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CHAPTER 11

BIOGAS PRODUCTION FROM LIGNOCELLULOSIC WASTE MATERIALS

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Introduction

Sustainable energy supply to industries and for domestic use is one of the major concerns of the world today. Many countries of the world are combating challenges of sustainable energy production. The world is heavily reliant on fossil fuel as the major source of energy and since this source is nonrenewable, other energy sources which are sustainable need to be explored to the full. Many nations are in search for a more reliable and sustainable source of energy and past researches have indicated that the use of biomass (a renewable source of energy) can provide a lasting solution to the energy problems of many nations sustainably.

Lady-finger banana is a type of small-size species banana cultivated in Thailand, Nigeria, and other tropical countries. Several factories in Southern Thailand produces banana snacks and packaged food made from these bananas, thereby generating several quantities of lady-finger banana peels, which can be used as raw materials or substrates in anaerobic digestion. Rambutan, longan, and longkong are also tropical fruits widely grown in Thailand and they are usually harvested in very large quantities during the peak of their seasons. During such periods, these fruits are sold at give-away prices due to surplus, some are even left to rot on the trees and most over ripe ones end up being dumped as organic wastes. These wastes release noxious gases, harmful to living organisms in the environment. These organic wastes can be stabilized and treated by anaerobic digestion and converted to useful products including methane gas. In addition, the burning of fossil fuels releases toxic gases into the atmosphere and causes environmental hazards (Haszeldine, 2009). These contributes to reasons why many countries are now in search for a cleaner and renewable source of energy, and are shifting towards the use of biomass (Tock et al., 2010).

Biogas (methane) is one of the most promising sources of energy that can be used as a substitute for fossil fuel in the energy sector. It is derived as an end product of the final phase of anaerobic digestion of organic biomass. It is produced by acetoclastic and hydrogenotrophic methane producing bacteria. Acetoclastic bacteria split acetate into methane and carbon dioxide, and hydrogenotrophic bacteria use hydrogen as electron donor and carbon dioxide as acceptor to produce methane (Nayono, 2009). The aim of this paper is to reveal the biochemical and theoretical methane potentials of wastes derived from selected tropical fruits from Thailand.

Materials and Methods

Inoculum

Anaerobic sludge from a concentrated rubber latex industry and a commercial pig farm both in Songkhla province of Thailand was collected separately and mixed at a 1:1 ratio based on total solid (TS) contents for inoculation.

Materials

Fresh and ripe lady-finger banana, rambutan, longan and longkong fruits were purchased from the fruit market in Hat Yai, Thailand. The fruit peels were carefully removed and chopped to a particle size of approximately 5mm and lesser. Also, Napier grass was harvested from a nearby farm and cut into small sizes of about 5mm.

The physical properties of each sample were determined and the elemental composition analysis was done. All samples were prepared and kept at 4 ^{0}C until use.

Biochemical methane potential assay

The agricultural produce was fermented using Biochemical Methane Potential (BMP) assay. 120 mL serum bottle with an effective volume of 60 mL was used for BMP assay in four replicates for each of the sample. pH (potential hydrogen) was controlled to fall within the optimum range for anaerobic fermentation (6.8-7.2). Inoculum to substrate ratio was fixed at 3 based on total solids (TS). Experiment was carried out in an incubator shaker operated at mesophilic temperature of 35 ^{0}C. 0.3 g TS of biomass was added in 60 mL working volume which was attained by addition of inoculum, nutrients, trace elements, buffer solution and distilled water.

Biogas quantity and composition was measured periodically using gas chromatography, GC 7820 valve system, Agilent technologies equipped with thermal conductivity detector (TCD) and helium as carrier gas. The temperature of injector port and detector were 50 and 100^{o}C respectively.

At the end of 60 days of BMP assay, the pH, alkalinity and volatile fatty acids of effluents from the serum bottles were investigated.

Pretreatment

Physical pretreatment (size reduction) was carried out for lady-finger banana peel by blending the peels and comparison was made between chopped and blended banana peel.

Thermal or heat pretreatment by boiling was also carried out for blended lady-finger banana peel (about less than 1mm sizes). Four pre-treated samples of the blended lady-finger banana peel was derived. The samples were heated to boiling point using electrical hot plate and separated by the duration of boiling; 5 minutes, 10 minutes, 15 minutes and 20 minutes.

Kinetics of Methane Production

The modified Gompertz equation (Eq. 1 below) was used to calculate the kinetics of methane production from the graph of cumulative methane production (mL) versus time in days. Microsoft Excel datasheet version 2013 was used in plotting the graphs and to fit the data with the modified Gompertz equation. Methane production potential (H_{max}), methane production rate (Rm) and lag phase (λ) were determined.

$$H = H_{max} \times exp\{- exp\left[\frac{R_m e}{H_{max}}(\lambda - 1) + 1\right]\} \qquad \text{Eq. 1}$$

where H is the cumulative volume of methane production (mL), H_{max} is the methane production potential (mL), R_m is the maximum production rate (mL/day), t is the time of fermentation (day), λ is the time of lag phase (day) and e is a constant (2.71828).

Analytical Methods

Total solids (TS) and volatile solids (VS) were determined using standard methods for the examination of water and wastewater. Elemental composition (carbon, hydrogen, nitrogen, oxygen, and sulphur) was determined using dynamic flash combustion technique. Cellulose, hemicellulose and lignin contents were determined using detergent fiber technique based on Van Soest et al., (1991). Alkalinity, pH & total volatile fatty acids (VFAs) analyses was carried out using direct titration methods.

The theoretical methane potential was calculated from element composition analysis using modified Buswell stoichiometric equation (Lubken et al., 2010; Park & Li, 2012). The proportion of methane in biogas can be used to evaluate the state of methanogenic biocoenosis. Theoretical methane potential (TMP) was used to investigate the maximum theoretical methane production by the complete degradation of each sample. TMP was calculated from the CHNS-O elemental compositions using the modified Buswell and Mueller (1952) equation according to Boyle who included nitrogen and sulfur to obtain the fraction of ammonia and hydrogen sulfide in the produced biogas as shown in Eq. 2 (Buswell and Mueller, 1952).

$$C_c H_h O_o N_n S_s + 0.250\,(4c-h-2o+3n+2s)\,H_2O \rightarrow 0.125\,(4c+h-2o-3n-2s)\,CH_4 + 0.125\,(4c-h+2o+3n+2s)\,CO_2 + nNH_3 + sH_2S \quad \text{Eq. 2}$$

where c, h, n, s, and o are the moles of Carbon, Hydrogen, Nitrogen, Sulphur and Oxygen, respectively, and the assumption of s=1 was used in calculation of TMP. The theoretical methane yield is reported in litres of methane per gram VS added. The experimental data were calculated and analyzed using Microsoft excel 2013.

Results and Discussion

Material characteristics

The anaerobic digester sludge used for inoculation had pH within the range of 7.5-8.5. The pig farm anaerobic sludge has 84,330 mg/L TS, 51,040 mg/L VS, while the rubber latex digester sludge has 47,500 mg/L TS, 38,360 mg/L VS. As revealed from the solid contents and in addition to physical observations, the rubber latex digester sludge contains more soluble organics and is more watery than the pig farm digester sludge which contains more particulate organic matter and is thicker.

The physical and chemical characteristics of all the materials used as substrates in this research were investigated to include the moisture contents (MC), total solids (TS), volatile solids (VS), fixed solids (FS), elemental compositions (CHNS-O), cellulose, hemicellulose and lignin contents, and shown in Table 1. The percentages of moisture content and total solid content (TS) of all the substrates added up to 100%. It was observed that the VS of the substrates is mainly made up of carbon, hydrogen, oxygen, nitrogen and sulfur contents (CHNS-O), this is shown by summing up the CHNS-O elemental composition to a total value which was almost equal to the VS content of the substrates as shown in Table 1. The remaining percentage of VS not accounted for by the CHNS-O elemental composition could be the value for minority mineral contents consisting of potassium, manganese, sodium, calcium and iron which were not investigated in this research.

Cellulose and hemicellulose contents are higher than lignin content in all the substrates, indicating that the substrates have a high percentage of biodegradable organic matter. However, this statement needs further verification through research because cellulose comprises partly of crystalline and partly of amorphous morphology, and therefore undergoes partial hydrolysis as compared with hemicellulose containing amorphous structure which is easily hydrolyzed. More research findings should be carried out to further investigate the effect of crystallinity on the hydrolysis of biomass. Cellulose, hemicellulose and lignin contents for all the substrates considered in this research did not sum up to 100%, however, the remaining percentage may account for pectin content present in the middle lamella of the cell walls of the substrate materials which was not investigated in this study. Substrates having high cellulose contents for example Napier grass (as shown in Table 1) may require pre-treatment to disrupt the crystalline structure and aid the efficiency of enzymatic hydrolysis and acidification in anaerobic digestion. The carbon to nitrogen ratio (C/N) for all the substrates is above 20:1 as shown in Table 1.

Lady-finger banana peel was further pretreated using physical pretreatment and thermal pretreatment because the fruit is usually available all year round unlike rambutan, longan, and longkong. Napier grass often serves as food for grazing animals such as cattle, goat, sheep, and so on, thus has a variety of uses and may not be categorized as a waste.

Table 1: Substrate Characteristics

Parameters of Analysis	**Lady-finger banana peel**	**Rambutan peel and seed**	**Longan peel and seed**	**Longkong peel and seed**	**Napier grass**
MC (% wet)	82.3	69.8	34.5	79.1	80.8
TS (g/kg wet)	177.0	302.2	655.4	209.2	191.9
VS (g/kg wet)	155.9	292.6	603.0	191.1	174.8
VS (% TS)	88.1	96.8	92.0	91.3	91.1
Cellulose (%)	13.5	13.4	0.4	17.1	44.5
Hemicellulose (%)	39.7	10.0	1.1	14.7	24.9
Lignin (%)	18.6	5.8	0.0	17.2	13.2
C (%w/w dry)	39.2	48.6	44.3	46.4	41.8
H (%w/w dry)	5.3	5.9	5.5	6.0	5.3
O (%w/w dry)	39.6	37.6	39.7	36.8	28.4
N (%w/w dry)	1.6	1.1	1.5	1.6	1.5
S (%w/w dry)	0.1	0.1	0.2	0.2	0.1
Sum CHONS (%)	85.8	93.2	91.2	90.9	77.2
C/N ratio	23.7	44.6	29.7	28.6	27.8

Theoretical biogas production

The theoretical methane yield in L CH4/g VS of all the substrates are shown in Fig. 1. From Fig. 1, longkong waste and rambutan waste have the highest theoretical methane potential, followed by Napier grass, longan peel with seed and lady-finger banana peel. Apart from Napier grass which mainly serve as food for herbivorous animals like cow, horses, goats, etc., all the other substrates considered in this research wastes derived from the consumption of the fruits. These wastes possess biodegradable properties and can be used for biogas production. Also, rambutan, longan and longkong are very seasonal and are not available all year round but can only be found in their seasons unlike the other substrates (lady-finger banana and Napier grass). Napier grass is also considered as an energy crop, having short growth time of 2 to 3 months to maturity and possesses high theoretical methane production potential of > 0.46 L CH_4 per g of volatile solids.

The theoretical ammonia content of lady-finger banana peel is not inhibitory to the anaerobic digestion process because the carbon to nitrogen (C/N) ratio is 23.7 which falls within the optimum condition for anaerobic digestion. The stoichiometric equation derived for each substrate is as follows:

Lady-finger banana peel

$C_{1055}H_{1704}O_{801}N_{38}S_1 + 258H_2O \rightarrow 526CH_4 + 529CO_2 + 38NH_3 + H_2S$ Eq. 3

Rambutan wastes

$C_{954}H_{1368}O_{553}N_{18}S_1 + 350H_2O \rightarrow 503CH_4 + 452CO_2 + 18NH_3 + H_2S$ Eq. 4

Longan wastes

$C_{619}H_{918}O_{417}N_{18}S_1 + 195H_2O \rightarrow 313CH_4 + 306CO_2 + 18NH_3 + H_2S$ Eq. 5

Longkong wastes

$C_{728}H_{1124}O_{434}N_{22}S_1 + 247H_2O \rightarrow 388CH_4 + 340CO_2 + 22NH_3 + H_2S$ Eq. 6

Napier grass

$C_{619}H_{918}O_{417}N_{18}S_1 + 195H_2O \rightarrow 313CH_4 + 306CO_2 + 18NH_3 + H_2S$ Eq. 7

Biochemical methane potential of substrates

The methane content of all the samples fluctuated during the first few days of commencement of BMP assay. This is probably as a result of microorganism adaptation and growth time. For chopped lady-finger banana peel, methane percentage was near stable from day 22 to day 60 within the narrow range of 53-58 %CH_4. For Napier grass it was observed that methane content was near stable from day 22 to 60 within the range of 51-57 %CH_4. Rambutan peel and seed was near stable at day 18 within 47-52% CH_4. Logan peel from day 2 to day 18, methane percentage was stable at 39% but increased to the range of 41-52% afterwards. Longkong peel methane content variation was observed to be similar to that of Logan peel. Comparison between theoretical methane yield calculated from theoretical methane potential and specific methane yield measured from biochemical methane potential assay is shown in Fig 1.

Alkalinity, pH and volatile fatty acids of effluents at the end of 60 days of BMP assay was investigated for all substrates as shown in Table 2. The pH for all the substrates was in the range of 7.30-8.11, alkalinity in the range of 3,375-4,625 mg/L as $CaCO_3$, and volatile fatty acids in the range of 212.5-462.5 mg/L as CH_3COOH. TVFA/alkalinity ratios were in the range of 0.047-0.11. These ranges are suitable for methane production in anaerobic digestion (Pisutpaisal et al., 2014). Alkalinity within the range of 3,000-5,000 mg/L $CaCO_3$, shows a properly operating anaerobic digestion system according to Standard Methods for the Examination of Water and Wastewater.

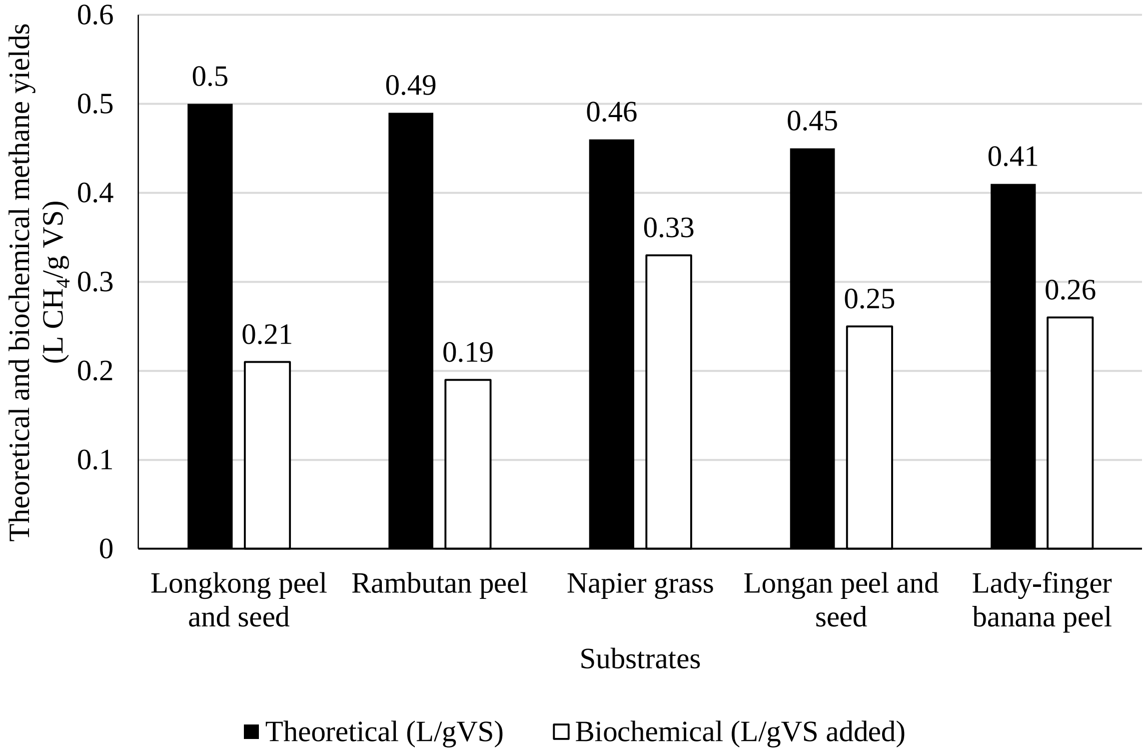

Fig. 1: The theoretical methane yield of substrates materials

Table 2: pH, alkalinity and VFA analysis of effluents at the end of BMP assay

No.	Substrates	pH	Alkalinity (mg/L as $CaCO_3$)	Total volatile fatty acid (mg/L as CH_3COOH)	TVFA/ Alkalinity	% CH_4 (maximum)
1	Lady-finger banana peel	8.10	4,438	250	0.06	60
2	Rambutan peel	8.11	3,875	244	0.06	52
3	Longan peel	7.42	3,500	212	0.06	54
4	Longkong peel	7.47	3,750	206	0.06	43
5	Napier grass	8.06	4,250	244	0.06	58

Effect of size reduction on the BMP of lady-finger banana peel

The cumulative methane yield obtained from chopped and ground lady-finger banana peel were 262.8 ml CH_4/ gVS and 341.8 ml CH_4/gVS, respectively. Above 80% of the ultimate methane yield was attained much earlier at about day 5 of runnunig BMP assay for ground lady-finger banana peel than it did for chopped lady-finger banana peel at about day 20 as shown in Fig. 2 and Table 3. However, the percentage of cumulative volume of methane production at day 20 to the cumulative methane volume at day 60 in both substrate sizes is higher than 80 % as shown in Table 3. Therefore, it can be concluded that size reduction of lady-finger banana peel by blending into slurry gives an increase in methane yield and in how fast the methane is produced.

The maximum methane yield obtained from ground lady-finger banana peel in this study (341.8 ml CH_4/g VS added) is higher than the maximum methane yield published by Gunaseelan (2004) for different varieties of ground ripe banana peels (243-322 mL CH_4/g VS added) and maximum methane yield reported by Nathoa et al. (2014) for banana peel (251.3 mL CH_4/g VS added). Moreover, the rate of methane and biogas production was faster for lady-finger banana peel than for the other different varieties reported by Gunaseelan (2004). The reason for this can be traceable to the peel genotype and biochemical composition. The properties of lady-finger banana peel is different from the properties of the banana peels used in past research work, the difference is mostly due to the genetic composition of the varieties. For example banana genotype AAA, Grande Naine contains higher cellulose than hemicellulose contents as published by Happi Emaga et al. (2008) and this was also similar to the properties of banana peels published by Bardiya et al. (1996). On the other hand for lady-finger banana peels, the hemicellulose content is higher than the cellulose content.

The stability of reaction from solid substrate to gaseous end product was investigated by measurement of pH, total alkalinity (TA) and total volatile fatty acids (TVFA) of the digestate at the end of 60 days of BMP assay. The pH narrowly fluctuated in the range of 7.3 – 8.11, which is suitable for methane production under mesophilic condition. Alkalinity was in the range of 3,000-5,000 mg/L $CaCO_3$, which shows properly operating anaerobic digestion according to Standard methods for the examination of water and wastewater. Small amount of VFA is present in the reactor in the range of 205-465 mg/L CH_3COOH. The ratio of total VFA/alkalinity is less than 0.1 for all the substrate samples, except for that of rambutan fruit of 0.11, which is within the optimum range for methane production (Pisutpaisal et al., 2014).

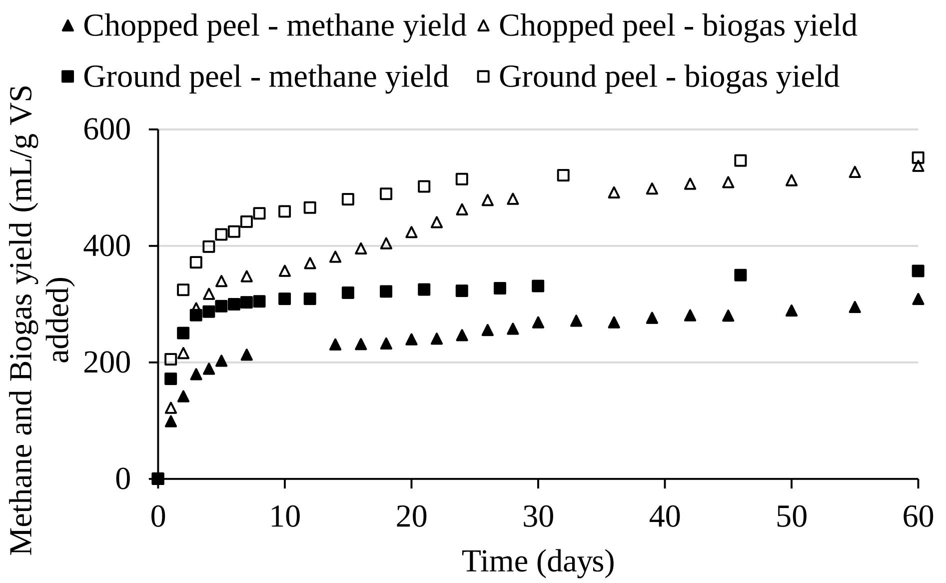

Fig. 2: Methane and biogas yield of chopped and ground lady-finger banana peel

Table 3: Milestone percentage of cumulative methane yields attained at day 60 in BMP assays of chopped and ground lady-finger banana peel

Milestone	Duration (days)	
	Chopped peels	Ground peels
> 50%	2	2
> 60%	3	2
> 70%	5	3
> 80%	20	5
> 90%	42	32

Effect of heat pre-treatment on BMP of lady-finger banana peel

The heat-pretreatment by boiling showed no significant effect on the total methane yield of ground lady-finger banana peel statistically. Although it has little effect on the rate of biogas production (R_m) as it increased from 5 minutes boiling to 20 minutes boiling as shown in Table 4. Considering the overal performance, heat pre-treatment of ground lady-finger banana peel by boiling is not economical and not suitable because energy will be used and this does not improve the overall methane yield. This could be as a result of the soft tecture of lady-finger bananna peels, for example if physically compared to Napier grass which has harder structure and higher cellulose content.

Table 4: Effect of heat pre-treatment on the BMP of lady-finger banana peel according to Gompertz equation

Varied duration of boiling ground lady-finger banana peel	Gompertz equation		
	λ (days)	R_m (mL/day)	H_m (mL CH_4/g VS added)
5 minutes	-0.4 ± 0.1	101.9 ± 14.8	334.4 ± 4.9
10 minutes	-0.2 ± 0.4	124.6 ± 29.0	323.5 ± 20.9
15 minutes	0.03 ± 0.02	185.0 ± 12.7	306.5 ± 6.9
20 minutes	0.03 ± 0.02	203.5 ± 23.9	327.7 ± 13.9

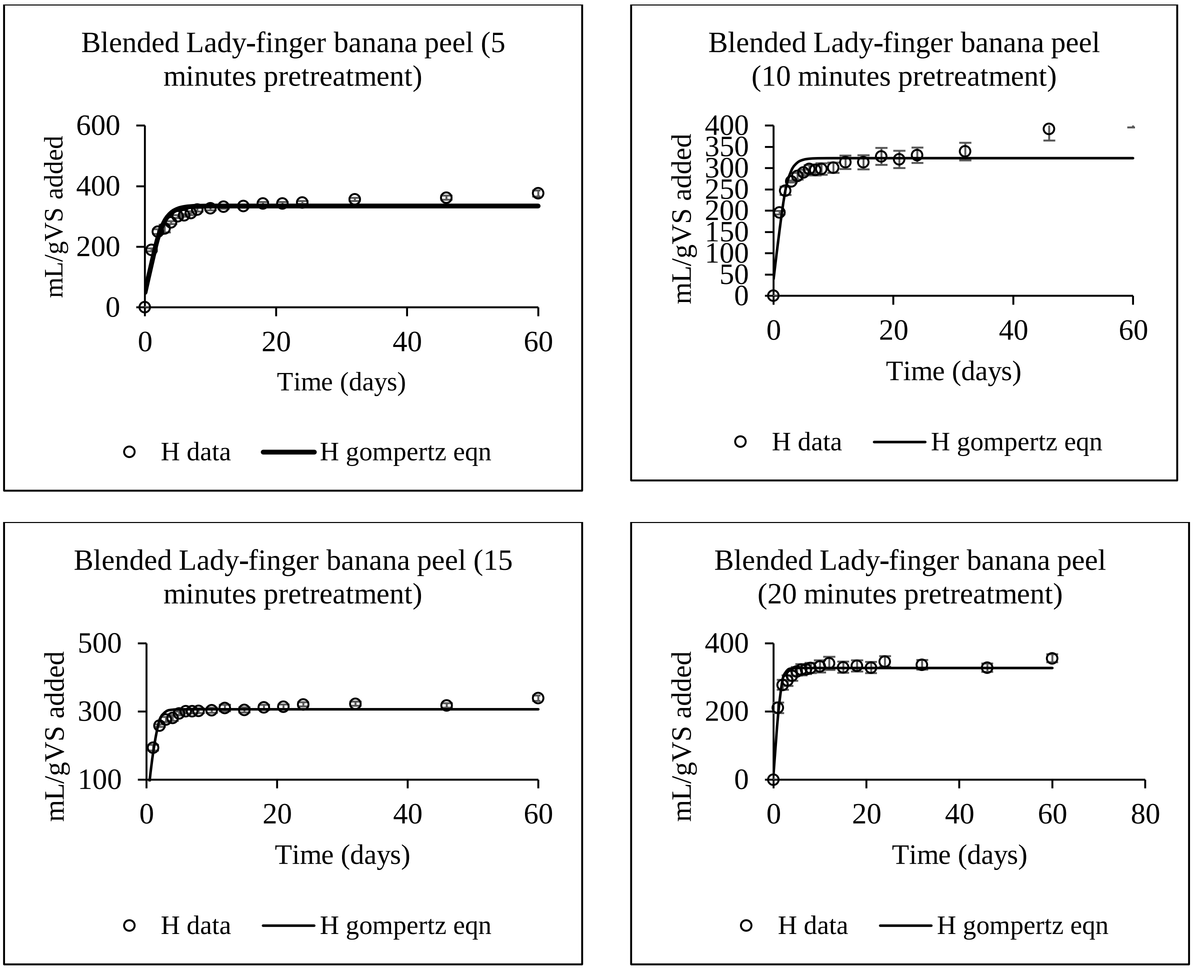

Fig. 3: Methane and biogas yield of heat-pretreated lady-finger banana peel

Conclusion

Dumping of organic wastes including peels and seed derived from agricultural produce (such as lady-finger banana, rambutan, longan, longkong, etc.) is harmful to living organisms in the environment as decomposition releases noxious gases to the environment (Haszeldine, 2009). This study reveals that these organic materials can be treated and stabilized using anaerobic digestion technology as a tool, and biogas (methane) can be produced. The residue of the digestion process can also be used as soil amendments. This gives a three-way benefit: organic waste treatment, biogas generation, and soil supplements production.

Physical pretreatment by size reduction increases the methane production and yield as well as biodegradability of lady finger banana peel in BMP assay, and biodegradability increased from 63% to 83% for chopped and blended peels. Observation was also made that thermal or heat pretreatment (by boiling) is not suitable for substrates high in hemicellulose contents such as lady-finger banana peel because the structure comprises more of hemicellulose and pectin which easily undergoes hydrolysis.

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