520	10,720
Longan waste (chopped)	0.454	0.234	52.0	18,216/19,531	18,160	9360
Rambutan waste (chopped)	0.493	0.193	39.4	19,591/20,932	19,720	7720

TMP = theoretical methane potential.
BMP = biochemical methane potential.
LHV = lower heating value.
HHV = higher heating value.
Biodegradability = (BMP/TMP) × 100%.

nately, banana is a year round plant. Therefore, it was chosen as a substrate to run the continuous digestion system.

3.5. Performance of single stage versus two stage anaerobic digestion of banana peel

3.5.1. pH

Two continuous digester systems were run side by side over varied total solid (TS) concentration. Both mesophilic reactors, mesophilic single stage (designated as MS) and mesophilic second stage (designated as M2) were run at equal HRT of 20 days. The small pretreatment unit of thermophilic reactor (designated as T1) was placed in front of M2 in order to observe its effects in terms of biogas production and system stability. At 1%TS feed concentration, pH in MS reactor and M2 reactor were stable at around 6.6, and in T1 reactor, pH was rather stable around 4.4 as shown in Fig. 2. The rapid pH drops in the T1 reactor at the initial operation (not shown on the graph) was a result of fermentation intermediates buildup as volatile fatty acids (VFAs). At feed concentration of 2% TS, pH in the M2 reactor could still stabilized near neutral, around 6.8 while pH in T1 reactor narrowly fluctuated in a range of 4.3–4.6. However, pH in MS reactor dropped significantly during 2% TS feed. This resulted from accumulation of VFAs at that loading. Addition of alkaline solution (NaOH 1 M) for pH adjustment was administered to MS reactor starting from day 88 to bring pH value within the range of 6.8–7.0 as indicated by arrows in Fig. 2. Despite the addition of alkaline solution, further pH drops continued. Biogas production was consequently affected during this feeding level (day 70 to day 103), with significant reduction in biogas production rate (442–71 mL/d) and methane content (50.4–4.5%). For the purpose of system and energy recovery, the feed concentration was decreased to the third feed concentration of 1.5%TS. Again, pH in T1 and M2 reactors did not seem to be affected at this level. Slow recovery of pH in MS reactor was marked with only one time pH adjustment at day 115 and became stable around 6.5–6.7, after 40 days of operation at this loading. Low pH was self-maintained in T1 reactor across all three feed concentrations tested due to the accumulated VFA from intense hydrolysis and acidogenesis reactions taking place. The T1 reactor was operated at a short HRT of only 4 days and 55 °C aiming to only pre-treat the substrate by facilitating the hydrolysis of LF banana peel. Methanogens are not expected to be active in the acidic environment of T1 reactor. The organic acids produced and a more crumbled solid substrate were transferred to the second reactor, making it easier to digest and methanogenesis can be enhanced.

3.5.2. Alkalinity and VFA accumulation

Alkalinity and total volatile fatty acid (TVFA) trends are shown in Fig. 3a and b. It should be realized that although the range of solid loading or OLR used in this study is not considered high compared to other solid digestion studies, the fast degradation nature of this banana peel, represented by the fast evolution of methane in BMP assay (Fig. 1 and Table 2), could release organic content available to the culture medium quickly. In contrast, if the substrate is hard to degrade, even at high OLR, the system would not be overloaded. At feed concentration 1% TS (OLR of MS 0.42 kg/m^3·d), alkalinity in the MS reactor stabilized around 1000 mg/L as $CaCO_3$, and VFA of 135 mg/L as CH_3COOH was present within the reactor, giving TVFA/ALK of 0.14. Similarly, in the M2 reactor, alkalinity and VFA stabilized around 1200 mg/L as $CaCO_3$ and 135 mg/L as CH_3COOH, TVFA/ALK 0.10 as shown in Fig. 3. It was noted that M2 reactor received OLR of only 0.36 kg/m^3.d. Low alkalinity existed in T1 reactor as a result of high concentration of organic acids, yielding high TVFA/ALK of 3.5 at feed concentration of 1% TS (OLR of T1 2.11 kg/m^3.d). At 2% TS (OLR of MS 0.81 kg/m^3·d), alkalinity in MS reactor increased to 1350 mg/L but with high VFAs of up to 1400 mg/L pushing TVFA/ALK above 0.8 which is a threshold of methanogenic system failure (Khanal, 2008) and showing drastic pH drop. Meanwhile, in M2 reactor at feed concentration of 2% TS (OLR of M2 0.66 kg/m^3·d), TVFA/ALK ratio was still below 0.4, which was an upper recommended level for stable methanogenic digester (Khanal, 2008). In T1 reactor at feed concentration of 2% TS (OLR of T1 4.03 kg/m^3.d), TVFA/ALK remained well above 2.5. It was interesting to observe the decline of TVFA/ALK in MS to below 0.4 after switching from 2% to 1.5% TS feed concentration where MS reactor received OLR at 0.6 kg/m^3.d. VFA accumulation reduced with time as the excess VFAs were utilized for biogas production sending TVFA/ALK to below 0.4 signifying a higher rate of VFA degradation and stability of the system (Fernández-Rodríguez et al., 2016). This low OLR bearing capacity found in our study has an implication on the economics of full scale implementation in banana peel mono-digestion. At low OLR, the biogas output is also small, making the return of investment longer or even becoming an unfeasible option to treat banana waste. Co-digestion with other agricultural wastes available in the area could be further researched.

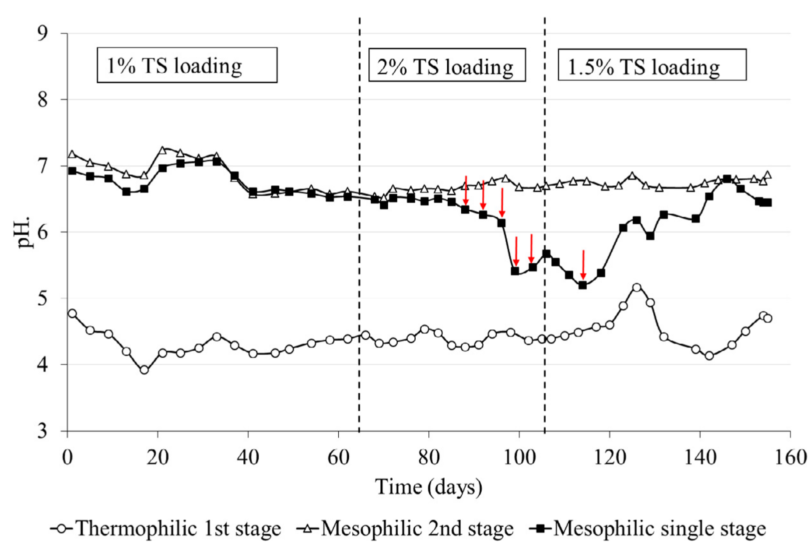

Fig. 2. pH of effluents from single stage and two stage digester system at different feed concentrations.

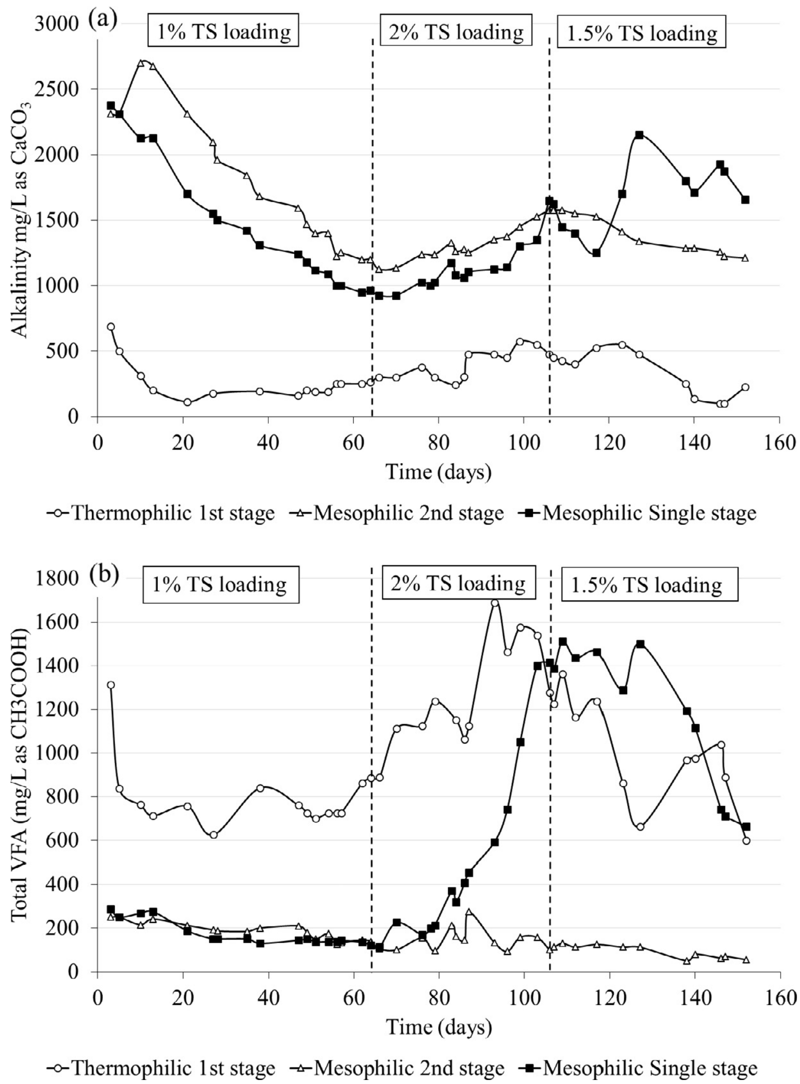

Fig. 3. (a) Alkalinity and (b) total VFA of effluents from single stage and two stage digester systems at different feed concentrations.

Nevertheless, this phenomena clearly showed that adding a pretreating stage to the methanogenic digester (T1-M2) could provide stability and process efficiency to the methanogenic digester. For longan and rambutant wastes with lesser degradation speed (Table 3) and methane potential (Fig. 1), single stage digestion could bear higher organic loading compared to ground banana peel case. However, if pretreated sufficiently, they would become more rapidly biodegradable and the necessity of two stage configuration shall be obvious. It has long been known that pH is a major factor influencing the pathways of fermentation, and the pH range of 4–6 derived in T1 reactor had prompted the production of hydrogen (Fernández-Rodríguez et al., 2015; Riau et al., 2010) which will be discussed in Section 3.5.3.

In this study, acetic acid and butyric acid were the main VFA species produced in the T1 reactor, and isovaleric acid and valeric acid were detected only at times not regularly. Acetic and propionic acids were the only VFAs detected in the M2 effluent. This was similar to the findings of Riau et al. (2010) whereby acetate and propionate only were found in the mesophilic second stage effluent in a temperature phased anaerobic digestion (thermophilic versus mesophilic) of sludge from wastewater treatment plant. In the MS reactor at 1% TS feed concentration, acetic and propionic acids were the only VFA species, but at 2% TS when VFAs build up within the reactor, acetic, propionic, butyric, iso-valeric and valeric acids were detected.

3.5.3. Biogas production

Fig. 4 shows the total biogas production across the three feed concentrations in the mesophilic single stage and the combined two-stage digestion systems. At 1% TS feed, composition of the biogas from bioreactors was not measured. During 2% TS feed, biogas production from MS reactor started to drop at around day 71 corresponding to an increase in VFA concentration (Fig. 3a) despite stable pH (Fig. 2). At day 85, pH started to decline as insufficient alkalinity was available to counter the VFA production. Multiple pH adjustments each time to around 7.0 was performed only to see no sign of recovery. Methane content dropped from 50.4% to around 4.5% during this period. The average methane yield during the 2% TS feed was 17.14 L CH_4/kgVS added in MS reactor. Although the VS destroyed increased in the MS reactor, it was accumulated in the form of VFAs, not converted to biogas, and this had an inhibitory effect on the reactor performance. System recovery occurred at feed concentration of 1.5% TS feed and methane percentage varied within the range of 51.5–57.3% with an average of 53.2%. Biogas production was higher at 1.5% TS (254.8 mL/d) than at 2% TS (179.2 mL/d) in the MS reactor as shown in Fig. 4.

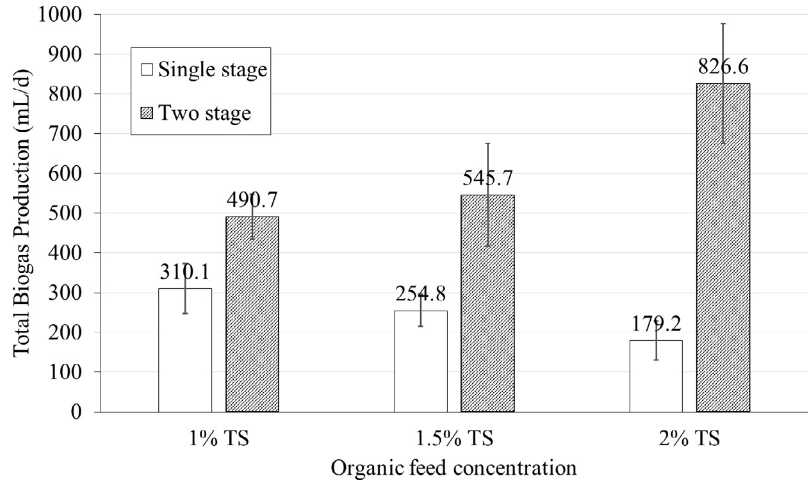

Fig. 4. Comparison of total biogas production in single stage and two stage digester systems.

A more balanced VFA production and consumption was restored. At stable condition of 1.5%TS feed, the average methane yield in MS reactor was 46.0 L CH_4/kgVS added.

Statistically, the overall biogas production in the two-stage digestion was higher than the biogas production in the single stage digestion across the three feed concentrations as shown in Fig. 4. This makes certain that the thermophilic first stage (T1) reactor provided hydrolysis and acidification of the feedstock effectively and actually helped cut down some organic load to the second stage by converting it to gaseous products. As a result, the biogas from T1 composed of H_2 and CO_2 as typical in the hydrolytic-acidogenic phases, and CH_4 was only detected occasionally and in very small concentrations, i.e. less than 1.5%. Methane yield was insignificant and therefore not determined in T1 reactor. High loading in anaerobic digester has potentially placed the system to the inhibitory environment for methonogenesis, particularly those operated at high temperature where more solids could be quickly hydrolysed and acidified such as organic fraction of municipal solid wastes (Fernández-Rodríguez et al., 2015). The biogas production from single stage digestion was the same statistically at 1% TS and 1.5% TS feed, but statistically lower at 2% TS feed. On the other hand, the overall biogas production of the two-stage digestion was statistically the same at 1% TS and 1.5% TS feed concentration and statistically higher at 2% TS feed concentration at $\alpha = 0.05$.

In two stage system, the hydrogen yield in T1 reactor at 1.5% and 2% TS feed was 17.5 and 15.2 L H_2/kg VS, at a corresponding hydrogen content of 33.0% and 33.4%, respectively. Methane production in M2 reactor increased as the VS destruction (the difference in the VS concentration of feed and effluent) increased as shown in Fig. 5 and Table 5. This clearly showed higher conversion of VS to biogas generation. The average methane yields in M2 reactor during feed concentrations of 1.5% and 2% TS were 43.3 and 70.3 L CH_4/ kg VS corresponding to 48.8% and 61.3% VS destroyed, respectively. In addition, the biogas yield in the two-stage digestion across the three feeding concentration is greater than the biogas yield in the mesophilic single stage (Table 5). The overall VS destroyed in the two-stage digestion was also higher than the VS destroyed in mesophilic single stage at the three organic loadings (Table 5). This is consistent with other work where the preceding thermophilic reactor in the two stage configuration, also known as temperature phased anaerobic digestion (TPAD) system, could improve solid substrate digestion, for instance at least 1.38 and 1.60 folds in sewage sludge and sugar beet pulp co-digestion compared to single stage thermophilic and single stage mesophilic, respectively (Montañés Alonso et al., 2016).

3.5.4. Energy yields

Energy yields obtained from the methane and hydrogen production per kilogram of VS was shown in Table 5. The densities of hydrogen and methane used in calculations were 0.09 kg/m^3 and 0.72 kg/m^3, respectively. The gravimetric heating values of hydrogen and methane used in calculations were 142 kJ/g and

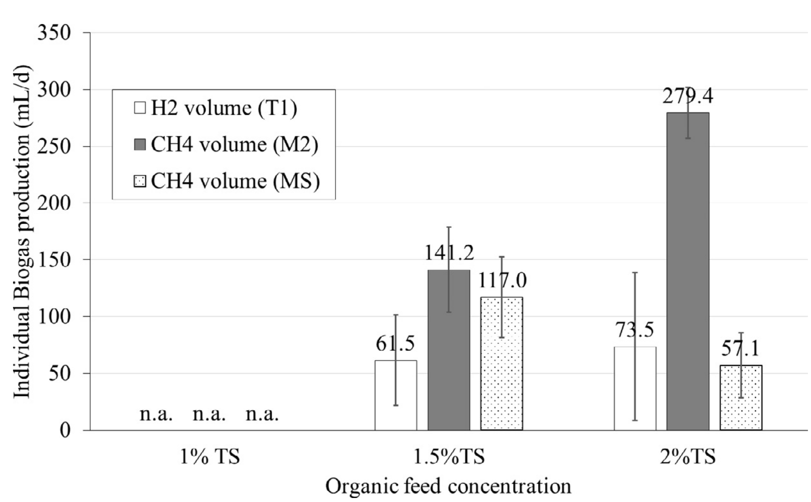

Fig. 5. Individual biogas production and composition in continuous anaerobic digesters.

Table 5
Volatile solids destroyed, biogas composition, and total energy yields in the continuous AD systems at steady states.

Reactors		Feed (% TS)	OLR (kg VS/m^3·d)	VS destroyed (%)	Avg. CH_4 (%)	Avg. H_2 (%)	Biogas yield (L/kg VS)	Energy yields (kJ/kg VS)
Mesophilic single stage (MS)		1.0	0.41	40.4	n.a.	n.a.	160.3	n.a.
		1.5	0.62	41.0	49.6	n.d.	91.2	1840.9
		2.0	0.83	53.8	33.3	n.d.	49.0	526.2
Two stage	Thermophilic prehydrolysis (T1)	1.0	2.07	19.2	n.a.	n.a.	47.4	n.a.
		1.5	3.11	22.2	<1.5%	33.0	44.7	1827.7[c]
		2.0	4.14	18.6	n.d.	33.4	42.4	2510.9[c]
	Mesophilic (M2)	1.0	0.35	24.0 (42.9[a])	n.a.	n.a.	165.1 (192.9[b])	
		1.5	0.54	48.8 (58.4[a])	36.1	n.d.	118.8 (151.2[b])	
		2.0	0.66	61.3 (68.5[a])	48.0	n.d.	147.7 (171.1[b])	

n.d. = not detected.
n.a. = not available.
[a] Overall VS destroyed calculated from influent to thermophilic first stage (T1) reactor and effluent from mesophilic second stage (M2) reactor.
[b] Overall biogas yield from T1 and M2 reactors.
[c] Overall energy yield from T1 and M2 reactors.

55.6 kJ/g, corresponding to 12.78 kJ/L and 40.0 kJ/L volumetric values, respectively (Zhu et al., 2008). At 1.5% TS feed, energy yields obtained from the single stage and the combined two-stage digestion systems are about the same level (Table 5), however, the overall energy yield in the combined two-stage digestion increased as the feed concentration increased. In contrast, the energy yield in mesophilic single stage decreased with increase in feed concentration. This suggests that at increased organic loading, the energy yields obtained from the two-stage system should be higher than the energy yield from mesophilic single stage and the gap will widen. Unfortunately, the biogas composition at 1% TS feed was not available but it should be inferred that the energy yield shall correspond to the degree of solid destruction, which there would be no significant difference in digestion efficiency at low OLR. Therefore, energy yield from both configurations should be quite similar at 1% TS feed. At high feed concentrations or high OLR, the pre-hydrolysis reactor will provide benefits of an efficient and sustained system stability as well as higher energy recovery. However, it must be noted that there is a positive influence of longer HRT when adding a thermophilic prehydrolysis tank to the mesophilic tank to form a two stage configuration. Further research on the effects and optimization of HRT of single stage versus two stage configurations at equal HRT especially for the highly degradable waste should give an insight on the practical design of the particular anaerobic digestion system.

4. Conclusion

Fruit wastes, banana peel, rambutan and longan wastes, were stabilized using anaerobic digestion where biogas and digestate can be utilized. Physical pretreatment, size reduction increased the biodegradability and methane yield/rate of banana peel in batch fermentation. The pre-hydrolysis thermophilic reactor as biological pretreatment helped stabilize the digestion process in ensuing mesophilic digester, particularly at high-solid feedstock. The overall VS destruction, biogas production and energy yield derived from the two-stage digestion were higher than in the single stage. In tandem, hydrogen, a more ideal energy source, was obtained from the pre-hydrolysis reactor, serving as an added advantage of the two-stage digestion.

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