Theoretical and Biochemical Methane Potential of Longkong Waste (Peel, Seed), Fruit Pulp and Napier Grass

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Received 15/07/2025; accepted 15/12/2025
https://doi.org/10.4152/pea.2027450512

Abstract

This study investigated theoretical methane potential (TMP)and biochemical methane potential (BMP) of Longkong (Lk) waste (peel and seed) and fruit pulp, and Napier grass (NG). TMP values were calculated using modified Buswell stoichiometric equation, while BMP assays were conducted to determine methane (CH_4) production. TMP results showed that Lk waste had higher theoretical CH_4 yield (0.5 L/g) of volatile solids (VS) than that from Lk pulp (0.39 L/g VS) and NG (0.46 L/g VS). BMP results showed that measured CH_4 yield was 0.21, 0.3 and 0.33 L/g VS for Lk waste, Lk pulp and NG, respectively. Lk peel is a more suitable substrate for biogas production compared to its pulp, due to high moisture content, low alkalinity and high volatile fatty acid of the latter. CH_4 potential of the three substrates compared favorably with those of others such as grapes and banana peels. The study recommends Lk peels and its leftover pulp for biogas production in regions with high cultivation.

Keywords: biochemical methane potential; biogas; Duolong's energy equation; modified Buswell stoichiometric equation; modified Gompertz equation; theoretical methane potentials.

Introduction•

Waste biomass materials are renewable energy sources that offer a sustainable solution to the prevalent global energy crisis deepened by population increase and the finite nature of fossil fuels [1]. Combustion of fossil fuels presents negative environmental impacts such as climate change, air pollution and water eutrophication. Biomass energy sources have become popular due to their relative abundance, versatility and carbon-neutral nature. Biomass can be converted into different energy products, including heat, electricity and fuels by combustion, anaerobic digestion, fermentation or gasification [2, 3]. Anaerobic digestion is a microbial process for breaking down organic matter without oxygen to produce biogas containing a mixture of CH_4 and CO_2 [4]. The biogas produced is a

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•The abbreviations list is in pages 545-46.

renewable energy source for heating and electrification. Furthermore, the digestate from anaerobic digestion process is rich in nutrients, and it can be applied as fertilizer for crop production [5]. However, the efficiency of the anaerobic digestion process depends on type and composition of biomass feedstock, operating conditions of the digester and choice of inoculum (microbial community). Research has shown that biomass feedstocks with high organic content, low lignin content and optimal carbon-to-nitrogen (C/N) ratios are generally more suitable for anaerobic digestion [6].

Researchers have explored various biomass feedstocks for anaerobic digestion such as agricultural residues, food waste, sewage sludge and energy crops. In their study, [7] have generated 64.4% CH_4 from co-digesting NG and cattle slurry under mesophilic conditions. Biogas potential of selected agricultural wastes, including papaya peel, bagasse, rice straw and soybean residue was examined by [8]. It was observed that soybean residue generated highest biogas yield of 263.04 mL at a hydraulic retention time of 25 days. The use of biomass feedstocks is limited by seasonal availability, transport cost and competition with food production [9]. This makes it important to evaluate the biogas potential of several biomass materials to ensure a sustainable feedstock supply for biogas production.

The study by [8] evaluated biogas production potential of both raw and processed agricultural wastes, highlighting their viability as renewable energy sources. The authors examined several agricultural residues, and compared their methane yields before and after undergoing pre-treatment processes such as grinding, soaking or chemical treatment. Findings revealed that processed agricultural wastes generally produced higher biogas yields compared to raw wastes, as pre-treatment enhanced biodegradability and accelerated microbial activity during anaerobic digestion. Among the tested materials, certain residues demonstrated particularly high methane potential, suggesting their suitability for large-scale biogas generation. The research underscores the importance of utilizing agricultural wastes not only as a sustainable waste management strategy, but also as a means of reducing reliance on fossil fuels.

Similarly, [5] investigated biochemical methane potential (BMP) and kinetic behavior of goat manure under different inoculum-to-substrate ratios (ISR). The study evaluated ISR of 1:1, 2:1 and 3:1 using anaerobic digestion batch tests to determine optimal conditions for methane yield. Results showed that highest cumulative methane production was achieved at ISR of 2:1, yielding approximately 220 mL CH_4/g VS, compared to 170 mL/g VS at 1:1 and 190 mL/g VS at 3:1. Kinetic modelling using modified Gompertz equation demonstrated strong fits ($R^2 > 0.95$) with 2:1 ISR exhibiting highest methane production rate and shortest lag phase, indicating efficient microbial activity. Findings highlight goat manure as a viable bioenergy feedstock that provides valuable to data for designing sustainable waste-to-energy systems. Despite this, there are limited studies on the use of certain biomass materials, such as Longkong (Lk) and Napier grass (NG) waste for biogas production.

Lk back peel is a waste generated from the industrial processing of Lk (*Lansium domesticum*) fruit, and NG (*Pennisetum purpueum*) is a tropical grass species. Lk is a popular tropical fruit grown in Southeast Asia. Its peel is rich in organic matter, and it has a high moisture content (MC) that makes it suitable as a feedstock for anaerobic digestion. CH_4 potential of Lk back peel has not been extensively explored. NG is a tropical grass widely grown in Africa and Asia as feedstock for livestock and bioenergy generation. It is known for its high biomass yield and its ability to thrive in marginal lands, making it an attractive feedstock for anaerobic digestion. However, CH_4 potential depends on location, harvesting age, MC and pretreatment methods [9].

This study aimed to investigate theoretical methane potential (TMP) and BMP using modified Buswell stoichiometric equation of Lk back peel and NG through anaerobic digestion. By investigating their CH_4 potentials, the study hopes to contribute to the development of sustainable biomass-based energy systems. Findings will significantly impact the development of anaerobic digestion systems in tropical regions where Lk fruit and NG are readily available.

Methodology

Inoculum

The microbial seed used for inoculation was a mixture of sludge collected from two full-scale anaerobic digesters: Chalong Concentrated Rubber Latex Industry and Liang Heng Lee Commercial Pig Farm in Songkhla Province, Thailand. Total solids (TS), volatile solids (VS) and pH of each sludge were determined, and mixing was done using a ratio of 50:50 TS for a diverse microbial population. Mixed anaerobic sludge was used majorly to obtain high diversity in the population of microorganisms. The population of microorganisms in anaerobic digester sludge from the two different industrial sources is usually not the same. Mixing from the two sources provides a diverse microbial species for inoculation [16]. The inoculum was kept in fridge at about 4 ºC, and used in the experiment within 72 h after collection. Mixed anaerobic sludge was used majorly to obtain high diversity in the population of microorganisms.

Materials collection and preparation

NG was collected from Satun Animal Nutrition Development Station, Satun province, Thailand. NG were about 2-3 months old, and were considered to be already matured. The plantation area was fertilized regularly by manure obtained from the animals housed in the station. Fresh NG was collected and milled into smaller sizes in the station using a rotary hammer milling machine with mesh size of 1 cm. Fig. 1a/b shows the picture of fresh and milled NG samples, respectively. Fresh Lk was obtained from Hadyai, Songkhla province, Thailand, and kept at 4 ºC until use. Two samples were derived to include Lk peel and seed as one sample and Lk pulp as the other. Although the seeds are not usually distinct, a few different of them were found and separated from the pulp. Fig. 1c shows Lk peel and pulp. NG and Lk samples were dried to constant weight and analyzed for TS, VS, C-H-N-S-O composition, cellulose, hemicellulose and lignin contents. Fresh samples were cut to smaller sizes before use in BMP assay.

Figure 1: Substrates of **(a)** fresh NG **(b)** milled NG and **(c)** Lk fruit.

Total solids and volatile solids tests

For TS analysis, each of the samples were grounded using mortar and pestle, weighed in ceramic crucibles in three replicates and dried in an oven at 103 ºC, for 24 h, or until a constant sample weight was obtained. TS was calculated using mathematical expression in Eq. (1) [3].

$$\mathrm{TS}(\%) = \frac{\text{weight of dried sample}}{\text{Weight of wet sample}} \times 100\% \qquad (1)$$

For VS analysis, each TS sample was placed in a furnace to a temperature of 550 ºC, and allowed to burn until a constant weight was achieved (1 h). Mathematical expression for VS calculation is stated in Eq. (2) [3].

$$\mathrm{VS}(\%) = \frac{\mathrm{TS}-\mathrm{A}}{\mathrm{TS}} \times 100 \qquad (2)$$

Theoretical methane potential

Data from element composition analysis was used to compute TMP using a modified Buswell stoichiometric equation [10]. Proportion of CH_4 in biogas can be used to evaluate the state of methanogenic biocenosis [4]. TMP was used to investigate maximum theoretical CH_4 production by complete degradation of each sample. From element composition analysis, the fraction of ammonia (NH_3) and hydrogen sulfide in produced biogas was obtained as shown in Eq. (3) [11].

$$C_c\,H_h\,O_o\,N_n\,S_s + 0.250\,(4c–h–2o+3n+2s)\,H_2O \rightarrow 0.125\,(4c+h–2o–3n–2s)\,CH_4 + 0.125\,(4c–h+2o+3n+2s)\,CO_2 + nNH_3 + sH_2S \qquad (3)$$

where c, h, o, n, s, stands for moles of carbon, hydrogen, oxygen, nitrogen and sulfur, respetively. Assumption of s = 1 was used in TMP computation. The assumption was made to simplify the model, and because Sulphur is usually present in organic waste in trace amounts. Theoretical CH_4 yield is given in L/g VS [12]. Microsoft Excel 2013 software was used in data analysis. Energy contents, i.e., lower heating value (LHV) and higher heating value (HHV) in kJ/g were calculated using modified Dulong's energy eq. [12], based on element compositions data, as shown in Eq. (4).

$$\text{Energy content (LHV, kJ/g)} = 38.2m_C + 84.9(m_H - m_O/8) - \Delta H_l \quad (4)$$

where m_C, m_H and m_O are contents of C, H and O, respectively, on a moisture-free basis, and ΔH_l is latent heat. Modified Dulong's formula can be used to compute heating values of gas, liquid and solid fuels by taking latent heat into consideration [13]. When the equation is applied to gaseous, liquid or solid fuels, ΔH_l is 0, 0.5 and 0.62 kJ/g, respectively [14, 15]. HHV was calculated by adding heat of water vaporization as in the following expression:

$$m_H\,(M_{\mathrm{H2O}}/M_{\mathrm{H2}})\,\Delta H_{\mathrm{boil}} = (18/2) * 2.44m_H = 22.0m_H) \quad (5)$$

where m_H, M_{H2O}, M_{H2} and ΔH_{boil} are content of hydrogen, molecular weight of water, molecular weight of hydrogen, and heat of vaporization, respectively (2.44 kJ/g) [14, 16].

Biochemical methane potential (BMP)

BMP method was carried out to assess the volume of CH_4 produced per gram/VS of substrates added. 120 mL glass reactors were set up with an effective volume of 60 mL, provided with 1% (v/v) nutrients, a buffer solution of 50 g/L $NaHCO_3$ at 10% (v/v) [14, 15] and a solution with the exact required amount of trace elements – iron(II) chloride monohydrate (0.5000 g), boric acid (0.0125 g), zinc chloride (0.0125 g), copper(II) chloride dehydrate (0.0095 g), manganese(II) chloride tetrahydrate (0.1250 g), ammonium heptamolybdate tetrahydrate (0.0125 g), aluminum chloride hexahydrate (0.0225 g) and cobalt(II) chloride hexahydrate (0.5000 g) – which were weighed in 50 mL beakers, dissolved with deionised water and added into a larger flask up to the effective volume required for the trace element solution.

Then, 0.5 mL of the trace element solution was added to prepare the stock solution (nutrients) by adding it to ammonium chloride (0.14 g), dipotassium hydrogen phosphate (0.125 g), magnesium sulfate monohydrate (0.05 g), calcium chloride dehydrate (0.005 g) and yeast extract (0.05 g). 1% nutrients and trace elements were added by adding 0.6 mL (1% of 60 mL effective volume for BMP) of homogenised stock solution. 6 mL of 50 g $NaHCO_3$ dissolved in 1 L distilled water were added, representing 10% (v/v). The pH was adjusted to 7.0 using small drops of 0.1 M sodium hydroxide or 0.1 M hydrochloric acid [16]. An effective volume of 60 mL was attained by adding deionised water. BMP reactors were flushed with nitrogen gas for some minutes, and immediately capped to maintain anaerobic conditions. The reactors were then positioned in the incubator with continuous revolution at 150 rpm, and at a temperature of 35 ± 1 °C. A control solution containing only 15 g TS/L of inoculum with no substrate was also prepared and filled to an effective volume of 60 mL with deionised water. The experiment was prepared in four replicates for the substrate samples and the control sample. The substrate samples were prepared using the ratio 1:3 (inoculum: substrate) to arrive at the effective volume. The pH, total alkalinity (TA) and total volatile fatty acids (VFA) of the digestate were analysed using standard methods at the end of the 60-day experiment to investigate the stability of reaction from the solid substrate to the gaseous result. The serum bottles were opened or uncapped, and the contents were analysed for pH, buffer capacity and VFA. The pH was measured using Mettler Toledo pH meter (model pH 1120x). Wet gas meter was used to measure total biogas volume (Master Flex, model 7554-95, Thermo-Fisher Scientific, USA). Gas samples were taken periodically with a graduated gastight syringe for composition analysis using a gas chromatography machine (GC 7820A Agilent Technologies) equipped with a thermal conductivity detector, 1/8-inch diameter stainless steel and a 0.25 mm by 30 m glass column using helium as carrier gas.

Methane production kinetics

Kinetics of CH_4 production was computed using modified Gompertz eq. (Eq. 6) from the graph of cumulative CH_4 production (mL) against time (days) [5, 17]. Microsoft Excel 2013 software was used for graph plotting and data analysis. Production potential (H_{max}), production rate (R_m) and lag phase (λ) were determined from the analysis.

$$H = H_{max} \times exp\left\{- exp\left[\frac{R_m e}{H_{max}}(\lambda - t) + 1\right]\right\} \tag{6}$$

where H = cumulative volume of CH_4 production (mL), H_{max} = CH_4 production potential (mL), R_m = maximum production rate (mL/day), t = time of fermentation (day), λ = time of lag phase (day) and e = constant (2.71828).

Discussion of results

Inoculum

The inoculum mixed-sludge had a pH falling within the range from 7.5 to 8.5. The pH and solid contents including TS, VS, mixed liquor suspended solids and mixed liquor suspended VS of pig farm digester and rubber latex industry sludge are shown in Table 1.

It is also shown that TS, VS, mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) for pig farm sludge were 84,330, 51,040, 85,467 and 52,230 mg/L, respectively. Rubber latex sludge recorded TS, VS, MLSS and MLVSS values of 47,500, 38,360, 48,632.86 and 39,983 mg/L, respectively. It is observed that the solid contents of pig farm sludge were higher than those from rubber latex sludge.

Table 1: Solid content analysis of the inoculum.

Parameters	Pig farm sludge	Rubber latex sludge
pH	7.5-8.5	7.5-8.5
TS (mg/L)	84,330	47,500
VS (mg/L)	51,040	38,360
MLSS (mg/L)	85,467	48,632.86
MLVSS	52,230	39,983

Substrates characteristics

Table 2 shows physicochemical compositions of the substrates. Lk pulp had the highest MC of 83.4%, while Lk peel had 79.1%. MC obtained was within the range of a typical biogas substrate, as reported by [18].

Table 2: Substrate characteristics.

Parameters of analysis	Lk pulp	Lk peel/ seed	NG
MC (% wet)	80.3	68.7	48.1
TS (g/kg wet)	196.8	313.5	519.2
VS (g/kg wet)	183.0	289.2	505.3
VS (% of TS)	93.0	92.2	97.3
Cellulose (%)	2.5	17.1	44.5
Hemicellulose (%)	3.4	14.7	24.9
Lignin (%)	0.6	17.2	13.2
C (%w/w dry)	38.4	46.4	41.8
H (%w/w dry)	6.4	6.0	5.3
O (%w/w dry)	46.7	36.8	28.4
N (%w/w dry)	0.9	1.6	1.5
S (%w/w dry)	0.0	0.2	0.1
Sum C-H-N-S-O (%)	92.4	90.9	77.2
C/N ratio	43.1	28.6	28.6

Lk peel had the highest TS (209.2 g/kg). VS content (wet) for Lk pulp and peel were 155.3 and 191.1 g/kg, respectively, while for NG it was 174.8 g/kg. Similarly, Lk pulp had the highest VS (% of TS) of 93.7, while that for Lk pulp and NG were similar, 91.3 and 91.1, respectively.

Cellulose and hemicellulose contents were highest for NG, 44.5% and 24.9%, respectively. Lk peel recorded highest lignin content (17.2%). C-H-N-S-O analysis showed that, except for Lk pulp, with 0% sulphur content, the other two samples had a trace amount (<0.3%) of S. Sulphur is not needed in a biogas plant because it tends to react with hydrogen and form H_2S, which can cause corrosion [19].

All three substrates are rich in carbon and oxygen: Lk pulp had 38.4% wt. C and 46.7 wt.% O; Lk peel had 46.4 wt.% C and 36.8 wt.% O; and NG had 41.8 wt.% C and 28.4 wt.% O. Carbon and oxygen are very beneficial to biogas production process. Carbon is the backbone of biogas production as it is the primary energy provider which directly influences the amount of CH_4 produced. On the other hand, oxygen is usually bound with carbohydrates and proteins in substrates, and it determines the amount of CO_2 that will be generated alongside CH_4 [8, 16]. NG had the lowest combined C-H-N-S-O weight of 77.2%, while Lk pulp and peel had 92.4 and 90.9%, respectively.

Lk pulp recorded highest C/N ratio of 43.1, while that of Lk peel and NG was 28.6. An optimal C/N ratio is required for efficient microbial metabolism, of which range, typically, is 20 from to 30 [20]. High C/N ratios imply more carbon content, which could impede microbial growth. Low C/N ratios show more nitrogen content and reduce inhibitory properties of microbes to NH_3 [21, 22]. Results showed that Lk peel and NG are suitable substrates for anaerobic digestion process.

Theoretical biogas production and energy production potential

Theoretical biogas production for the various substrates is represented in Eqs. (7- 9). Eq. (7) shows that 247 moles water are needed to digest a mole Lk waste, to produce 388 moles CH_4 gas, generating 340 moles CO_2 and 22 moles NH_3.

$$C_{728}H_{1124}O_{434}N_{22}S_1 + 247H_2O \rightarrow 388CH_4 + 340CO_2 + 22NH_3 + H_2S \quad (7)$$

Eq. (8) shows that 205 moles water are required to produce 1753 moles CH_4 gas, while generating 1663 moles CO_2 and 22 moles NH_3.

$$C_{3415}H_{6806}O_{3120}N_{68}S_1 + 205H_2O \rightarrow 1753CH_4 + 1663CO_2 + 68NH_3 + H_2S \quad (8)$$

Eq. (9) shows that 195 moles water are required to generate 313 moles CH_4 gas while also producing 306 moles CO_2 and 18 moles NH_3.

$$C_{619}H_{918}O_{417}N_{18}S_1 + 195H_2O \rightarrow 313CH_4 + 306CO_2 + 18NH_3 + H_2S \quad (9)$$

Results in Eqs. (7-9) show a relationship between MC of substrates and water requirement during anaerobic digestion. Lk fruit flesh has a higher MC compared to its waste, and it requires fewer moles of water for complete anaerobic digestion. Results also highlight the role of C/N ratio in CH_4 yield. A higher C/N ratio implies higher CH_4 yield with more CO_2 and NH_3 present [22, 26].

Theoretical analysis of biogas composition is presented in Table 3.

Table 3: Theoretical biogas composition (including ash in computation) and energy potential.

Item	Substrate	% CH_4	% CO_2	% NH_3	% H_2S	LHV (MJ/g)
1	Lk waste	51.81	45.40	2.73	0.06	118.63
2	Lk fruit	50.20	47.96	1.84	0.01	675.17
3	NG	49.38	47.92	2.62	0.07	97.16

It is shown that Lk peel and seed had highest percentage CH_4 content (51.81%). Both Lk peel and Lk pulp had higher CH_4 content compared to NG (49.38%). CO_2 content was highest in Lk pulp (47.96%). NG had a slightly lower value (47.92%) and Lk peel had 45.40%. In terms of NH_3 content, Lk peel (2.73%) and NG (2.73%) had higher values than those from Lk pulp (1.84%).

A similar trend is seen for H_2S content of biogas: Lk pulp recorded low H_2S content (0.01%), while Lk peel and NG had 0.06% and 0.07% H_2S, respectively. This may be due to MC present in the three substrates. The higher MC of Lk pulp allows for more microbial activity. This will reduce NH_3 and H_2S content of generated biogas, which aligns with findings from similar studies on biogas production from biomass residues [6].

BMP of substrates

Fig. 2 depicts daily production from the three substrates. Fig. 2(a) shows that Lk pulp has a higher CH_4 production capacity compared to Lk peel. Highest daily production for Lk fruit was 299.28 mL CH_4/g VS recorded on day 35, while that for Lk peel was 213.33 mL CH_4/g VS recorded on day 37. However, for NG, highest production was 308.29 mL CH_4/g VS recorded on day 55.

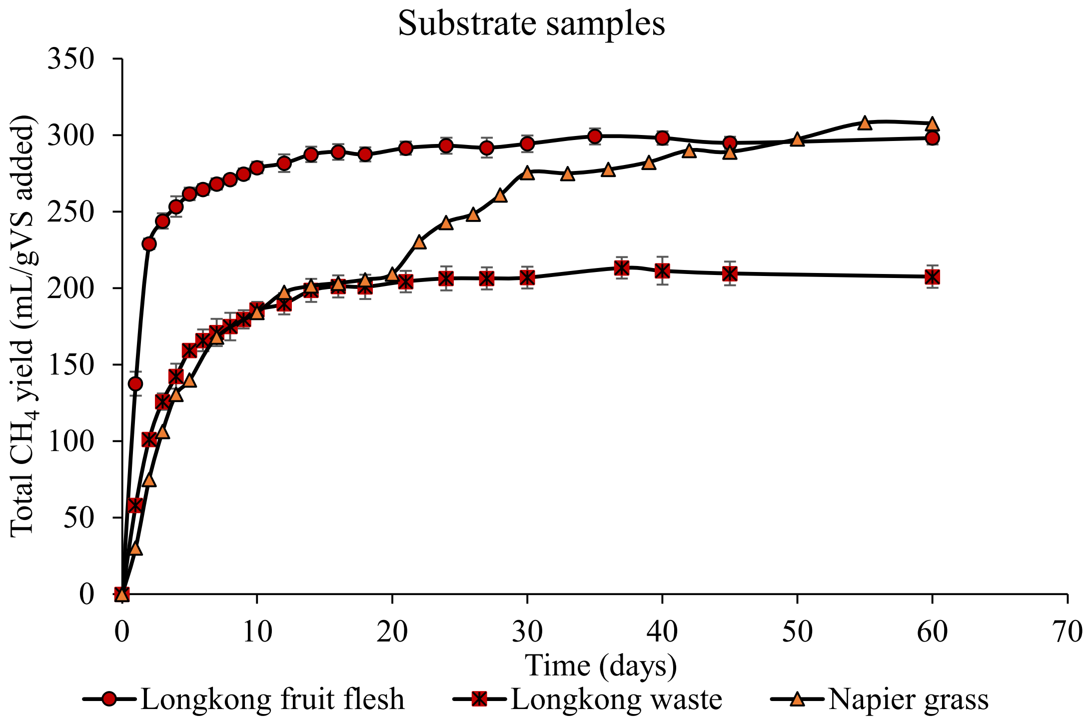

Fig. 2: CH_4 yields from BMP assay.

The results of properties of the digestate at the end of the 60-day experiment and data from Gompertz equation for the three substrates are compared in Table 4.

Table 4: Properties of digestates at the end of 60 days BMP experiment.

Substrates	pH	Alkalinity (mg/L $CaCO_3$)	VFA (mg/L CH_3COOH)	VFA/alkalinity	Max.% CH_4	H max (CH4/g VS)	Rm (mL/g VS-day)	Λ (d^{-1})	R^2
Lk pulp	7.48	3,625	219	0.060	47	283.26	124.64	-	0.96
Lk waste	7.47	3,750	206	0.055	43	202.31	30.62	-	0.97
NG	8.06	4,250	244	0.060	58	152.56	3.34	-	0.95

It is seen that pH values of the substrates were slightly alkaline. Lk pulp sample had a pH of 7.48, Lk peel had 7.47, while the one from of NG was 8.06. Alkalinity tests showed that NG had 4,250 mg/L $CaCO_3$, while that of Lk pulp and Lk peel were 3,625 and 3,750 mg/L, respectively. Higher alkalinity values indicate the capacity of the substrate to neutralize acids and maintain a stable pH. Total VFA analysis, which is measured in mg/L CH_3COOH, shows that Lk peel had lower VFA (206 mg/L CH_3COOH) values compared to Lk pulp and NG. High VFA is undesirable in anaerobic digestion, as it introduces instability that can cause the system to crash [24], and it inhibits methanogenic microorganisms [25]. Ratio of total VFA to alkalinity for the three substrates was 0.06. By BMP approach, NG has the potential to produce 58% CH_4, while Lk fruit and Lk peel can produce 47 and 43%, respectively. The finding is that, while Lk fruit can generate more CH_4 compared to Lk peel, its lower alkalinity and higher VFA means that the latter would be a better substrate for biogas production.

Fig. 3 shows CH_4 L/g VS for TMP *vs.* BMP assay. Lk peel had a higher theoretical CH_4 yield compared to NG, but a lower biochemical CH_4 yield. CH_4 yield for NG was slightly higher than that of Lk fruit. A similar calculated CH_4 yield from NG of 0.48 L/g VS was also reported by [26].

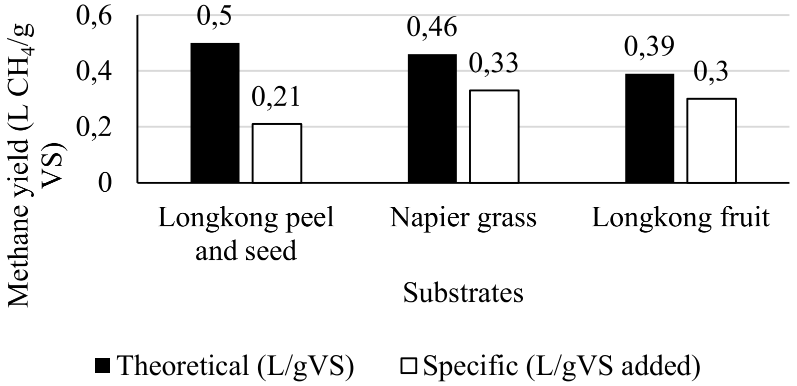

Figure 3: CH_4 yields (theoretical and BMP) of the substrates.

The results of the study compare favorably with those of previous studies, as shown in Table 5.

Table 5: Comparison of CH_4 potential with reference substrate.

Materials	Ultimate CH_4 yield (L CH_4/g VS added)	References
Sweet sorghum bagasse	0.262-0.33	(27)
NG	0.19-0.34	(15)
Pineapple peels	0.52	(28)
Cabbage, cauliflower and food waste	0.475	(20)
Cooked food waste	0.328	(10)
Banana peels	0.330	(29)
Lk (waste/fruit)	0.5/0.39	Current research
NG	0.46	Current research

Conclusions

The research demonstrated the viability of utilizing Lk back peel and leftover Lk pulp as substrates for anaerobic digestion and biogas production. NG was used as a reference point to validate findings. Results show that Lk peel has a higher TMP, while Lk pulp exhibits a higher BMP. However, lower alkalinity and higher total VFA content in Lk pulp may pose challenges for digester stability. Additionally, higher MC in Lk pulp may require larger digester volumes to achieve optimal biogas production. Nonetheless, CH_4 potential of Lk peel and waste Lk pulp compares favorably with other biogas substrates, making this fruit a promising feedstock for biogas production, particularly in regions where its cultivation is abundant.

Acknowledgments

The authors acknowlwdgwe Graduate School (Thailand Education Hub for ASEAN Countries Scholarship), Prince of Songkla University, Hatyai campus, Songkhla province, Thailand, for sponsorship of research-based studies.

Authors' contributions

M. J. Adeyemi: conceptualization; methodology; data analysis; writing-original draft. **T. A. Orhadahwe:** conceptualization; data analysis; writing-original draft; review and editing. **F. O. Adeyemi:** review and editing; software. **S. Chaiprapat:** conceptualization; methodology; supervision; review and editing.

Abbreviations

BMP: biochemical methane potential
$CaCO_3$: calcium carbonate
CH_3COOH: ethanoic acid
CH_4: methane
C-H-N-S-O: carbon, hydrogen, nitrogen, sulphur, oxygen
C/N: carbon-nitrogen ratio
H_2S: hydrogen sulphide
HHV: higher heating value
ISR: inoculum-to-substrate ratios
LHV: lower heating value
Lk: Longkong
MC: moisture content
MLSS: mixed liquor suspended solids
MLVSS: mixed liquor volatile suspended solids
$NaHCO_3$: sodium bicarbonate
NG: Napier grass
NH_3: ammonia
PH: potential hydrogen
TA: total alkalinity
TMP: theoretical methane potential

TS: total solid
VFA: volatile fatty acid
USA: United States of America
VS: volatile solids

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