Effect of mixture ratio on co-digestion of vegetable and fruit waste with macro-algae, chicken manure and tofu dregs

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Abstract. Fruit and vegetable wastes (FVW) are generated in many traditional markets in Indonesia and mostly disposed directly to landfill. Various studies highlighted that FVW could be further valorised either as bioenergy resources or other high-value products. In this study, the biochemical methane potential (BMP) and its electrical energy potential from co-digestion of FVW with macro-algae (MA), chicken manure (CM) and tofu dregs (TD) were investigated. The research design used was a Completely Randomised Design (CRD). All treatments were prepared in triplicate at a ratio of 100:0; 70:30 and 50:50, respectively. All individual anaerobic co-digestion tests of FVW with CM and TD were carried out at the organic loading rate (OLR) of 3 kg VS/m\textsuperscript{3}/day. The effects of different feedstock ratio on stability and performance indicators were further evaluated. The biomass characterisation analyses showed that FVW, MA, CM and TD samples have a high amount of carbohydrates, proteins, and fats, indicating their suitability as feedstock for biogas production. All tested samples have pH value within the ideal range of 6.8-8.0, indicating that there was no pH inhibition during the digestion process. Increasing co-digestion feed ratio of FVW: CM (70:30) or FVW: TD (70:30) was found to reduce biogas or methane potential. The theoretical estimation of electrical potential from co-digestion of FVW indicated that changing the feed to a ratio of 50:50, produced higher energy generation. This finding confirmed that co-digestion could be an option to enhance biogas and methane yield due to additional nutrient supply from the co-substrates.

1. Introduction
Municipal and agro-industrial solid waste problems in Indonesia have been highlighted as a great challenge to be tackled due to its degrading impact on environmental quality and safety [1]. Some measures to transform these waste into valuable products is highly essential [2]. The municipal and industrial solid waste
also contains a fraction of organic matter readily available to be transformed by physical, chemicals, biological or thermal methods into a more highly economic output, i.e. bioenergy [3, 4].

One of the municipal solid wastes is fruit and vegetable waste (FVW), generated from the traditional market and highly abundant in Indonesia [5]. Agro-industrial solid wastes, such as from poultry business (i.e. chicken manure/CM) and tofu industry (i.e. tofu dregs/TD) are also immensely available to be further valorised [2, 6]. Besides biomass from organic waste, marine biomass such as macroalgae (MA) has also been emphasised as future biomass resources for renewable energy [6].

These wastes contain high organic matters, therefore have the potential to be transformed into bioenergy and other bio-based products. Zhang et al. [7] stated that anaerobic digestion (AD) is one of the standard technologies widely implemented to transform organic matters, such as from food waste or another biomass, into biogas. The AD could also generate digestate, which was widely known for its positive benefits as a substitute for chemical fertilisers and soil conditioners.

Various studies reported that FVW could be converted into biogas in a single AD process [8, 9], but the biogas and methane production still need to be improved. Co-digestion of FVW is, therefore, was suggested as one of the improvement strategies [10, 11]. Similarly, MA, TD and CM have been reported as a potential feedstock for biogas production, either as single- or co-digestion process [12-15].

Feeding composition and ratio in the anaerobic co-digestion process is one of the factors which may influence the process’s stability, thus biogas and methane production [16, 17]. This study aimed to investigate the influence of feeding ratio on methane potential and electrical energy estimation from co-digestion of FVW with MA, TD and CM.

2. Materials and Methods

This research was performed from March to June 2019 at Laboratory of Bioindustry, Department of Agro-industrial Technology, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia.

2.1. Inoculum and feedstocks

Inoculum (digestate) was obtained from an anaerobic digester at Balai Besar Pelatihan Peternakan in Batu City, treating cattle slurry under mesophilic conditions. The inoculum was prepared based on the procedures explained in our previous studies [6, 12, 14, 18, 19]. Parameter analysed for inoculum included total solids (TS), volatile solids (VS), ash, and moisture content (MC). FVW was collected from traditional market Pasar Induk Gadang, Malang City, East Java Indonesia. TD was collected from the tofu industry in Kendalsari, Malang City. MA (Gracilaria sp.) was collected from Pulokerto village, Pasuruan Regency. MA was obtained from traditional chicken poultry from Batu City. Parameters analysed include TS, VS, ash, MC C/N ratio, crude protein, crude lipids, crude fibre, and calorific value (CV).

2.2. Feedstock preparation

The mixing ratio on the anaerobic co-digestion experimental was calculated on a wet weight basis. The main substrate used was FVW, which then mixed with TD, MA and CM at a ratio of 50:50 and 70:30. Sample with FVW as a single substrate in the AD process was also prepared. All samples were ground to reduce the particle size.

2.3. The Biochemical Methane Potential (BMP) test set-up

A completely randomised design (CRD) with factors of feeding ratio and various co-digestion feedstocks was employed in this study. Control blank (inoculum only) and positive control α-cellulose were prepared to measure the inoculum and microbial activity. Seven samples with different feeding ratio were set, with the inoculum to substrate ratio ($R_{IS}$ of 6), to investigate the effect of additional biomass feedstock on improving biogas or methane production. All samples were prepared in triplicate. The BMP test was
operated for 28 days at 37 °C, with the procedures based on our previous studies [6, 12, 14, 18, 19], following
some modification. The internal pressure was recorded on a daily basis using a digital manometer, which
then converted into biogas volume at standard temperature and pressure (STP) condition using the following
formula [20]:

\[
\text{Volume biogas (mL)} = \frac{P \times Vol \times Vm \times R \times T}{P \times Vol \times Vm \times R \times T}
\]  

Where: P is pressure (in kPa), Vol is headspace volume in the reactor (in mL), Vm is the molar volume of
an ideal gas (22.414 L/mol), T is the temperature (in K), and R is gas ideal constant (8.314 J/K mol).

2.4. Analysis
TS, VS, MC, and ash content were measured following the Standard Method 2540 G, and pH was analysed
based on the Standard Method 9040C [21]. CV was measured using ASTM D240-19 method (i.e. Bomb
calorimetry method) [22]. Buswell equation was used to calculate theoretical methane concentration in this
study, as follows [23]:

\[
C_{t}H_{h}O_{o}N_{n}S_{s} \cdot \frac{1}{4}(4c - h - 2o + 3n + 2s)H_{2}O = \frac{1}{8}(4c + h - 2o - 3n - 2s)CH_{4} + \frac{1}{8}(4c - h + 2o + 3n + 2s)CO_{2} + nNH_{2} + sH_{2}S
\]  

Where: C, H, O, N is the fraction of each element (on a TS basis); c, h, o, n and s are the molar proportion
of mass fraction (each represents for C, H, O, N and S element).

The specific methane production (SMP) was calculated using the formula described in Strömberg et al.
[23], while the estimation of electrical energy potential was following our previous study [6].

3. Results and Discussion
3.1. Inoculum and feedstock characteristics
Table 1 shows the characteristics of inoculum and feedstock as a single substrate. It can be seen that the
feedstock samples have VS values in the range of 39-99 %TS. Both FVW and TD have high VS values,
showing its suitability for feedstock in the AD system. Pavi et al. [25] stated that a high amount of VS
indicated a high potential of converting the organic fraction of biomass substrates into biogas in an AD
process.

| Parameters            | Inoculum | FVW | TD  | MA  | CM  |
|-----------------------|----------|-----|-----|-----|-----|
| TS (% of WW)          | 1.39     | 8.40| 9.63| 84.84| 73.61|
| VS (% of WW)          | 0.86     | 7.32| 9.52| 7.90 | 44.44|
| VS (% of TS)          | 62.33    | 87.16| 98.84| 39.35| 60.37|
| MC (% of WW)          | 98.61    | 91.59| 90.37| 16.34| 26.39|
| Ash (% of WW)         | 0.54     | 1.07| 0.11| 14.59| 29.17|
| CV (MJ/kg TS)         | n.m.     | 6.43| 5.25| 16.27| 6.32 |
| Carbohydrate          | n.m.     | 35.64| 60.52| 50.69| 18.63|
| Crude Protein         | n.m.     | 29.86| 14.28| 17.66| 22.34|
| Crude Fat             | n.m.     | 2.68| 4.54| 0.72 | 1.05 |
| C/N ratio             | n.m.     | 47.64| 16.11| 11.24| 9.72 |

Table 1. Characteristics of inoculum and feedstock as a single substrate.
3.2. Characteristics of co-digested substrates

The characteristics of various co-digested substrates based on different mixing ratio can be seen in Table 2. When combined with TD, the VS values were still high in the range of 88-91% TS. However, when combined with MA and CM, the VS values decreased with an increase in the ratio of co-digestion feedstock added, giving the VS values varied from 51-62 %TS. Despite the VS variation, a reduction of C/N ratio provides an insight that co-digested substrates combination is potential for enhancing the AD process [26]. These data also indicated that FVW added with different co-digestion feedstock at different ratio had influenced the organic material content, which may have an impact on biogas or methane production. This agrees with Bouallagui et al. [10], who reported that methane yield from mono-digestion of FVW and co-digestion of FVW with abattoir wastewater (AW) and waste activated sludge (WAS) was varied due to variation in the composition of the waste mixture.

Table 2. The characteristics of feedstock mixture at different feeding ratio and substrate combination.

| Parameters       | FVW: TD (50:50) | FVW: TD (70:30) | FVW: MA (50:50) | FVW: MA (70:30) | FVW: CM (50:50) | FVW: CM (70:30) |
|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| TS (% of WW)     | 9.62            | 8.74            | 42.14           | 29.83           | 39.47           | 28.17           |
| VS (% of WW)     | 8.71            | 7.72            | 21.69           | 16.11           | 23.15           | 17.35           |
| VS (% of TS)     | 90.59           | 88.32           | 51.49           | 54.03           | 58.65           | 61.61           |
| MC (% of WW)     | 90.38           | 91.26           | 57.86           | 70.17           | 60.53           | 71.83           |
| Ash (% of WW)    | 0.90            | 1.02            | 20.44           | 13.71           | 16.32           | 10.81           |
| pH               | 7.50            | 7.60            | 7.60            | 7.50            | 7.70            | 7.70            |
| C/N ratio        | 31.87           | 38.18           | 31.87           | 38.18           | 28.68           | 36.26           |

3.3. The characteristics of digestate

Digestate’s characteristics from all samples after completion of the BMP test are shown in Table 3. The pH values demonstrated a neutral range of 6.80 – 7.33. The pH values were decreasing with co-digestion of FVW with CM in accordance with the increasing FVW proportion. Ji et al. [27] stated that the low TS and the high VS value of FVW might lead to a faster hydrolysis stage in the AD process, causing both acidification and a reduction in the pH values of the inoculum. However, in general, all samples have pH values within the ideal range for both acidification and methanogenesis bacteria during the AD process [28]. The digestate samples from co-digestion of FVW with TD (70:30) and FVW with CM (50:50) have lower VS, with the value of 1.65 and 1.73 %w/w. A lower VS value in the remained digestate may have associated with higher biogas or methane production [10].

Table 3 also shows that the digestate still contain a high organic faction, which can be potential to be further valorised as bio fertiliser or soil conditioner, either by direct application to land or through composting [29]. Yet, further investigation is still needed to investigate the environmental risk of digestate application in correlation to the accumulation of macro-nutrient (i.e. N, P and K) in the soil, as highlighted by Nkoa [30].

3.4. Specific methane potential

The average specific methane potential (SMP) of all tested samples is shown in Figure 1. The mono-digestion of FVW resulted in an average SMP higher, followed by co-digestion of FVW with CM (50:50). However, the average of SMP from mono-digestion of FVW in this study (0.100 m³ CH₄/kg VS) was
slightly lower than the reported value of 0.160-0.430 m³ CH₄/kg VS [10, 31, 32]. Such different values were possibly due to different properties of FVW, as well as due to the influence of inoculum used, as previously emphasised in various studies [27, 33]. Further improvement is necessary, for example, through the addition of trace element.

In addition, the data shows that the mixing ratio between FVW and co-digested substrates influenced the SMP values. When FVW was co-digested with MA and CM, an increase in FVW proportion was followed by a decrease in the SMP values. For example, co-digestion of FVW with CM (50:50) has higher SMP than that of from co-digestion of FVW with MA (70:30), with the value of 0.084 and 0.038 m³ CH₄/kg VS. However, in the case of FVW co-digested with TD, increasing FVW proportion was parallel with an increase in the SMP values. The co-digestion of FVW with MTD at ratio of 50:50 and 70:30, gave the average SMP of 0.036 and 0.068 m³ CH₄/kg VS, respectively.

| Parameters           | pH  | MC (%ww) | Ash (%ww) | TS (%ww) | VS (%ww) | VS (%TS) |
|----------------------|-----|-----------|------------|-----------|-----------|-----------|
| Control blank        | 7.33| 98.82     | 0.82       | 2.12      | 1.25      | 58.96     |
| Control positive α-cellulose | 7.13| 98.26     | 0.91       | 2.74      | 1.83      | 66.79     |
| FVW (100)            | 7.00| 98.43     | 0.67       | 2.81      | 1.98      | 70.46     |
| FVW:TD (50:50)       | 7.00| 98.12     | 0.75       | 2.41      | 1.87      | 77.59     |
| FVW:TD (70:30)       | 7.17| 98.56     | 0.56       | 2.59      | 1.65      | 63.71     |
| FVW:MA (50:50)       | 7.07| 97.91     | 0.78       | 2.34      | 2.15      | 91.88     |
| FVW:MA (70:30)       | 7.20| 97.67     | 0.52       | 2.94      | 2.29      | 77.89     |
| FVW:CM (50:50)       | 6.95| 97.87     | 0.68       | 2.75      | 1.73      | 62.91     |
| FVW:CM (70:30)       | 6.80| 97.89     | 0.71       | 2.36      | 1.65      | 69.92     |

In addition, the data shows that the mixing ratio between FVW and co-digested substrates influenced the SMP values. When FVW was co-digested with MA and CM, an increase in FVW proportion was followed by a decrease in the SMP values. For example, co-digestion of FVW with CM (50:50) has higher SMP than that of from co-digestion of FVW with MA (70:30), with the value of 0.084 and 0.038 m³ CH₄/kg VS. However, in the case of FVW co-digested with TD, increasing FVW proportion was parallel with an increase in the SMP values. The co-digestion of FVW with MTD at ratio of 50:50 and 70:30, gave the average SMP of 0.036 and 0.068 m³ CH₄/kg VS, respectively.

Figure 1. The average SMP of mono-digestion of FVW and co-digestion of FVW with TD, MA and CM.
Various factors may influence the variation in SMP values from mono- or co-digestion of FVW. The organic fraction of biomass substrates can also be one of the influencing factors [25]. However, Figure 2a shows that this was not implied in this study. Therefore, the composition of co-digested substrates, such as carbohydrates, protein and fat (as shown in Table 1), possibly contributed to influencing the performance of the AD process, as have also been underlined in previous studies [34, 35]. Another possible cause is the C/N ratio of co-digestion mixture. As can be seen from Table 1, co-digestion of FVW with CM (50:50) has C/N ratio within the ideal values for AD process of 25-30 [10]. Therefore it produced higher SMP compared to other samples. Yet, Figure 2b indicates that despite a reduction in C/N ratio from 47.64 to 28-39.18, it still has insignificant impact in improving the SMP values.

Figure 3 shows the estimated electrical energy potential from mono- and co-digestion of FVW, which was in the range of ~317 – ~1947 kWh per 10,000 kg substrate. The substrates with higher SMP tend to produce higher methane volume, thus higher electrical potential [6]. However, the data indicates that energy potential obtained from mono- and co-digestion of FVW is influenced by the composition and amount of
co-digested substrates added, which agreed with previous study [36]. For instance, despite its higher SMP value, mono-digestion of FVW resulted in lower electrical potential (734 kWh) compared to that of from co-digestion of FVW with MA (70:30) at the value of ~995 kWh and FVW with CM (50:50) at value of ~1947 kWh, respectively. This was possibly due to higher moisture content in FVW. Menardo and Balsari [36] also reported that biomass with higher water content produced low methane yield and electrical energy potential [36]. Furthermore, the electrical energy potential was influenced by methane produced and the amount of organic fraction readily available to be transformed into biogas methane [37].

![Figure 3](image.png)

**Figure 3.** The estimated electrical potential on the basis on 10,000 kg of biomass sample (on a w/w basis).

4. Conclusions

This study concluded that the mixing ratio on co-digestion of FVW with TD, MA and CM have impact on biogas production, i.e. SMP values, as well as on the estimated electrical energy potential. Characteristics and composition of co-digested substrates may contribute to affect the stability and performance of an AD system, either in mono- or co-digestion operation. Co-digestion can be an option to enhance biogas and methane yield due to additional nutrient supply from the co-substrates. Yet, further study to investigate the effects of the mixing ratio on co-digestion of FVW with other biomass feedstock on continuous trials over a longer time period is suggested.

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