

Potential of *Gracilaria* sp. as single- or co-digestion feedstock for biogas production

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Abstract. High consumption of fossil fuels in Indonesia needs to be reduced by using renewable energy, such as from biomass. In Indonesia, as one of the largest maritime countries, macroalgae (i.e. seaweed) are also considered as potential renewable biomass feedstocks for substituting any land biomass. This study aimed to investigate biodegradability and biogas potential of macroalgae (*Gracilaria* sp.) as single- or co-digestion feedstock using anaerobic digestion technology. The biochemical methane potential (BMP) test was carried out for 28 days at temperature of 37 °C to investigate the specific methane potential. All samples were in triplicate. Combinations of *Gracilaria* sp. with various biomass feedstocks (i.e. food waste, tofu dregs) were also tested. The findings indicated a potential valorisation of macroalgae for biogas production either as single or co-digestion feedstock, as well as a potential for electricity generation. However, further optimisation process is required for better and higher degradation process.

1. Introduction

A decrease in supply of non-renewable energy including fossil fuels and coals has been highlighted globally [1]. However, energy demands continue to increase due to population explosion and developing industries, resulting in a high price of fossil fuel-based energy [2]. Indonesia is one of the countries experiencing energy dilemma. Therefore, the Indonesian Government has established the National Energy Policy (NEP) to develop and expand the production of new and renewable energy, such as from biomass [3], which include land and marine biomass.

Food waste (FW) and agro-industrial waste (i.e. tofu dregs or TD) have been considered as potential biomass feedstock for bioenergy generation [4, 5]. FW, for example, Kiran et al. [4] reported that the FW production in Indonesia in 2008 was more than 30.90 million tonnes, and it was projected to increase by ~50% in 2025 because of population and economic growth. Indonesian Statistics [6] reported that, in 2015, tofu industries consumed 963.14 thousand tonnes soybeans, which estimated to generate TD of approximately 215.74 thousand tonnes. However, FW contains high organic materials such as carbohydrate, lipids and proteins [7], while TD is high in proteins (33.4% total solids/TS) and crude fibre (54.3%TS) [8], making them suitable feedstock for bioenergy or high value-added products.



Furthermore, globally, due to a change and competition in land use, a shift to marine biomass such as macroalgae as bioenergy feedstock has been greatly acknowledged [9]. Indonesia, indeed, an archipelago country, has a great potential for marine biomass, such as macroalgae [10]. One of macroalgae species locally abundant in Indonesia is *Gracilaria* sp. [11]. *Gracilaria* sp. has high carbohydrate content [12], in which McDermid and Stuercke [13] found that carbohydrate content of *Gracilaria* sp. was mainly in the form of cellulose, thus it has potential as feedstock for bioenergy production such as biogas.

Anaerobic digestion (AD) is biological degradation process of organic material occurring under anaerobic conditions [14], resulting in the production of methane (CH₄), carbon dioxide (CO₂) and residual organic (i.e. digestate) [15]. Several studies have reported the use of macroalgae as feedstock in the AD system for biogas production. For example, *Ulva* sp. has been reported to have a great biogas potential [16-18]. While, Meinita et al. [19] found that *Gracilaria* sp. is also potential for bioenergy production include bioethanol.

Therefore, this study aimed to investigate biodegradability and biogas potential of *Gracilaria* sp., as a single- and co-digestion feedstock in AD, as well as to estimate its electricity potential.

2. Materials and Methods

2.1. Feedstocks and inoculums

FW was freshly collected from the Universitas Brawijaya canteen in Malang, East Java, Indonesia. TD was from a tofu small- and medium-scale enterprise (SME) in Kendalsari, Malang, East Java, Indonesia. FW was then grinded and stored in plastic containers, while TD was directly stored in plastic containers without any particle size reduction pre-treatment. Both samples were kept in a cold storage at Bioindustry Laboratory, before using for Biochemical Methane Potential (BMP) test. Wild macroalgae (WM) from the species of *Gracilaria* sp. was collected from Ujung pangkah Beach, Gresik City, East Java, Indonesia in the month of June in 2018. Upon arrival at Bioindustry Laboratory *Gracilaria* sp. was ground to reduce the particle size (to a size of xx mm) using commercial blender and kept under room temperature. The characterisation of those samples includes moisture content (MC), ash, total solids (TS), and volatile solids (VS).

Inoculum (i.e. organic residue or digestate) for the BMP test was collected from a mesophilic digester treating cattle slurry at *Balai Besar Pelatihan Peternakan* in Batu City, East Java, Indonesia. The inoculum was prepared by sieving the digestate through a 1 mm screen to remove larger particles. The characterisation of inoculum was carried out for the following parameters: pH, temperature (°C), MC, ash, TS, and VS.

2.2. BMP test set-up

A manual BMP system using water bath (37 °C) was used for BMP test, which operated for 28 days in batch condition. Control blank samples were prepared to measure the indigenous methane production from the inoculums. The positive control (α -cellulose) samples were prepared to test the activity of the inoculum. Samples of *Gracilaria* sp. (WM) alone (100% WM), FW alone (100% FW), TD alone (100% TD), and co-digestion of WM with FW and TD (at ratio of 50:50 on a wet weight basis) were tested in this study. All samples were prepared in triplicates with an inoculum to substrate ratio (I/S ratio) of 6. The BMP test was carried out using 250-mL serum bottle with working volume of 40 mL. The biogas production was measured as pressure using a Digitron 2026P absolute pressure meter (Electron Technology, UK) on a daily basis.

2.3. Analysis

TS, VS, MC and ash determination was based on Standard Method 2540 G [20]. pH was measured using a digital pH meter, calibrated in buffers at pH 7 and 9.2. Biogas production was calculated by converting pressure readings to gas volume in the headspace at standard temperature and pressure (STP) of 273.15 K and 101.325 kPa. Elemental analysis was carried out using elemental analyser (628 Series Elemental Determinator, LECO). The theoretical methane concentration was calculated using

Buswell equation [21], with the assumption of 85% organic biomass breakdown. The specific methane potential (SMP) was calculated using the equation below [22]:

$$SMP = \frac{V_S - V_B \frac{m_{IS}}{m_{IB}}}{m_{VS,SS}} \quad (1)$$

Where: V_S is the mean value of accumulated methane volume from reactor with inoculum and substrate, V_B is the mean value of methane volume from reactor with inoculum only (blank), m_{IS} represents the mass of VS of inoculum added in the sample, m_{IB} represents the mass of VS of inoculum added in the blank sample, and $m_{VS,SS}$ represents the mass of added substrate in the reactor.

The theoretical SMP were compared with the measured SMP. Electricity potential estimation was calculated with the assumption of 1 m³ biogas has a calorific value of 22 MJ, and 1 m³CH₄ is equal with 36 MJ. With assumption of electrical conversion efficiency of 35%, therefore 1 m³ biogas will yield 2.14 kWh (electricity) and 1 m³CH₄ will yield 10 kWh.

3. Results and Discussion

3.1. Substrates characteristics

The study showed that FW contains highest organic contents as shown by VS value of more than 24.83 % wet weight (ww), while *Gracilaria* sp. has the lowest organic contents at value of 7.90 % ww (Table 1). In terms of the mixture of WM:FW (50:50) contains a high organic contents of 16.65 % ww, compared to that of WM:TD (50:50) of 12.78 % ww. This result indicated that *Gracilaria* sp., FW and TD are suitable to be used as feedstock in AD process for generating biogas. According to Xue et al. [23], the organic content in biomass feedstock as indicated by the VS concentration could affect the production of biogas.

Table 1. Substrates characteristics

Parameter	WM	FW	TD	WM:FW (50:50)	WM:TD (50:50)
TS (% WW)	84.84	25.69	9.63	22.17	18.38
VS (% WW)	7.90	24.83	9.52	16.65	12.78
VS/TS (% TS)	39.35	96.65	98.84	75.12	69.50
MC (% WW)	16.34	74.31	90.37	77.83	81.62
Ash (% WW)	14.59	1.70	0.11	5.52	0.56

3.2. BMP test results

The BMP test results showed that cumulative biogas production of inoculum sample has only been 26.23 ml per 40 mL of samples used (Figure 1). The figure also indicates that at TD 100%, a rapid biogas production was occurred after a 2-day lag time and reached a plateau after 8 days. A similar trend in a short lag time for biogas production was also evident in other samples. However, for samples of WM:TD (50:50), and WM:FW (50:50) were continued to experience a slight increase until day 20. WM as single- and co-digestion feedstock with TD has similar trend on the cumulative biogas production. The final cumulative biogas production from AD of TD after 28 days incubation at an I/S ratio of 6:1 were as follows 50.20 mL (WM 100), 74.18 mL (TD 100), 64.17 mL (FW 100), 50.02 mL (WM:TD (50:50)), and 60.34 mL (WM:FW (50:50)), respectively. Using the theoretical methane concentration from the Buswell equation, the cumulative methane potential was found to be 25.35 mL (WM 100), 37.46 mL (TD 100), 34.20 mL (FW 100), 25.26 mL (WM:TD (50:50)), and 32.16 mL (WM:FW (50:50)).

The specific methane production (Figure 2) indicated that TD and FW as a single digestion feedstock, both biomass has superior SMP value than WM alone. While digesting *Gracilaria* sp. alone

producing a low SMP. Furthermore, the methane for WM alone was produced only after 9-day incubation period. During day 0 to day 5, the methane production was negative, indicating that there was a problem in the initial digestion, where microorganism consortia need longer time for adaptation. This was possibly due to a high lignin content [24] and salinity concentration [25] in the macroalgae samples.

However, when mixing *Gracilaria* sp. with other biomass feedstock, in this case FW and TD, the methane production started on day 1 and continuously to increase until day 9, then remain stable up to day 28. Methane production was also much higher when using *Gracilaria* sp. as co-digestion feedstock compared to that of using *Gracilaria* sp. alone. This study may potentially indicate that the amount and type of other biomass feedstock affected the biogas production. For example, at the same ratio of 50:50, mixing *Gracilaria* sp. with FW produced more biogas than that of with TD.

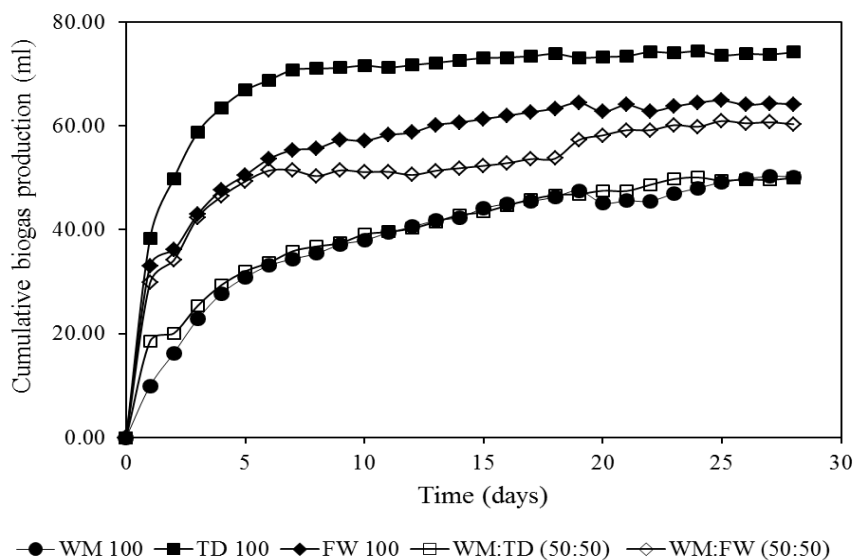


Figure 1. Cumulative biogas production of *Gracilaria* sp. as single- or co-digestion feedstocks compared to FW and TD alone.

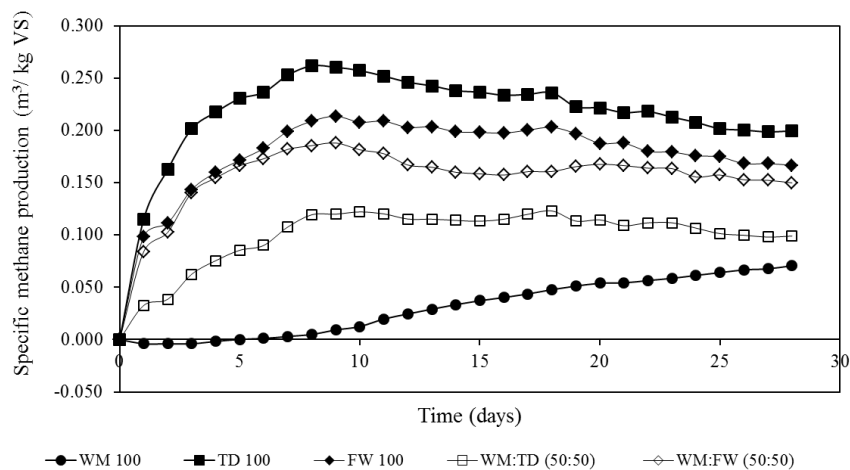


Figure 2. SMP of *Gracilaria* sp. as single- or co-digestion feedstocks compared to FW and TD alone.

In this study, the average SMP value for *Gracilaria* sp. as single feedstock was the lowest compared to other samples, giving the value of $0.060 \text{ m}^3 \text{ CH}_4/\text{kg VS}$ (or $0.043 \text{ m}^3 \text{ CH}_4/\text{kg ww}$). However, this result was higher than the value reported by Kawaroe et al. [26], that digestion of *Gracilaria* sp. alone under batch condition produced $11.6 \text{ L CH}_4/\text{kg ww}$ (or $0.0116 \text{ m}^3 \text{ CH}_4/\text{kg ww}$). The average SMP of other biomass samples were as follows: $0.230 \text{ m}^3 \text{ CH}_4/\text{kg VS}$ (TD 100), $0.192 \text{ m}^3 \text{ CH}_4/\text{kg VS}$ (FW 100), $0.112 \text{ m}^3 \text{ CH}_4/\text{kg VS}$ (WM:TD 50:50), and $0.165 \text{ m}^3 \text{ CH}_4/\text{kg VS}$ (WM:FW 50:50), respectively (Figure 3). The results indicated that using *Gracilaria* sp. as co-digestion feedstock could enhance the SMP of *Gracilaria* sp. as a single-digestion feedstock. Therefore, *Gracilaria* sp. has a great potential to substitute the main feedstock should there are an optimisation of the AD operating parameters. However, further in-depth study is required.

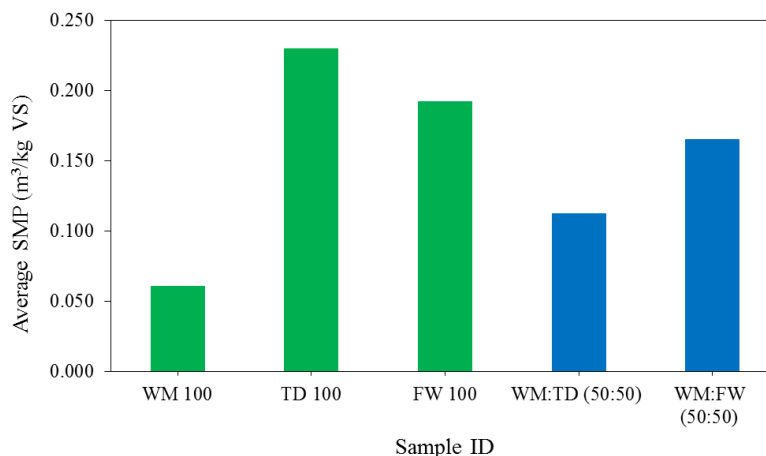


Figure 3. Average SMP values of *Gracilaria* sp. as single- or co-digestion feedstocks compared to FW and TD alone.

3.3. Electricity Potential Estimation

Table 2 shows that the estimation of electricity potential from calculated from 1000 kg of fresh feedstock. The energy potential depends on SMP of each feedstock added in AD process, where the higher SMP generated higher methane volume so as the energy produced. This study demonstrated that *Gracilaria* sp. can also be used as co-digestion feedstock in the AD process. As a single-digestion feedstock, a pre-treatment may be needed to reduce the salinity content of *Gracilaria* sp., to introduce a more stable and faster biodegradation process. Furthermore, based on the electrical potential, FW also has a great potential for AD feedstock. Also, *Gracilaria* sp. when co-digested with FW has exceeded the electrical potency obtained from single- or co-digestion of TD with *Gracilaria* sp. This was possibly due to the high VS content in fresh FW samples. However, an in-depth and comprehensive assessment is further required, including energy and mass balance to obtain net energy production from AD of *Gracilaria* sp. a single- or co-digestion with TD or FW, or possibly other feedstocks.

Table 2. Electrical potential from single and co-digestion of *Gracilaria* sp.

Sample ID	Electrical energy (kWh)
WM 100	186.05
TD 100	218.43
FW 100	460.60
WM:TD (50:50)	143.37
WM:FW (50:50)	275.26

Note: assumption of 1 tonne biomass feedstock

4. Conclusion

The study confirmed that the highest methane potential was obtained from using 100% of TD followed by 100% of FW. Co-digesting *Gracilaria* sp. either with FW and TD has higher SMP compared to that of *Gracilaria* sp. alone, indicating that *Gracilaria* sp. has potential for biogas/methane production as substituting biomass feedstock in the AD system. However, further improvement in AD process of *Gracilaria* sp. is therefore necessary, such as by implementing pre-treatment prior AD process and by adjusting the operational conditions. This study also confirmed that FW has potential to be valorised as electricity via AD pathway route, either as single- or co-digestion feedstock with *Gracilaria* sp.

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