Co-digestion of cocoa pods and cocoa leaves: Effect of C/N ratio to biogas and the energy potential

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Abstract. In Indonesia, energy needs are mostly fulfilled by non-renewable fuels. In the longer term, such practices may lead to an energy crisis due to problems of resource continuity and availability. Therefore, the development of renewable energy remains a priority for the Indonesian Government, one of which is from biomass sources. Indonesia has vast and abundant biomass sources available for further transformation to bioenergy. Waste from the cocoa plantation (e.g. cocoa pods and cocoa leaves), for instance, contain organic materials that are potential for biogas production. This research aimed to evaluate the effect of C/N ratio on biogas production and energy potential from the co-digestion of cocoa husk with cocoa leaves. The Biochemical Methane Potential (BMP) test operated under batch and mesophilic condition for 28 days were carried out. The samples tested include blank control, positive control (α-cellulose), and co-digestion samples at different C/N ratio in the range of 22 to 25, prepared in triplicate. The results indicated that pH inhibition was not evident during the digestion process. The findings also confirmed that the co-digestion of cocoa pods with cocoa leaves at a much higher C/N ratio was able to enhance biogas production. Further estimation on the electricity potential showed a potential application of this co-digestion strategy for providing alternative renewable energy sources.

1. Introduction
Energy is a crucial need for supporting all daily necessities and activities. Therefore, global energy demands are continuing to increase alongside the increase in the population [1]. Furthermore, growing energy needs also cause a continuous use of non-renewable energy sources, i.e. fossil fuels based energy, which becoming a threat due to climate change effects [2]. Directorate of Energy, Mineral and Mining Resources [3] stated that Indonesia imports fossil fuels energy (e.g. oil and gas) approximately 356.1 thousand tons per year until 2016, and this number is continuing to increase. Therefore, the development of renewable energy is critical to fulfilling energy needs in Indonesia.

One of the potential resources for renewable energy is biomass, which can be transformed into various bioenergy such as biogas, bioethanol, biohydrogen, and biodiesel. [4]. One of the common and widely
applied technologies is anaerobic digestion (AD) for producing biogas. The AD is operated under mesophilic or thermophilic temperature [5]. AD not only can be implemented for treating wastewater [6] or solid wastes [7]; but also, various biomasses include cocoa pods and cocoa leaves [8]. In 2017, Indonesia’s cocoa production was accounted for 593.8 thousand tons of cocoa fruits, in which 75% of the total production was cocoa pods [9]. While the production of cocoa leaves waste was about 3.14 tonnes/ha/year [9]. Both cocoa pods and cocoa leaves contain materials (i.e. carbon, nitrogen, protein, fat, crude fibre) needed by anaerobic consortia [10], thus the potential for biogas feedstock. Moreover, cocoa leaves contain a high C/N ratio of 48, thus can be used for co-digestion feedstock [11]. C/N ratio of cocoa pods was much lower at the value of 20.7 [12]. There have been various studies highlighted that C/N ratio has influenced the methane or biogas production potential from cocoa husks either in a single- or co-anaerobic digestion system (such as with swine manure, poultry manure) [13-15, 27]. However, the limited study was found on focusing co-digestion of cocoa pods with cocoa leaves which concern on the impact of the C/N ratio.

In AD, the C/N ratio is one of the operational parameters important for ensuring stable biodegradation with the optimal range of 20-30 [16]. Inadequate C/N ratio may inhibit the microbial activity in degrading the organic matter into biogas, which potentially reduces biogas or methane production. Therefore, this study aimed to evaluate the effect of the C/N ratio in the co-digestion of cocoa pods with cocoa leaves.

2. Materials and Methods
This research was carried out at the Laboratory of Bioindustry, Department of Agro-industrial Technology, Faculty of Agricultural Technology, Universitas Brawijaya from June – December 2019.

2.1. Cocoa pods, cocoa leaves and inoculum
Cocoa pods and cocoa leaves were collected from PT. Kampung Coklat in Blitar City by using the grab sampling method. The cocoa pods with yellow colour and the cocoa leaves with brown and dry characteristics were used in this study. No further pre-treatment was applied to both cocoa samples. All samples were analysed upon arrival at the Laboratory of Bioindustry. Parameters analysed include C/N ratio, total solids (TS), volatile solids (VS), ash, moisture content (MC), crude protein, crude lipids, crude fibre, and calorific value (CV).

Digestate collected from a mesophilic digester treating cattle slurry at Balai Besar Pelatihan Peternakan in Batu City was used as inoculum in this study. The inoculum was prepared based on the procedures explained in our previous studies [17-20]. Parameters analysed for inoculum include TS, VS, ash, and MC.

2.2. Mixture substrate preparation
The mixing ratio of cocoa pods and cocoa leaves added was based on the level of C/N ratio used in this study. The carbon and nitrogen concentration of cocoa pods and cocoa leaves were previously analysed. Then, using the formula from Ismayana et al. [21], the number of cocoa pods and cocoa leaves added into the mixture was obtained (See Table 1).

\[
\frac{C}{N} = \frac{\left((%C \text{ cocoa pods} \times W_1)+\left(%C \text{ cocoa leaves} \times W_2\right)\right)}{\left((%N \text{ cocoa pods} \times W_1)+\left(%N \text{ cocoa leaves} \times W_2\right)\right)}
\]  

Where C is the percentage of carbon in biomass samples, N is the percentage of nitrogen in biomass samples, Wn is the percentage of cocoa pods or cocoa leaves added in the mixture to reach the targeted C/N ratio.
Table 1. Substrate added to each mixture.

| Sample ID       | Cocoa pods (%) | Cocoa leaves (%) |
|-----------------|---------------|-----------------|
| C/N ratio 22 (P1) | 0             | 100             |
| C/N ratio 23 (P2) | 33            | 67              |
| C/N ratio 24 (P3) | 67            | 33              |
| C/N ratio 25 (P4) | 100           | 0               |

2.3. Experimental set-up for BMP test

The experimental design used a completely randomised design (CRD) with one factor of the C/N ratio composed of four levels (i.e. C/N ratio of 22 (P1), 23 (P2), 24 (P3), and 25 (P4)). Two control samples used included the control inoculum (inoculum only) and the control positive (inoculum with α-cellulose). All samples were prepared in triplicate. The procedure for the BMP test was, according to Suhartini et al. [17-20], with some modification. The BMP test was operated for 30 days at a mesophilic condition (37°C) with a total volume of 175 mL in each reactor. Biogas production was daily measured as the internal pressure using a digital manometer. In details, the experimental design for the BMP test can be seen in Table 2.

Table 2. Experimental design for the BMP test.

| Sample ID       | Cocoa pods: cocoa leaves (g) | Inoculum added (g) | R\textsubscript{FS} |
|-----------------|------------------------------|--------------------|---------------------|
| Blank sample (inoculum only) | 0                            | 175.00             |                     |
| Positive control sample (α-cellulose) | 0.34                         | 174.66             | 6:1                 |
| P1              | 0.47                         | 174.53             | 6:1                 |
| P2              | 0.41                         | 174.59             | 6:1                 |
| P3              | 0.48                         | 174.53             | 6:1                 |
| P4              | 0.41                         | 174.59             | 6:1                 |

2.4. Analysis

The proximate analysis includes total solids (TS), volatile solids (VS), moisture content (MC), and Ash content were analyses using the Standard Method 2540 G [22]. The pH value was measured following the Standard Method 9040C [22]. CV was measure using the Bomb Calorimetry method [23]. The biogas volume calculated following the procedure and formula explained in our previous study [18], reported in a standard temperature and pressure (STP) condition. Theoretical methane concentration was calculated using the Buswell formula [24], while the specific methane production (SMP) was calculated based on the formula explained in a study by Strömberg et al. [25]. The calculation for estimating the electrical potential was based on Suhartini et al. [17]. Data were analysed using statistical analysis R software to measure the reliability of the BMP test.

3. Results and Discussion

3.1. Characteristics of cocoa pods and cocoa leaves

Table 3 shows the characteristics of cocoa pods and cocoa leaves as a single biomass feedstock. The data indicated that both substrates contain high organic matters. When cocoa pods mixed with cocoa leaves to reach C/N ratios of 23 and 24, the proximate analysis results showed that the mixtures have quite similar organic matter as shown by the VS values on the basis of % wet weight (WW) and %TS (Table 3). Furthermore, previous studies reported that cocoa pods contain fermentable sugars, making it faster to be
This result shows that cocoa pods and cocoa leaves have potential as a feedstock for biogas production, either as mono- or co-digestion feedstock. However, the amount of lignin needs to be considered as both substrates were categorised as lignocellulosic biomass. Antwi et al. [27] found cocoa pods contain high lignin concentration (21.29%), thus difficult to break down by the anaerobic microorganism to its strong bond with cellulose and hemicellulose. Therefore, further pre-treatment is advisable following this study.

### Table 3. Characteristics of cocoa pods and cocoa leaves as a single substrate.

| Parameters       | Cocoa pods | Cocoa leaves |
|------------------|------------|--------------|
| TS (% of WW)     | 88.33      | 89.09        |
| VS (% of WW)     | 77.42      | 74.05        |
| VS (% of TS)     | 87.66      | 83.12        |
| MC (% of WW)     | 11.67      | 10.91        |
| Ash (% of WW)    | 10.90      | 15.04        |
| CV (MJ/kg TS)    | 27.2       | 21.95        |
| Crude fibre      | 50.83      | 42.68        |
| Crude Protein    | 9.40       | 9.17         |
| Crude Fat        | 0.58       | 1.13         |
| Total N (%)      | 1.18       | 1.49         |
| C organic (%)    | 29.94      | 32.37        |
| C/N ratio        | 25         | 22           |

### Table 4. Characteristics of a mixture of cocoa pods and cocoa leaves.

| Parameters                       | Cocoa pods: cocoa leaves (33:67) | Cocoa pods: cocoa leaves (67:33) |
|----------------------------------|----------------------------------|----------------------------------|
| TS (% of WW)                     | 90.57                            | 89.88                            |
| VS (% of WW)                     | 78.25                            | 77.61                            |
| VS (% of TS)                     | 86.40                            | 86.35                            |
| MC (% of WW)                     | 9.43                             | 10.12                            |
| Ash (% of WW)                    | 12.32                            | 12.27                            |
| C/N ratio*                       | 23                               | 24                               |

Note: *results of calculation [18]

### 3.2. BMP test results
#### 3.2.1. pH
Table 5 shows that the pH of all reactor samples before and after the BMP test was well within the average optimum pH value of 6.5-8.0 [28]. The finding also suggests that no inhibition was evident due to pH in the anaerobic mono- and co-digestion of cocoa pods with cocoa leaves.
Table 5. Characteristics of samples before and after the BMP test.

| Sample ID                  | pH Before | pH After | MC (%WW) Before | MC (%WW) After | Ash (%WW) Before | Ash (%WW) After | TS (%WW) Before | TS (%WW) After | VS (%WW) Before | VS (%WW) After | VS (%TS) Before |
|----------------------------|-----------|----------|-----------------|----------------|-----------------|-----------------|----------------|----------------|----------------|----------------|-----------------|
| Blank sample (inoculum only) | 7.4       | 7.3      | 98.37           | 0.73           | 1.63            | 0.9             | 55.21          |                |                |                |                  |
| Positive control sample (α-cellulose) | 7.5       | 6.8      | 96.72           | 0.55           | 3.28            | 2.73            | 83.23          |                |                |                |                  |
| P1                         | 7.3       | 7.2      | 97.75           | 0.42           | 2.25            | 1.83            | 81.33          |                |                |                |                  |
| P2                         | 7.2       | 7.3      | 98.26           | 0.20           | 0.74            | 0.54            | 72.97          |                |                |                |                  |
| P3                         | 7.3       | 6.8      | 98.38           | 0.34           | 1.61            | 1.28            | 79.50          |                |                |                |                  |
| P4                         | 7.3       | 7.2      | 98.86           | 0.28           | 1.14            | 0.86            | 75.44          |                |                |                |                  |

3.2.2. Proximate analysis results - digestate

The characteristics of digestate after the BMP test are shown in Table 5. The data demonstrates that the organic fraction (VS) remained in the digestate (Table 3). The presence of organic fraction was found to provide significant benefits if applied into the soil, either directly or pre-processed with composting. A previous study by Makádi et al. [29] found that organic fraction in the digestate can amend the soil organic matter (SOM), thus influenced soil fertility.

3.2.3. Specific methane production

Figure 1 shows the SMP values of mono- and co-digestion of cocoa pods with cocoa leaves under different C/N ratios. The figure shows that all treatments have a lag phase started from day 2 to day 18 or day 20. This was followed by constant biogas/methane production up to day 27; then these started to decline. Such behaviour was evident to treatment P1, P2, and P4, respectively. While for treatment P3, a decrease in SMP was initiated on day 20. The average SMP of all treatments was in the range of 0.154 – 0.164 m³ CH₄/kg VS (Figure 2), with P2 produced the highest SMP compared to that of other treatments. Such behaviour was possibly due to the VS content of the mixture in P2 treatment was higher than other treatments, as shown in Table 3. According to Orhorhoro et al. [30] that biomass substrates contain high VS value will generate more biogas as it acts as food sources for the microorganism to consume.

![Figure 1. The SMP of mono- and co-digestion of cocoa pods with cocoa leaves under different C/N ratio.](image)

The data also indicated that SMP of cocoa pods and cocoa leaves, either as mono of co-digestion, were still low, which was possibly due to its organic compositions, such as fibres or its lignin content. Cocoa
Pods and cocoa husks were found to have high lignin content [27, 31], thus need a longer time to breakdown [32]. Another factor that may cause a low SMP production is due to no pre-treatment was subjected to biomass samples for reducing the lignin content, i.e. breaking the lignin strongly bound to cellulose and hemicellulose. Tian et al. [33] stated that pre-treatment to lignocellulosic biomass could enhance microbial degradation.

Figure 2. Correlation between SMP and C/N ratio. Lower case letter indicates a not significant difference (BNT at α=0.05). Error bars represent standard errors from three replication.

Figure 2 also shows the correlation between the C/N ratio and SMP of all treatments. The results indicated that changes in the C/N ratio in the range of 22-25 have no significant influence on the SMP values. The data shows that P2 (with a C/N ratio of 23) has the highest SMP, followed by P4, P3, and P1. This was indicating that digesting cocoa pods alone generated lower biogas and methane potential, compared to that of digestion cocoa leaves alone or co-digestion of cocoa pods with cocoa leaves. The data also indicated that the mixing ratio of cocoa pods and cocoa leaves (100:0, 33:67, 67:33, and 0:100), to reach the targeted C/N ratio, generated similar methane potential. Thus, the finding further confirmed that both cocoa pods and cocoa leaves are potential to be used in the AD system, either as single- or co-digestion feedstock. Co-digesting cocoa pods with cocoa leaves were found to increase biogas and methane production. A previous study also reported that co-digestion could generate higher biogas or methane production [34].

From the SMP values and on the basis of 10,000 kg WW substrates samples, the electrical potential was estimated to be in the range of 1,192-1,284 kWh (see Table 5). Feedstock samples with higher SMP values tend to produce more electrical energy potential. The data also indicated that co-digestion of cocoa pods with cocoa leaves, as shown in P2 and P3 treatment, generated much higher energy potential compared to single digestion of cocoa pods or cocoa leaves. This finding demonstrated that co-digestion could be a promising method in improving the methane potential and energy potential. A previous study by Dahunsi et al. [13] also showed that co-digestion of cocoa pod husks with poultry manure, and combined with H2O2 pre-treatment, improve the electrical energy balances and economy feasibility (i.e. profitability). Similarly, Acosta et al. [14] also suggested that co-digestion of cocoa waste may result in higher energy potential compared to that of single-digestion.
Table 6. Estimation of the electrical potential of AD of cocoa pods and cocoa leaves.

| Treatment ID | SMP  | Electrical potential (kWh) |
|-------------|------|----------------------------|
| P1          | 0.154| 1192.27                    |
| P2          | 0.164| 1283.30                    |
| P3          | 0.161| 1249.52                    |
| P4          | 0.163| 1207.07                    |

3.3. Options for valorisation of cocoa pods and cocoa leaves

Various studies have emphasised that cocoa pods are potential biomass resource for further valorisation into bioenergy and biobased products with the biorefinery approach [35-37]. Ghysels et al. [35], for instance, stated that combining AD with slow pyrolysis technology generated not only biogas but also biochar and bio-oil. Another study by Elleta et al. [36] also reported that cocoa pods could be transformed into natural adsorbent for water treatment. Lu et al. [37] added that cocoa pods could be used as biofertiliser through composting, soap making, animal feed, activated carbon, paper making, bioenergy, chemicals dietary fibres, and dietary antioxidants (phenolics). Other potential valorisation of cocoa pods includes antioxidant compounds (i.e. flavanols, phenolic), theobromine, caffeine, and cocoa butter (i.e. fatty acid) [38]. While for cocoa leaves, reported that cocoa leaves are potential sources for the production of antioxidants (i.e. polyphenol extract) [39, 40], compost [41], purine alkaloid (i.e. theobromine) [42]. Yet, in Indonesia’s case, further research, development, and implementation of valorisation of cocoa pods and cocoa leaves are critically needed.

4. Conclusions

The findings in this study revealed that cocoa pods and cocoa leaves are potential biomass for further valorisation as biogas in a single- or co-digestion system. Low SMP values were may be due to fibre content (i.e. lignin) in the sample feedstock. C/N ratio from 22-25 has no significant impact of SMP from the AD of cocoa pods and cocoa leaves. Co-digestion of cocoa pods with cocoa leave enhances biogas production. Thus it contributes to higher electrical potential.

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