IOP Conf. Series: Earth and Environmental Science **475** (2020) 012064 doi:10.1088/1755-1315/475/1/012064

Effect of pre-treatment on anaerobic biodegradability of *Gracilaria verrucosa*

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Abstract. In global world, increasing needs of energy is parallel to the increase of population. The limitation of land availability and change of land use has turning the focus into marine biomass, especially macroalgae, as renewable energy feedstocks. Indonesia is one of the biggest countries producing marine macroalgae. The aim of this research was to evaluate the impact of washing pre-treatment on the characteristics of macroalgae and its biodegradability in anaerobic digestion process. In this study, a biochemical methane potential (BMP) test was carried out using fresh macroalgae, specifically *Gracilaria verrucosa*. The ratio of inoculum to substrate was 6:1 with operation temperature of 37 °C and incubated for 28 days. Prior the BMP test, the *G. verrucosa* samples were washed with flowing water for 10 minutes. The findings confirmed for washing pre-treatment was significantly enhance cumulative biogas and methane production by more than 50%. The specific methane potential (SMP) of washed *G. verrucosa* increased by six-fold, with the value of 0.108 m^3 CH_4 / kg VS. In this case, reduction of salinity concentration after washing may play an important role in increasing the anaerobic degradability. It was estimated that a high electricity potential is generated from anaerobic digestion of *G. verrucosa* after washing pre-treatment.

1. Introduction

Energy security has been highlighted as one of the main issues faced by Indonesia, where access to affordable and renewable energy is limited [1]. Therefore, the government established Indonesia's National Energy Plan with target of 23% renewable energy production, including from biomass resource [2]. Furthermore, as one of the archipelago countries and due to land availability limitation, Indonesia has a great potential for marine biomass as feedstock for renewable energy production, called as bioenergy such as biogas, bioethanol and biodiesel [3].

Potential marine biomass for generation of bioenergy in Indonesia is macroalgae, also known as seaweed [4], with total production of 10 million tonnes in 2015 and estimated to continuously increase [5]. Local macroalgae abundantly available in Indonesia is mainly from the genus of *Gracilaria* and *Eucheuma* [6]. Krisye et al. [7] reported that *Gracilaria* sp. can potentially be used as feedstock for biogas production using anaerobic digestion (AD) technology, with the value of 32.7 L biogas/kg.

Previous study has also investigated the use macroalgae for bioenergy routes [8]. However, some limitations in the anaerobic digestion of macroalgae were also reported, possibly due to salinity concentration which resulted in an inhibition occurrence during the process [9].

Pre-treatment prior anaerobic digestion process can be one of the feasible options to enhance the biogas or methane production. A study by Tedesco et al. [10] reported that particle size reduction as a way of macroalgae pre-treatment, increase the generation of biogas and methane. Another way is to wash macroalgae aiming to reduce the salinity. Milledge et al. [11] studied the impact of washing on methane production from *Sargassum muticum,* which greatly found in the UK. They found that washed *S. muticum* produced lower biomethane (0.225 m^3 CH_4/kg VS) than unwashed *S. muticum* (0.117 m^3 CH_4/kg VS), which indicating washing pre-treatment may delayed methane production. Lestari et al. [12] found that washing pre-treatment of *Ulva sp.* can greatly reduce the sand, mud as well as the fermentable soluble metabolites, causing a decrease in biogas and methane production. However, there has been a limited study on effect on washing pre-treatment on *Gracilaria verrucosa* prior to AD process, especially in the Indonesia context. Therefore, this study was aimed to evaluate the impact of washing pre-treatment on biogas and methane production from *Gracilaria verrucosa.*

2. Materials and Methods

2.1. Feedstocks and inoculums

G. verrucosa was freshly collected from Ujung Pangkah Beach, Gresik Regency – Indonesia. Fresh *G. verrucosa* was kept at cold storage upon arrival at Laboratory of Bioindustri, Dept. of Agro-industrial Technology, Universitas Brawijaya. Washed and unwashed *G. verrucosa* samples were grinded using a commercial blender, and directly stored in plastic containers and kept at cold temperature, prior the BMP test. The characterisation of both samples was carried out for the following parameters: elemental analysis (C, H, O N), proximate analysis (i.e. moisture content/MC, ash, total solids/TS, and volatile solids/VS), and the calorific value (CV). Inoculum for the biochemical methane potential (BMP) test was prepared from digestate taken from a mesophilic digester treating cattle slurry at *Balai Besar Pelatihan Peternakan* in Batu City. Digestate was collected in a 5-litre container. The digestate was sieved through a 1 mm screen for removing any larger particles. The inoculum was degassed for 48 hours prior the BMP test aimed to eliminate any residual biogas production. The characterisation of inoculum was based on the following parameters: pH, temperature (^oC), MC, ash, TS, and VS.

2.2. Pre-treatment procedures

Washing pre-treatment employed in this study was according to Lestari et al. [12]. Fresh *G. verrucosa* was washed using flowing water for 10 minutes. The washed *G. verrucosa* was drained. Both unwashed and washed *G. verrucosa* was grinded to reduce the particle size of < 0.5 cm.

2.3. *Experimental set-up for BMP test*

A manual BMP system was used for the BMP test in this study. The test was carried out under batch condition with the inoculum to substrate ratio (I/S ratio) was 6: 1. All samples were prepared in triplicates, including blank, positive controls (α-cellulose), unwashed and washed *G. verrucosa.* The blank samples are included in the test to measure the indigenous methane production from the inoculums. The positive controls (α-cellulose) are used to test the activity of the inoculum. The BMP test was carried out using 250-mL serum bottle with working volume of 40 ml. The serum bottles were placed in a water bath at 37 ^oC without shaking for 28 days. Pressure was measured on a daily basis using a digital manometer (i.e. Digitron 2026P absolute pressure meter) (Electron Technology, UK).

2.4. Analysis

TS, VS, MC and Ash content were determined based on the Standard Method 2540 G [10]. pH was measured using a digital pH meter, previously calibrated in buffers at pH 7 and 9.2. Biogas production was calculated at standard temperature and pressure (STP) of 273.15 K and 101.325 kPa by converting pressure readings to gas volume in the headspace. Elemental analysis was performed using elemental analyser (628 Series Elemental Determinator, LECO). CV was measured using a bomb calorimeter, and compared with theoretical CV calculated using Boie formula, as shown in equation 1 [13].

$$HCV\ (MJ\ /\ kg\ TS) = 35.160\ C + 116.225\ H - 11.090\ O + 6.280\ N + 10.465\ S \quad (1)$$

Where, HCV represents higher calorific value in MJ/kg, while C, H, N, S and O are expressed as a mass fraction.

The theoretical methane concentration calculated using Buswell equation [14], was used to calculate specific methane potential (SMP). The SMP was calculated using the formula as reported by Strömberg et al. [15], as follows:

$$BMP = \frac{V_S - V_B \frac{m_{IS}}{m_{IB}}}{m_{VS,sS}} \quad (2)$$

Where, SMP is the normalised methane volume (m^3 CH_4/kg VS), V_S is the accumulated methane volume from reactor with inoculum and substrate, V_B is the methane volume from reactor with blank sample (inoculum only), m_{IS} is the mass of VS of inoculum added in the sample, m_{IB} is the mass of VS of inoculum added in the blank sample, and $m_{VS,sS}$ is the mass of substrate added in the reactor

Electrical potential estimation was calculated based on the assumption that 1 m^3 biogas has a calorific value of 22 MJ, where 1 m^3 methane is equivalent to 36 MJ. In this study, the electrical conversion efficiency was assumed at the value of 35%, therefore 1 m^3 methane is equal with 10 kWh.

3. Results and Discussion

3.1. Characteristics of G. verrucosa

Table 1 shows the characterisation of *G. verrucosa* using physic-chemical analysis. The results indicated that *G. verrucosa* has a high MC and a low VS concentration, giving the content of 39.32 %TS. The C/N ratio was ~10.7, much lower than the favorable value for digestion at 20-25. However, more than 50% of the solids in the *G. verrucosa* were present as carbohydrate, which is a potential material for biogas production. The measured CV (16.20 MJ/ kg TS) was higher than theoretical value of 13.70 MJ/ kg TS, which may possibly due to the organic materials contained in *G. verrucosa*, as previously described by Niessen [16]. Despite a low VS concentration and C/N ratio, a high carbohydrate content in *G. verrucosa* may indicating its potential to be used as biomass resource for biogas production.

3.2. The BMP test results

Figure 1 shows the cumulative biogas and methane production of *G. verrucosa* and positive control against inoculum samples. The methane production was calculated using the assumption of 50% theoretical methane concentration based on Buswell calculation. It can be seen that the inoculum samples were remained stable after day 1, with a slight increase started from day 15 to day 28. For positive control (α-cellulose), a rapid biogas and methane production was achieved after a short time, and reaches a stable production after 17 days, giving the average values of 73.483 mL (biogas) and 36.742 mL (methane). The figure also indicates that washing pre-treatment (washed *G. verrucosa*) caused an increase in cumulative biogas and methane production, with the average values of 44.607 ml and 22.304 mL, respectively. While unwashed *G. verrucosa* showed the lower cumulative biogas (22.789 mL) and methane (11.395 mL) than that of the inoculum samples (17.544 mL - biogas and 8.772 mL - methane). Such behaviour indicated that some inhibition was occurred in the anaerobic digestion that hinders the biogas and methane production. One of the inhibition factors in digesting macroalgae is the salinity, as mentioned by Tabassum et al. [9]. This study demonstrates that washing pre-treatment was assumed to able to remove the salinity of *G. verrucosa*, thus increasing the anaerobic biodegradability by approximately 50%.

Table 1. Characteristics of *G. verrucosa*

Parameters	Values
TS (% of WW)	20.09
VS (% of WW)	7.90
VS (% of TS)	39.32
MC (% of WW)	79.91
Ash (% of WW)	12.18
Biochemical composition (% of TS)	
Carbohydrate	50.96
Protein	17.66
Fat	0.72
Elemental analysis (% of TS)	
C	31.03
H	6.19
O (by difference)	60.02
N	2.90
Other substrate parameters	
Measured CV (MJ/kg TS)	16.20
Theoretical CV (MJ/kg TS) (Boie)	13.70

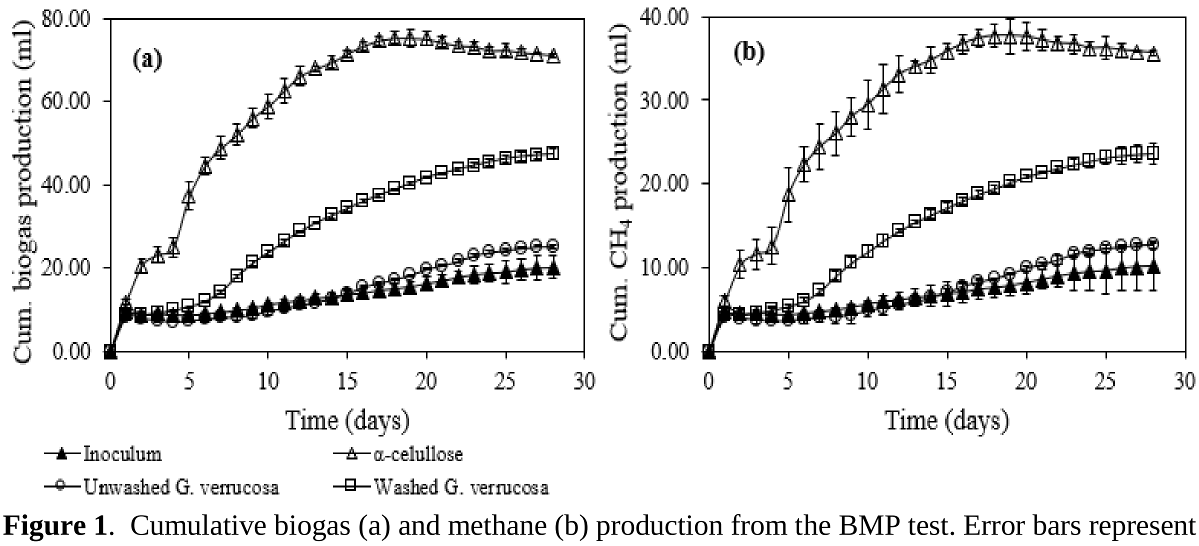

Figure 1. Cumulative biogas (a) and methane (b) production from the BMP test. Error bars represent standard deviation on 3 measurements in the relevant period

Figure 2 shows the net biogas and methane production of *G. verrucosa* and positive control against inoculum samples. The value was calculated by subtracting their cumulative biogas/methane production with that of the inoculum. The results indicated that the trends in net biogas and methane of all samples were similar to the trends in cumulative biogas and methane production. The inoculum sample only has an average value of 17.554 mL (biogas) and 8.772 mL(methane). While the positive control has the average net biogas and methane values of 55.939 mL and 27.970 mL, respectively. Unwashed *G. verrucosa* has the average net biogas production and methane production of 4.389 mLand 2.195 mL. The figure also shows that for unwashed samples, a longer time was required for the consortia microorganism to adapt with the biomass samples used. Only after 15 day both net biogas and methane production gave positives values, which indicating that an inhibition to anaerobic digestion process was occurred. Again, washed *G. verrucosa* had higher net biogas and methane production, with the average values of 26.207 mL and 13.0104 mL, respectively. This indicated that washing pre-treatment has positive effect on improving the anaerobic digestion performance as indicated by the increase value in biogas and methane generation. This was possibly because the washing pre-treatment may reduce the salinity concentration in fresh *G. verrucosa*.

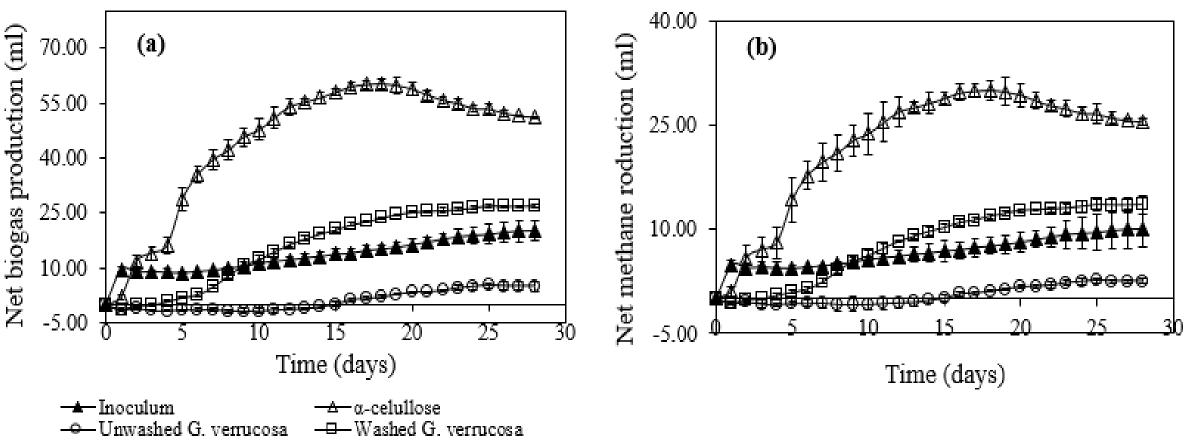

Figure 2. Net biogas (a) and methane (b) production from the BMP test of *G. verrucosa* and tpositive control against inoculum samples. Error bars represent standard deviation on 3 measurements in the relevant period

The SMP values of unwashed and washed *G. verrucosa* against positive control and inoculum can be seen in Figure 3. The inoculum samples, reached a stable value after approximately 17 days, with the average SMP of 0.014 m^3 CH_4 / kg VS. This value indicated that there was consortia activity in the inoculum to produce biogas/methane. The highest SMP was resulted from the use of positive control at the average values of 0.225 m^3 CH_4 / kg VS, indicating that the microorganism consortia have a quite good performance in breakdown the organic materials. However, this value was still much lower than that of values reported in other studies [17, 18]. This also indicated that, further treatment is needed to enhance the performance of the inoculum in the BMP test, such as by addition of trace element or else as explained in Angelidaki et al. [19]. Unwashed *G. verrucosa* has SMP of 0.018 m^3 CH_4 / kg VS just slightly higher than inoculum. After washing pre-treatment, *G. verrucosa* samples produce a six-fold higher in biogas and methane production.

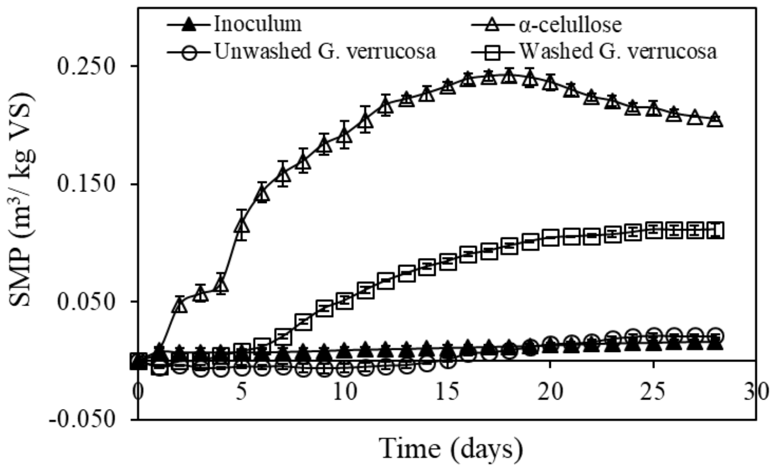

Figure 3. Specific methane production from the BMP test: unwashed and washed *G. verrucosa* against inoculum and the α-cellulose positive control. Error bars represent standard deviation on 3 measurements in the relevant period

3.3. Estimation of electricity potential

Table 2 shows that the electricity potential from unwashed and washed *G. verrucosa* samples was 14.296 kWh/ton and 85.262 kWh/ton, respectively. These amounts were depended on both methane potential and the amount of organic content (VS) in the biomass samples. The findings in this study are therefore, indicate that washed *G. verrucosa* has a great potential to be valorised for biogas which then converted into electricity and heat sources, by combining a Combined Heat and Power (CHP) unit with the AD unit.

Table 2. Electricity potential estimation single digestion of unwashed and washed *G. verrucosa*

Substrate	Energy/ day (MJ/day)	Methane volume (m^3CH_4)	Electricity Potential (kWh)
Unwashed *G. verrucosa*	0.018	1.428	14.279
Washed *G. verrucosa*	0.109	8.526	85.262

Note: calculation was based on 1 ton of *G. verrucosa*

4. Conclusions

Washing pre-treatment on *G. verrucosa* prior anaerobic digestion process was found to increase the biogas production, as well as the methane potential. The washing pre-treatment may reduce the salinity concentration of *G. verrucosa*, thus enhancing the anaerobic degradability. An increase in biogas and/or methane potential was parallel to an increase in the electricity potential. The findings confirm that a high electricity potential is estimated to be generated from anaerobic digestion of washed *G. verrucosa*. Further studies are required to enhance the biogas and methane production, possibly to use other operational parameters under a long-term operation to investigate the anaerobic digestion process stability.

Acknowledgement

Authors would like to thank for the financial support from Menristekdikti through *Kompetisi Nasional Skema Penelitian Dasar* Grant 2019, as part of research collaboration under Newton Fund Institutional Link programme 2019.

IOP Conf. Series: Earth and Environmental Science **475** (2020) 012064 doi:10.1088/1755-1315/475/1/012064

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