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Bioconversion of fruit wastes and effects of thermophilic hydrolysis for banana peel digestion

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Abstract

Methane production potential of tropical fruit wastes were compared by anaerobic digestion assay and stoichiometric Buswell's equation. Methane yields were in the order of banana peel, longan waste, and rambutan waste (330.6, 268.3, 234.6 and 193.2 $mLCH_4/gVS$). Their calculated biodegradability related well with structural composition and properties. In continuous system operations on banana peel at feed concentrations 1%TS and 2%TS, mesophilic single stage digester (20-day HRT) failed at 2%TS. Prehydrolysis thermophilic reactor (4-d HRT) was placed as pre-treatment to mesophilic reactor (20-d HRT). Higher biogas and energy yields were obtained and greater system stability was achieved over the single stage digestion. The energy yield of 2510.9 kJ/kgVS was achieved at a feed concentration of 2%TS with thermophilic prehydrolysis.

Keywords: Banana peel, Fruit waste, Pre-hydrolysis, Methane, Hydrogen

1 Introduction

Banana has a variety of species in genus *Musa*. It is widely grown in tropical countries in Asia and Africa. When ripens, the color changes to yellow as the fruit softens. The ripe banana contains a skin known as peel at approximately 30-40% of the fruit's total weight and it is a highly biodegradable biomass (1). Rambutan (*Nephelium lappaceum*) and longan (*Euphoria longan*) are also tropical fruits largely cultivated in Thailand and other Southeast Asian counties with a sizable portion being supplied to the canning industry. The wastes of these fruits could be a feedstock for anaerobic digester (AD). Most studies taken, however, had focused on batch assays (2-4). In the first part of this work, we emphasized on the methanation potential of these main tropical fruit wastes (either containing peel or peel+seed) in order to compare and identify the suitable feedstock for AD, and subsequently the continuous anaerobic digester systems were run in single stage versus two stage modes for comparison.

In anaerobic digestion of solid substrate, hydrolysis is often a rate limiting step. Once solubilized to soluble monomers, volatile fatty acids (VFA), hydrogen, and carbon dioxide can be produced before further converted to acetate and methane. Digester acidification is often a cause of system failure at high organic loadings (5). Fast degradability substrates could also cause over acidification. In a multiple stage system, anaerobic reactions are separated to different reactors to achieve efficiency and stability. Since temperature imposes great impact on enzymatic hydrolysis (6), thermophilic pretreatment stage could be beneficial in AD of this biomass.

2 Methodology

2.1 Inocula

Inoculum used in this experiment was collected from two full scale anaerobic digesters. The two fresh anaerobic digester sludges, having pH 7.5-8.5, were kept to settle overnight in separate containers to remove scum and thicken the sludges for use. Solid contents of the sludges were determined prior to the experiments after screening and sedimentation. And then settled sludges were mixed at 1:1 ratio based on TS to provide microbial diversity. Rigorous biogas production was observed early during the sludge storage for degasing as to remove organics in the sludge. This occurrence indicates high microbial activity. The degased sludge after 20 days was used as BMP inoculum.

2.2 Substrate

Ripe lady-finger banana (*Lusa sapientum Linn.*) peel was collected from southern Thailand, prepared and analyzed for TS and VS contents. The fresh peel was chopped to 5 mm in size. Ripe longan and rambutan fruits were collected from the local market in Songkhla province. Chopped longan and rambutan wastes, composed of peel and seed, of 5 mm size were prepared to be used in biochemical methane potential (BMP) assay. All samples were dried at 60 ºC and ground using mortar and pestle for analysis of the elemental composition.

2.3 Biochemical methane potential assay

Anaerobic digestion or BMP assay was set up according to the procedure based on (7), in 120 mL glass bottles with effective volume of 60 mL. Seed inoculum of 15 g TS/L was added with the fresh fruit materials of 2-5 g/L TS. The pH was adjusted to 7.0 using small drops of 0.1 M NaOH or 0.1 M HCl. DI water was added to make a 60-mL effective volume and flushed with N_2 gas. The glass reactors were incubated at 35 ± 1 °C with continuous swirling at 150 rpm. These assays were run in four replicates.

2.4 Calculations of theoretical methane production and energy yield

Maximum theoretical methane potential (TMP) was determined from the complete degradation of the samples based on the modified Buswell and Mueller equation using elemental compositions (C, H, O, N, and S) as shown in Eq. 1 and Eq. 2 (8).

$$C_c H_h O_o N_n S_s + yH_2O \rightarrow xCH_4 + zCO_2 + nNH_3 + sH_2S \quad (1)$$

$$x = 0.125\,(4c + h - 2o - 3n - 2s),$$
$$y = 0.250\,(4c - h - 2o + 3n + 2s),$$
$$z = 0.125\,(4c - h + 2o + 3n + 2s) \quad (2)$$

where c, h, n, s, and o are the moles of carbon, hydrogen, nitrogen, sulfur and oxygen, respectively, assigning s = 1.

2.5 Anaerobic digester operations

The continuous digestion experiment was carried out using two bioreactor systems; a single stage mesophilic digester (35±1 ºC), designated as MS reactor, and a two-stage system comprising of a first stage thermophilic digester (55±1 ºC), designated as T1 reactor, serving as a pretreatment step followed by a second stage mesophilic digester (35±1 ºC), designated as M2 reactor. The bioreactors were made of glass and covered with gastight cap-seal. The reactor temperatures were controlled by water baths with electrical heaters. The reactors were inoculated at 44,000 mg/L MLSS of the anaerobic sludge.

Ground lady-finger banana peel was prepared into slurry every 2-3 days to a concentration of 1 and 2%TS, and stored in at 4 ºC until use. MS reactor (4L effective volume) was operated at HRT 20 days. In two stage system, T1 reactor (1.2L effective volume) was operated at HRT 4 days at the same solid feed concentrations while M2 reactor (2.8L effective volume) was operated at HRT 20 days. A portion of effluent from T1 was fed to M2 reactor while the rest was used for chemical analyses. T1 reactor was employed as a thermal hydrolytic pre-treatment of the substrate. pH adjustment to mesophilic single stage reactor was administered when significant pH drop occurred.

2.6 Analytical methods

Total solids (TS) and volatile solids (VS) were determined using Standard Methods for the Examination of Water and Wastewater (9). Samples of ripe lady-finger banana peels, longan wastes and rambutan wastes were dried at 60 ^{o}C to a constant weight and grinded for the analysis of carbon, hydrogen, oxygen, nitrogen, and sulfur (CHONS) elemental compositions.

Biogas volume was measured on daily basis using a drum-type wet gas meter Model TG0/5, Ritter (Germany). Biogas sampling was done through a septum gas sampling point. Biogas was injected to a gas chromatography (GC Agilent™ 7820A Agilent Technologies), equipped with a thermal conductivity detector (TCD) using helium (He) as carrier gas to determine methane composition. For hydrogen composition measurement, GC (Agilent™ 7820A) with a Packed Column: Shin Carbon ST100/120 (2 m × 1 mm) was used with argon (Ar) as carrier gas.

Alkalinity and total volatile fatty acids (TVFA) analyses of effluents were done using direct titration methods (10), and pH was measured following Standard Methods (9).

3 Results and discussions

3.1 Substrate characteristics

Characteristics of the substrates including ripe lady finger (LF) banana peel, rambutan waste and longan waste are shown in Table 1. Rambutan and longan wastes comprise of the fruit peel and seed. The VS in rambutan and longan wastes are 96.8% and 92.0% dry weight, respectively. C/N ratio in LF banana peel (23.1) and longan waste (29.5) are within the optimal range of 20-30 suitable for anaerobic digestion. Rambutan waste has high C/N (44.2). If the rambutan waste is used as mono-substrate in continuous anaerobic digester, nitrogen deficiency might entail. Mixing it with the other low C/N material could help alleviate this nutrient deficiency.

Table 1 Substrate characteristics in this study

Parameters	Banana waste	Rambutan waste	Longan waste
Volatile solids	88.1	96.8	92.0
Fixed solids	11.9	3.2	8.0
Carbon	39.2	48.6	44.3
Hydrogen	5.3	5.9	5.5
Nitrogen	1.7	1.1	1.5
Sulfur	0.1	0.1	0.2
Oxygen	39.6	37.6	39.7

3.2 Anaerobic digestion (BMP) assay of fruit wastes

In digestability test, the three fruit wastes were subjected to anaerobic digestion assessment. Methane production developed quickly at the start for all substrates (Fig. 1). This could be a result of the mixed active inocula from two sources. The cumulative methane yield at day 30 obtained for chopped LF banana peel, chopped longan waste and chopped rambutan waste were 268.3, 234.6 and 193.2 $mLCH_4/gVS_{added}$, respectively. Results clearly showed that the banana peel has the highest methane yield despite the lower VS content. Among other compositions, high hemicellulose could play an important role in anaerobic digestibility of fruit waste. Banana waste, therefore, seems more promising by its great volume and less seasonal variation. Although seeds of rambutan and mangosteen have reportedly high methane potential attributing to high fat content (2), their weight contribution is rather small compared to the combined waste stream (seed+peel). Higher VS content in rambutan and longan wastes did not translate to the higher biogas yield.

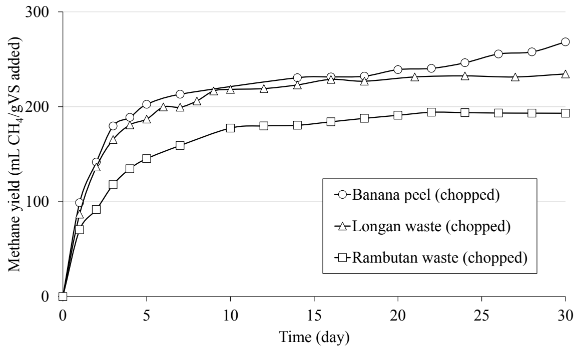

Figure 1 Methane evolution from anaerobic digestion assay

3.3 Theoretical methane potential (TMP) and biodegradability of substrates

There is always a portion of plant cells that would not be converted biologically under test conditions. The degree of conversion in any particular pathway can be represented by "biodegradability". To estimate the biodegradability of the fruit wastes, TMP was calculated from the modified equation of Buswell and Mueller (1952), and the results are shown in Eq. 3-5. This method assumes that all of the VS in the substrate is 100% converted. The fixed solids content in the substrate was already used to average down the methane yield to L/gTS. The theoretical methane yield of LF banana peel, rambutan and longan wastes are 413.2, 493.1, and 453.7 L CH_4/kg VS, respectively.

LF banana peel:
$C_{1055} H_{1704} O_{801} N_{38} S + 257.9H_2O \rightarrow 526.0CH_4 + 529.4CO_2 + 38.1NH_3 + H_2S$ (3)

Rambutan waste:
$C_{954} H_{1368} O_{553} N_{18} S + 349.7H_2O \rightarrow 502.7CH_4 + 451.6CO_2 + 18.3NH_3 + H_2S$ (4)

Longan waste:
$C_{619} H_{918} O_{417} N_{18} S + 194.8H_2O \rightarrow 312.9CH_4 + 305.8CO_2 + 17.9NH_3 + H_2S$ (5)

Biodegradability was the ratio of total cumulative methane yield (in L CH_4/g VS_{added}) of BMP assay at 30 days to the TMP (in L CH_4/g VS_{added}). The biodegradability of LF banana peel (65.4%) was obviously higher than those of longan waste (52.0%) and rambutan waste (39.4%). Our tests showed that the LF banana peel contains higher hemicellulose content (39.7%) compared to the other two materials (10.0% for rambutan waste and 24.2% for longan waste). This differentiation may be associated with hemicellulose since it is more biodegradable than cellulose and lignin.

3.4 pH, alkalinity and VFA accumulation in single and two-stage digestion

Two continuous digester systems were run side by side over varied total solid (TS) concentrations in feed and hence organic loading rate (OLR, kgVS/m^3.d). While both mesophilic reactors, mesophilic single stage (MS) and mesophilic second stage (M2) were run at HRT 20 days, a smaller unit of thermophilic reactor (T1) was placed as a pretreatment unit of M2 so as to evaluate its impacts. At 1%TS feed concentration, pH in MS reactor and M2 reactor were stable at around 6.6, and in T1 reactor, pH was stable around 4.4 as shown in Fig. 2. The rapid pH drops in the T1 reactor at the initial operation (not shown) was a result of the buildup of 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 the range of 4.3-4.6. Nevertheless, pH in MS reactor dropped significantly during 2% TS feed. Substantial accumulation of VFAs in MS occurred at this loading, which is considered low for AD. Addition of alkaline solution (NaOH 1M) for pH adjustment was administered (marked as arrows in the figure) to MS reactor starting from day 88 to adjust pH to 6.8-7.0 (Fig. 2). Despites, further pH drops continued. Significant biogas and methane reduction was clearly shown during day 70 to 103.

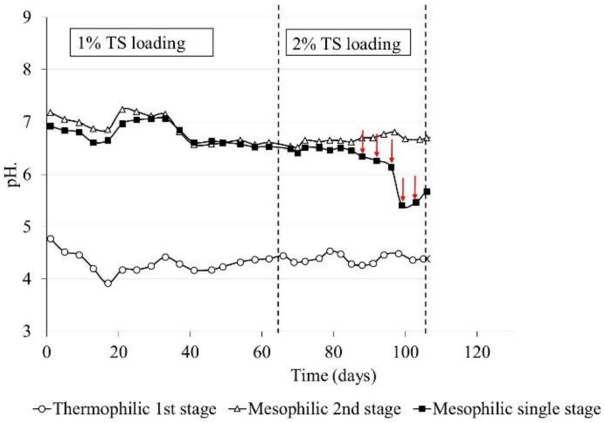

Figure 2 pH of effluent from banana peel digestion

Changes in total volatile fatty acid (TVFA) level are shown in Fig. 3. 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), could release organic content available to the culture medium quickly. In contrast, if the substrate is more recalcitrant to biodegradation, the system would not be overloaded even at high OLR.

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 TVFA of only 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 VFA of up to 1400 mg/L pushing TVFA/ALK above 0.8 which is a threshold of methanogenic system failure (11) and showing drastic pH drop. Meanwhile, in the 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 (11). In the T1 reactor at feed concentration of 2% TS (OLR of T1 4.03 kg/m^3.d), TVFA/ALK remained well above 2.5.

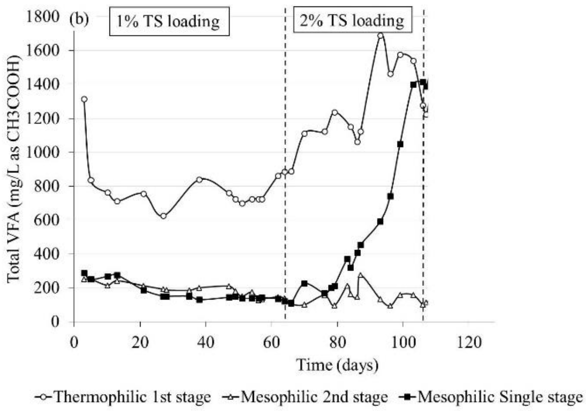

Figure 3 TVFA concentration during the experiment at 2 organic loadings

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. 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 (12).

3.5 Biogas production and energy yields in single stage and two-stage digestion

Fig. 4 shows the total biogas production at two feed concentrations in the mesophilic single stage and the two-stage digestion system. 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. Methane content dropped to around 4.5% during this period (starting from 50.4%). The average methane yield during the 2% TS feed was 17.14 L CH_4/kgVS added in MS reactor. The energy yield at 2%TS of the two-stage system was 2,511 kJ/kg VS which is significantly higher than single stage MS (526.2 kJ/kg VS). At this loading around 0.83 kg VS/m^3.d

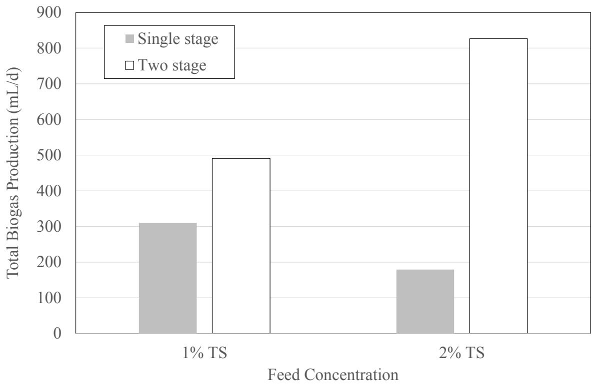

Figure 4 Total biogas production at 2 organic loadings of different TS in feed

4 Conclusion

Fruit wastes; banana peel, rambutan and longan wastes, were tested using anaerobic digestion that indicated banana peel has higher degree of biodegradability and methane yield. The thermophilic pre-hydrolysis of banana peel added to a normal mesophilic reactor gave positive impacts with its higher biogas and energy yield as well as process stability. Hydrogen, a more ideal source of energy, was produced in the first reactor. Combined hydrogen and methane production from banana peel digestion could serve as an alternative to a typical single stage anaerobic digestion.

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