

Original Research Article

Anaerobic Co-digestion of Pineapple Wastes with Cow Dung: Effect of Different Total Solid Content on Bio-methane Yield

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Abstract: The abundance of agricultural wastes produced from pineapple processing and livestock industries has resulted in the difficulties of disposing of a large amount of waste. Anaerobic digestion is a way to reduce waste and generate renewable energy sources including biogas. In this study, pineapple waste is co-digested with cow dung in batch experiments under mesophilic temperature at $38\pm 1^\circ\text{C}$ at a working volume of 100 ml in 125 ml serum bottle. The effects of the total solid on methane yields are investigated at a different substrate ratio. The batch study is conducted at 3 different total solid which are 12%, 20% and 28% and at three different substrate ratio cow dung to pineapple waste (CD: PW) (1:1, 1:2 and 1:3). Daily biogas collection for 28% total solid at 1:1 ratio results in the highest cumulative biogas production of 313 ml, followed by 28% total solid at 1:3 ratio with 246 ml biogas yield. The highest methane yield is achieved at 12% total solid with a 1:2 ratio (17.19 $\text{CH}_4/\text{g VS}$). Results show that at 12% total solid produces the highest methane yield at all ratios compared to other total solid percentages. Moreover, methane yield decreases as the total solid percentage increases from 12% to 28%. Overall, the production of methane from pineapple wastes co-digested with cow dung is proven to be a good strategy to minimise solid wastes.

Keywords: Biogas; co-digestion; cow dung; methane; pineapple; total solid

Received: 8th August 2020

Accepted: 10th September 2020

Published: 30th September 2020

Citation: Aili Hamzah AF, Hamzah MH, Ahmad Mazlan FN, *et al.* Anaerobic co-digestion of pineapple wastes with cow dung: Effect of different total solid content on bio-methane yield. *Adv Agri Food Res J* 2020; 1(1): a0000109. <https://doi.org/10.36877/aafj.a0000109>

1. Introduction

Malaysians generated about 38,142 tons of waste daily in 2018 according to The Star Online and there was a tremendous upsurge in the amount of waste generated in Malaysia in 2018 compared to 19,000 tons waste per day in 2005 in which 44.5% of them were food waste (Chu, 2019). Through anaerobic digestion, renewable resources especially food waste can be utilised as energy and nutrient. In an anaerobic environment, anaerobic digestion process works by decomposing the organic matter by microbes and produces 50–75% methane (CH₄), 19–34 % carbon dioxide (CO₂) and less than 1% biohydrogen (H₂) (Elina, 2016; Jehan *et al.*, 2017). During anaerobic digestion, through the metabolic action of methanogenic bacteria, wastes are converted biochemically into methane. The reactions are carried out by various groups of microbes at four different stages named hydrolysis, acidogenesis, acetogenesis and methanogenesis (Deressa *et al.*, 2015; Mata-Alvarez *et al.*, 2000; Ogunleye *et al.*, 2016).

Nowadays, there are few methods that are subjected to anaerobic digestion: single-phase digestion, two-phase digestion, dry fermentation and co-digestion (Deressa *et al.*, 2015). Three major issues or difficulties have been reported, namely; the effect operational factors, the nutritional imbalance and lack of diversified microorganisms via direct utilisation of substrates or mono-substrates in anaerobic digestion (Hagos *et al.*, 2017). Here, it is suggested that the co-digestion process can be performed to reduce the aforementioned problem, like incorporating agricultural by-products with livestock manure. Several lines of evidence have established that co-digestion is an economically feasible and promising technology in terms of good synergism in the digestion reactor and the ability to improve stability of the process, as well as to improve the biogas production rates (Astals *et al.*, 2013; Giuliano, 2013; Mata-Alvarez *et al.*, 2000). Anaerobic digestion is often categorised based on total solids (TS) content classes. Three of the foremost classes based on substrate total TS content — wet for TS lower than 15%; dry for TS lower than 25%; and solid-state operated at up to 40% TS content (Abbassi-Guendouz *et al.*, 2012; Rabii *et al.*, 2019; Yi *et al.*, 2014).

In the pineapple sector, a lot of agricultural waste from pineapple is produced from various harvesting activities (Chakravarty, 2016; Chan, 2000). Currently, according to Rani and Nand (2004), lack of the appropriate method of managing these wastes has become an environmental concern. Converting pineapple wastes into value-added products is one of the promising ways to handle these wastes without destroying the environment (Khai *et al.*, 2014; Maneeintr *et al.*, 2018). Biogas can be produced from peel, core, pulp and crown of pineapple waste, but the amount of nitrogen in these wastes is insufficient to produce a high amount of biogas. A high amount of carbohydrates in pineapple wastes can be used as a carbon source

for microbial fermentation to produce bio-methane (Khai *et al.*, 2014). Livestock such as cows and buffaloes produce lots of manure and most of the manure ends up as waste. Livestock manure is the main organic waste that contains a high quantity of nitrogen (N) and phosphorus (P), a high percentage of these substances causes nutrient imbalance and failure to properly manage these wastes leads to environmental pollution (Chakravarty, 2016; Deressa *et al.*, 2015). Bacteria found in cow dung are known to produce a high yield of methane gas (Gupta *et al.*, 2016). According to Neshat *et al.* (2017), facultative and obligative bacteria are responsible for hydrolysis and acidogenesis and several bacterial genera including *Corynebacterium*, *Lactobacillus*, *Escherichia coli* and *Actinomyces* were isolated from an anaerobic digester.

Lignocellulosic wastes are ineffective to be used as the sole substrate and hard to digest due to their complex structure that hinders their biodegradability. While manure contains useful microorganisms that can enhance biological activity and about 10 pounds of nitrogen were found in per ton of cow dung (Neshat *et al.*, 2017). However, the carbon insufficiency in cow dung cannot completely fulfil the anaerobic digestion requirements. Temperature is one of the important parameters in anaerobic co-digestion. Thus, it has been suggested to be incorporated with carbon-rich wastes. Higher operational temperature requires higher energy demand and that makes mesophilic temperature preferable compared to thermophilic (Alemahdi *et al.*, 2015). The mesophilic temperature should be maintained at 20°C to 40°C. Hydraulic retention time, total solid (TS), volatile solids (VS), initial cultivation pH, temperature, C/N ratio and the ratio of pineapple waste to cow dung are some of the common operational parameters that need to be considered when operating anaerobic digester. To ensure high biogas yield, optimisation of these parameters is necessary, but it depends on the type of waste and operation used. Thus, all of these facts suggest for us to optimise the biogas production process from pineapple waste and cow dung using anaerobic co-digestion in mesophilic condition but due to several limitations, only TS factor is chosen as a preliminary study. The current study aims at investigating the effect of TS added in anaerobic co-digestion of pineapple waste with cow dung at different ratios. Hence, incorporating lignocellulosic materials such as pineapple waste and nitrogen-rich waste like cow dung has been seen as a good strategy in managing waste.

2. Materials and Methods

2.1. Substrate Preparation

The wastes of Sarawak pineapple were collected from sundry supermarkets around Serdang, Selangor area. The pineapple wastes consisting of pulp, core, peel and crown were ground using an electric blender. The cow dung was collected from Ladang 16 at UPM, Selangor. The cow dung and pineapple wastes were stored at 4°C and later used as substrates

for anaerobic co-digestion. The substrates were first characterised on their total solid (TS), volatile solids (VS), carbon (C) and nitrogen content (N) and pH. The carbon and nitrogen were analysed by using CHN628 Series Carbon, Hydrogen, Nitrogen Determinator (LECO, United States) and the values were used to determine the C/N ratio of the substrate. Meanwhile, TS and VS were conducted based on the standard method (APHA, 1998). Then, the pH of the substrates was measured using pH5SS Spear pH Tester (IONIX, Singapore).

2.2. Experimental Setup

The anaerobic co-digestion experiment was conducted at 100 ml working volume with 25 ml headspace in 125 ml serum bottles as shown in Figure 1. Pure nitrogen gas was flushed into the serum bottles for 2 minutes to remove oxygen traces as well as to ensure the anaerobic condition and the bottles were enclosed with rubber stoppers. Co-digestion was conducted at mesophilic temperature ($38\pm 1^\circ\text{C}$) in water bath WNB 45 (Mettler, Germany) for 30 days. The gas was collected for 30 days to observe the difference of gas produced in each day. The water displacement method was used to measure cumulative biogas production (Jehan *et al.*, 2017). Abbassi-Guendouz *et al.* (2012) stated that the threshold value of the TS solid of the feedstock is 30% since the methanogenic activity was strongly inhibited above that value. Meanwhile Fernández *et al.* (2008) observed that the reactor had significantly high performance when TS content was adjusted to 20%. Ardaji *et al.* (2016) observed that biogas production increased when TS increased from 5 to 25%. Thus, this study was conducted at TS at 12% to 28%. The bottles containing the mixture of pineapple waste and cow dung were at 3 different ratio CD: PW of 1:1, 1:2 and 1:3, respectively. The pineapple waste slurry was first diluted to 3 different total solids (TS) of 12%, 20 % and 28 % of pineapple waste added using distilled water (Abbassi-Guendouz *et al.*, 2012; Ardaji *et al.*, 2016; Fernández *et al.*, 2008). The initial pH of both substrates was low; the final pH after mixing both substrates was still insufficient to be maintained around the optimal pH. Thus, the pH of substrates was adjusted to 7 ± 1.0 using sodium hydroxide (NaOH) (Jehan *et al.*, 2017). In order to produce methane and biogas effectively, it is crucial to maintain pH, C/N ratio and temperature at the optimal condition to provide better growth conditions for methanogenic bacteria. Hence, pH value must be maintained at 6.5 to 8.0 (Ogunleye *et al.*, 2016; Sibiya *et al.*, 2014). The experiments were triplicate, and biogas was collected daily and its composition was analysed.

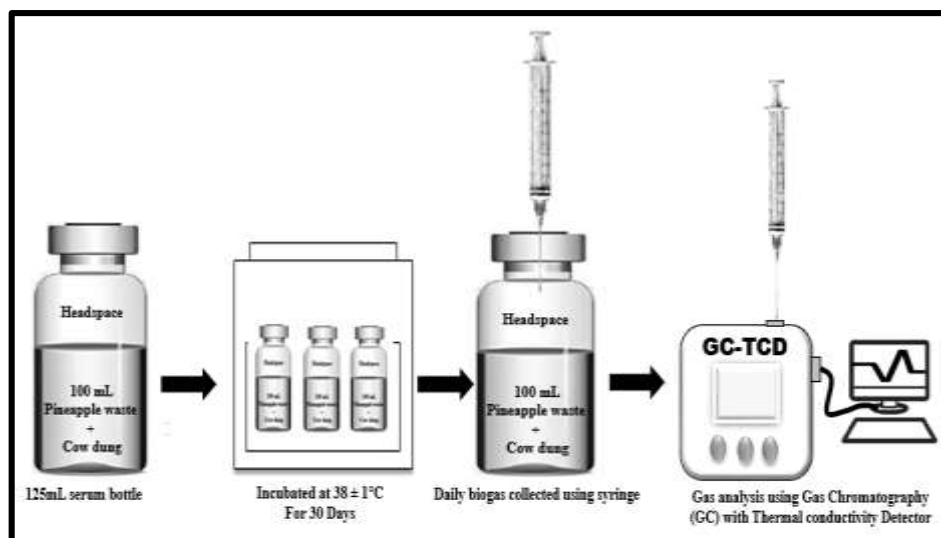


Figure 1. The co-digestion of pineapple waste and cow dung process.

2.3. Biogas Measurement and Analysis

The composition of the daily biogas produced was determined by a gas chromatograph (GC) (Agilent 6890) with thermal conductivity detector (TCD). Methane (CH₄) gas quantity in 1 ml sample was calculated using Equation 1 (Alemahdi *et al.*, 2015; Khairul *et al.*, 2018). The methane yield was expressed as the volume of methane produced based on the initial total VS of the feedstock.

$$\text{CH}_4 \text{ area} / 2291.67 = \text{qCH}_4 \text{ quantity in 1ml gas sample} \quad (1)$$

3. Results and Discussions

3.1. Characteristics of Pineapple Waste and Cow Dung

It is crucial to examine the characteristic of the waste before conducting the co-digestion process, pineapple waste and cow dung were first characterised based on their TS, VS, carbon content, nitrogen content, C/N ratio and pH, and the results are summarised in Table 1. It can be seen from the characterisation results, the TS, VS and pH of the pineapple were 79.35%, 4.95% and 4.82%, respectively while 81.86%, 6.25 % and 6.81% for cow dung, respectively. In accordance with that, cow dung had the highest TS, VS and pH compared to pineapple waste. Formerly, Rani and Nand (2004) reported that the TS, VS and pH of pineapple as 72.57%, 95.90% and 4.7% whereas Anhuradha and Mullai (2010) stated that TS and VS of cow dung were 15.32% and 77.50 %, respectively. High TS value suggests that there is low water content in the wastes (Ardaji *et al.*, 2016; Liotta *et al.*, 2014). Apart from that, pineapple waste has high carbon content compared to cow dung. The C/N ratio of pineapple waste and cow dung were 44.95 and 55.51, respectively, in which the ratio was

determined from the carbon and nitrogen content from the CHNS analyser. C/N ratio in this study was relatively higher than other studies (Anhuradha & Mullai, 2010; Arelli *et al.*, 2018; Bardiya *et al.*, 1996; Dahunsi, 2019). Low nitrogen value in waste contributes to the higher C/N ratio (Arelli *et al.*, 2018; Dahunsi, 2019). Based on the literature, C/N of anaerobic digestion should be maintained at a ratio range of 20–30, and unsuitable C/N ratio can contribute to the increase of total ammonia nitrogen (TAN), free ammonia or high volatile fatty acids (VFAs) accumulation (Kainthola, Kalamdhad, & Goud, 2019). Excessive ammonia concentration inside anaerobic digester contributes to elevated pH in the digester, which is toxic to methanogens as well as leads to microbial growth inhibition (Rabii *et al.*, 2019).

Table 1. Characterisation of pineapple waste and cow dung.

Parameter	Pineapple Waste	Cow Dung
Total Solid (TS)	79.35%	81.86%
Volatile Solid (VS)	4.95%	6.25%
Carbon content	39.79%	8.90%
Nitrogen Content	0.89%	0.16%
C/N ratio	44.95	55.51
pH	4.82	6.81

3.2. Cumulative Biogas Production

The cumulative biogas production for all TS at different CD: PW ratio is shown in Figure 2 (a)–(c). According to the results, the highest cumulative biogas was produced by 28% TS at 1:1 ratio of CD: PW followed by 28% TS at 1:3 ratio. In contrast, the lowest cumulative biogas was produced by 12% TS at 1:3 ratio of the substrate. Comparing the cumulative biogas production for 12%, 20% and 28% at all CD: PW ratio of the substrate, 28% of TS produced the highest biogas after 30 days compared to the other TS. According to Ardaji *et al.* (2016), at high TS, the amount of organic matter increase could benefit the degradation process. The higher the TS percentage added, the higher the biogas produced. As observed in Figure 2(a), initially the biogas produced by 28% was lower than 20% and 12% but biogas produced kept increasing after day 3 as more substrates were degraded by the microbes. The same trends were showed by 28% TS in Figure 2 (b), where the amount of biogas produced was lower than 20% but the biogas produced was higher than all TS after day 23. Meanwhile, in 1:3 ratio as shown in Figure 2 (c), the biogas produced in 28% was always higher than all TS from the beginning until the end of the fermentation period. Even so, the composition of biogas is essential to be examined as the biogas produced contains methane and other gas components.

This finding was in accordance with those reported by Ardaji *et al.* (2016) who observed the effect of TS adjustment on biogas production from co-digestion of pomegranate waste,

poultry manure and cow dung sludge. They found out that biogas production increased from 0.273 L/day to 0.736 L/day with an increase in TS from 5% to 25% and the highest TS which was 25% produced the highest biogas production. Complementary to this, it has also been reported that co-digestion of pineapple, orange, apple, banana and jackfruits produce more biogas (975ml) compared to pineapple alone (900 ml) due to the high concentration of solids and total viable count of microbes than the sole fruit substrate (Chakravarty, 2016).

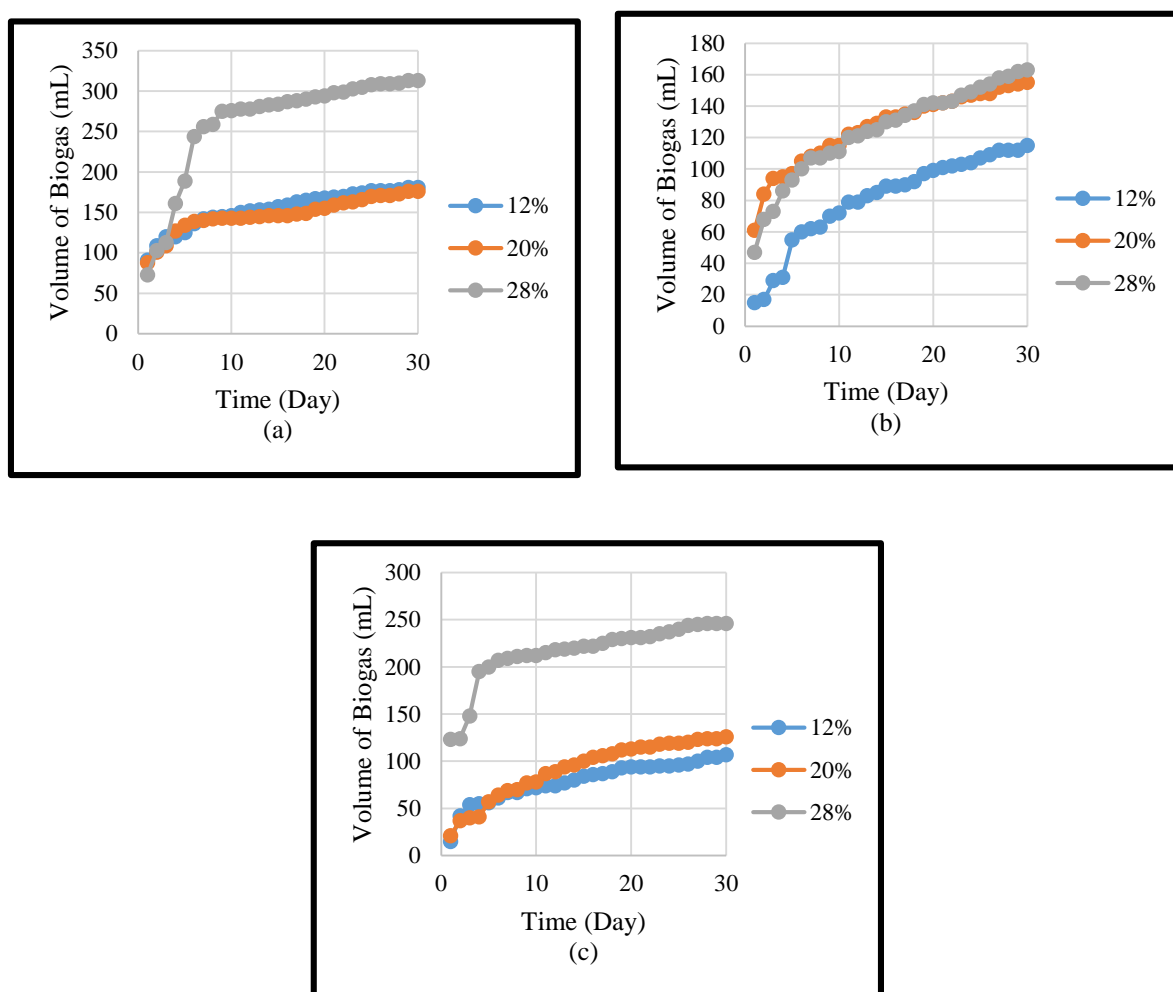


Figure 2. Cumulative biogas production for CD:PW ratio (a) 1:1; (b) 1:2; (c) 1:3.

3.3. Methane Yield from Batch Fermentation

The effect of TS on the cumulative methane yield at different ratio over 30 days of fermentation is shown in Figure 3 (a)–(c). According to Figure 3 (a), at 1:1 CD:PW ratio, 12% TS showed a constant increase in methane yield over 30 days of the fermentation period. Whereas at 20 % and 28% TS, methane production was slower towards the end after 5 days. At 1:2 ratio CD: PW, all TS showed increasing trends in methane production (Figure 3 [b]). For 12% TS, methane produced higher methane compared to 20% and 28% TS and increased

to the maximum yield after 5 days. Then, as illustrated in Figure 3 (c), 12% and 20% TS showed a constant increase. However, after 23 days, there was no methane produced for 20% TS while for 12% TS, methane was still being produced after 27 days. On the other hand, the amount of methane produced was the lowest and slow since the start for 28% TS since, at high TS, the substrate content was high in lignocellulosic materials thus making it harder for the microbes to degrade it (Abbassi-Guendouz *et al.*, 2012; Fernández *et al.*, 2008).

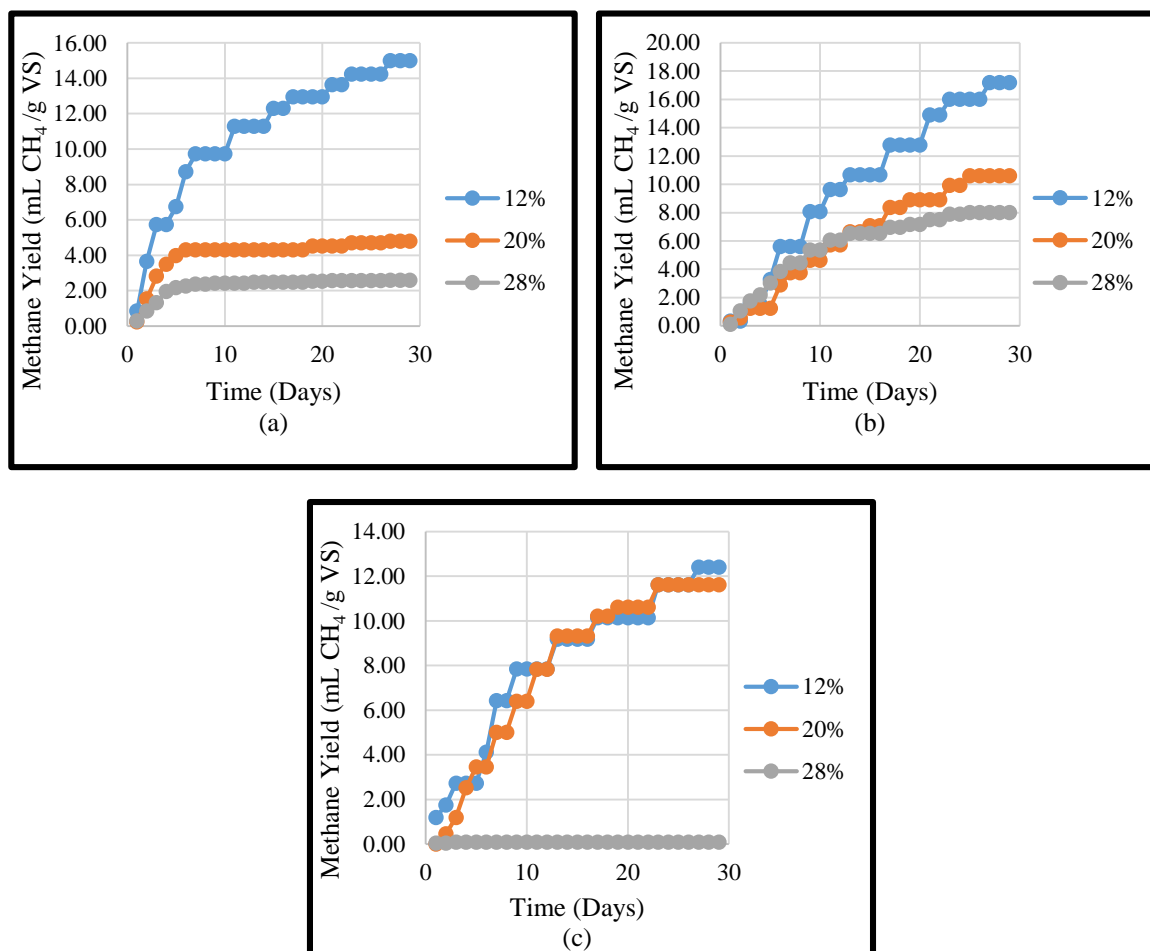


Figure 3. Cumulative methane production for CD:PW ratio (a) 1:1; (b) 1:2; (c) 1:3.

Low methane content suggests slow hydrolysis and low biodegradability of the substrate. To which at higher TS content, the amount of substrate increases while water content decreases, thus contributing to the aforementioned problems (Ardaji *et al.*, 2016; Orhorhoro, Egunilo & Sadjere, 2017). High pineapple wastes in the substrate mixture also make it hard for the microbes to degrade the wastes. According to Jehan *et al.* (2017), a slow rate of degradation of the substrates is due to the complex structure of lignocellulosic biomass such as pineapple that makes it hard for the microbe to digest the substrate. Dahunsi (2019) also pointed out that due to the lignin-cellulose matrix in lignocellulosic biomass, there are limits in the process efficacy during hydrolysis where microbial enzymes fail to degrade these substrates. Lignin-cellulose matrix presence in the lignocellulosic wastes itself makes its

structure very complex and resistant to deconstruction. The exposure of cellulose and hemicellulose to microbial attack during hydrolysis to convert it into simple sugar is interrupted by its cell structure support named lignin (Karuppiah & Azariah, 2019). The lignin layer protects the cellulose and hemicellulose and makes it inaccessible for degradation.

Overall, 12% TS of 1:2 ratio CD:PW resulted in the highest methane yield with 17.19 ml CH₄/g VS followed by 12 % TS at 1:1 ratio with 15 ml CH₄ /g VS after 30 days. Conversely, the lowest methane yield was produced by 28% TS at a 1:3 ratio with only 0.08 ml CH₄/g VS. In this study, it was observed that methane yield decreases as the TS percentage increases at all ratio in which TS of 28 % contributes to the lowest methane yield at all ratio. Even if 28% TS gives the highest biogas produced compared to 12% and 20%, the methane content is low. This is due to the other biogas composition such as carbon dioxide and hydrogen that contribute more in terms of its composition (Khairul *et al.*, 2018; Neshat *et al.*, 2017). Biogas that contains high methane makes it a useful fuel to replace natural gas or can be transformed into heat and electricity (Jafari-Sejahrood *et al.*, 2019).

Similar findings were reported by Ogunleye *et al.* (2016) using animal waste and fruit wastes where the yield of methane decreased with an increase in TS content from 8% to 40%. Liotta *et al.* (2014) also mentioned that increasing TS of food waste from 4.5% to 19.2% contributes to the reduction in methane production. The drop in methane yield as TS increases is due to the amount of water. Water reduces the restriction of mass transfer of the particulate substrate and promotes hydrolysis, making it possible for bacteria growth and movement as well as nutrient transportation (Ejiroghene *et al.*, 2017; Liotta *et al.*, 2014). Thus, at higher TS, low water content causes the transport process to be slower which on the other hand, reduces the level of microbial activity, hence, lowering the methane produced (Liotta *et al.*, 2014).

4. Conclusion

It can be concluded that the co-digestion of pineapple waste and cow dung has proved that the addition of pineapple waste has the potential of increasing biogas. This study focused on the effect of the TS percentage on the biogas and methane yield from the co-digestion process. Co-digestion of pineapple waste at high TS content produces high biogas yield. On the contrary, high methane yield is observed at low TS percentage although, at low TS content, the amount of biogas is low. A decreasing trend of methane yield is observed when increasing the TS content. Low methane content suggests low water content at high TS which inhibits the microbial activity, thus, reducing the digestion process. Moreover, the presence of complex structure due to the lignin layer that binds cellulose and hemicellulose in pineapple wastes makes it harder for microbes to degrade the substrate and consequently slows down the hydrolysis process. Further improvement should be implemented and developed to improve the efficacy of the process such as pretreatment of pineapple waste itself and make it accessible for the microbe to degrade the wastes. Thus, it was proven that

successful implementation of the anaerobic co-digestion as a method of waste treatment has the potential to change the concept of ‘waste to wealth’ which will lead to total utilisation of renewable energy resources in reducing energy requirement, making it readily available and minimising environmental pollution.

Author Contributions: Conceptualization, AFAH and FNAM; Formal analysis, AFAH and FNAM; Investigation, AFAH and FNAM; Methodology, AFAH and FNAM; Supervision, MHH, HCM, NSJ and SIS; Validation, MHH, HCM, NSJ and SIS; Writing — original draft, AFAH; Writing — review & editing, MHH and HCM All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by Geran Putra — Inisiatif Putra Muda (GP-IPM) through Universiti Putra Malaysia Grant (Grant No: GP-IPM/2018/9669700).

Acknowledgments: Thanks and appreciation are acknowledged to the Department of Biological and Agricultural Engineering, Department of Chemical and Environmental Engineering and Smart Farming Technology Research Centre, Faculty of Engineering, Universiti Putra Malaysia, Selangor, Malaysia.

Conflicts of Interest: The authors declare that there is no conflict of interest in this work.

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