Great Potency of Rejected Macroalgae *Gracilaria verrucosa* for Biogas Production by Anaerobic Digestion and H$_2$S Scrubber

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**Abstract:** Unutilized *Gracilaria verrucosa* in aquaculture provides an opportunity in bioenergy development such as biogas production and reducing the environmental pollution risk. The existence of trace gas product for example hydrogen sulfide in biogas is considered because of its odor, emissions and corrosive effect. The purposes of this study were to analyze biogas production of red macroalgae *G. verrucosa*. The study was carried out for 31 days for each batch cycle after acclimatization. The digester capacity was 1500 land equipped by H$_2$S scrubber. The result showed that the maximum biogas volume was produced on day 10-19 and the daily production volume was 72 l/day. Biogas consisted of 66-69% methane, 17-31% carbon dioxide and <1% hydrogen sulfide. The scrubber decreased H$_2$S until undetected after passing the filter. Using H2S scrubbers to reduce H2S content that cause corrosion effect can maximize biogas production of macroalgae.

**Key words:** Biogas - Macroalgae - *Gracilaria verrucosa* - Anaerobic Digestion - H$_2$S Scrubber

**INTRODUCTION**

Energy crisis spawned considerable interest in renewable energy exploration resources. Bioenergy become the most significant source of energy demand and offer economic value on a large-scale development [1, 2]. Energy based on biomass has been widely studied to fill energy requirement that is increasing day by day. Algae gained attention as a source of renewable energy to produce fuels and chemicals compounds [3]. Macroalgae has many advantages in renewable energy application as a high photon conversion efficiency then synthesize biomass through assimilation that utilize abundant sunlight, carbon dioxide and inorganic nutrients quickly [4, 5].

The capacity of macroalgae production per unit area was significantly higher than terrestrial biomass [4, 6]. Macroalgae can fix carbon dioxide levels higher than terrestrial biomass and allow restoring the larger carbon [6-8]. The lignin content of macroalgae is lower than the land plants lignin [9, 10] so it is easier to be polymerized and hydrolyzed [11, 12]. In addition, macroalgae have the ability to live in the wastewater, which is very important for sustainable energy production to avoid the competition with food crops [10]. In Indonesia, the most dominant and commonly red macroalgae cultured in pond are *G. verrucosa*. Because their availability is abundant and it can be found throughout the Indonesian archipelago [13]. Macroalgae biomass can be converted become biogas through anaerobic digestion [14-19]. This process occurs because of bacteria activities in substrate to produce methane. Rumen microbes such as bacteria, protozoa, fungi and bacteriophages act as decomposers of organic material [20]. Previous research analyzed total
gas and methane production of some macroalgae species using rumen bacteria mixture [21]. Manure has an ideal anaerobic biodegradability level because it contains degraded bacteria as inoculum [22, 23]. Bacteria require acclimatization in order to adapt in different substrates.

Anaerobic digestion includes several stages and reactions. First stage is hydrolysis, in which the complex organic structure of the substrate is broken down into simpler structure. In acidogenesis, the simple organic compounds formed at the end of first stage. There are converted into volatile organic acids, carbon dioxide and hydrogen. Subsequently, acetate, hydrogen and carbon dioxide are synthesized from the organic acids during the acetogenesis. In methanogenesis, the products of third stage are converted into biogas, which mainly consists of methane and carbon dioxide as its major composition.

Biogas consists of 55-75% methane (CH\textsubscript{4}), 25-45% carbon dioxide (CO\textsubscript{2}), hydrogen (H\textsubscript{2}), nitrogen (N\textsubscript{2}) and hydrogen sulfide (H\textsubscript{2}S) form the rest of the gas. Methane is flammable gas and can be used as a renewable energy source. While, hydrogen sulfide cause unpleasant odors and become distinctive characteristics of biogas. This gas become a troubled effect because poisoned and corrosive for several equipment. The combustion of H\textsubscript{2}S produce sulfur dioxide emission and dangerous for environment.

The filtration using biological filter is one of solution for increasing the quality of biogas and reduce H\textsubscript{2}S content. The purposes of this research were to analyzed biogas production and composition then compared before and after filtering by H\textsubscript{2}S scrubber. Beside that this research also analyzed biogas utilization for lamp and stove application to estimate biogas conversion to bioenergy.

**MATERIALS AND METHODS**

**Digester Installation:** The digester with 1500 L capacity was made from fiber and equipped with two tanks as substrate input and sludge output. It is planted on the land so called as fix dome digester [24]. There were two tap or valves to flow biogas from digester, one connected to flow meter and H\textsubscript{2}S scrubber, other just closed and used to take biogas sample. Flow meter measured the volume of gas production (Specification Itron ACD G1.6 (Q\textsubscript{max}=3m\textsuperscript{3}h\textsuperscript{-1}; Q\textsubscript{nom}=0.16m\textsuperscript{3}h\textsuperscript{-1})) and installed before scrubber. H\textsubscript{2}S scrubber was made from cylinder polyvinylchloride (PVC) material, complement of biogas digester and connected with the gas output. The material of plastic was resistant from corrosion. The size of H\textsubscript{2}S scrubber was 30 cm diameter and 160 cm height. This scrubber consists of some marbles and coir as the medium for bacteria that decompose H\textsubscript{2}S. The mass of marbles spread the prevalent air around the cylinder. Then the air will pass the coir, the bacteria catch the sulfate for their metabolism and reduce the concentration of H\textsubscript{2}S in biogas. The digester was operated at mesophilic temperature and depends on environmental temperature (Figure 1).

**Substrate Preparation:** There were no specific treatments and storage for macroalgae in the field. Macroalgae that were used in this research collected around digester location and washed to remove the sand and mud [13, 14] then dried. The first stage in substrate preparation was soaked 25 kg dried macroalgae on 400 l fresh water for 2 hours until wet and their form back to previous shape. The second stage was mixing wet macroalgae and water in ratio 1:2 until homogen and ready as a substrate.

**Inoculum Preparation:** Inoculum preparation was started by mixing cow manure (Starter) with water (100 l and 800 l) and loaded into digester and waited until produce biogas for several days. The substrate and inoculum were then mixed to acclimate them as an inoculum and done in semi continues system. Substrate were loaded gradually 2% of digester capacity every 4 day, followed by releasing the same amount of slurry from outlet. Substrate was acclimatized to adapt bacteria from cow manure with macroalgae until the inoculum was ready to be processed. The mixture components were 800 l of water, 100 l of inoculum. Substrate was loaded gradually 2% of capacity in every 4 day, followed by releasing the same amount of slurry. The inoculum was observed until it reached a neutral pH and produced biogas. During acclimatization, the inoculum was stirred and analyzed by measuring the pH and temperature [23].
Anaerobic Digestion in Batch Method: Anaerobic digestion process in batch method was started when the pH has stabilized in the neutral condition. Total substrates (300 l) were loaded directly to the digester at once and at the same time removed the same amount of slurry. Field experiment was held during 31 days for two replications. Temperature and pH were not controlled; depend on environmental condition. Daily and cumulative gas production was measured using a flow meter every day, while Chemical Oxygen Demand (COD), Total Solid (TS), Volatile Solid (VS), water content and gas composition were analyzed every 10 day. COD value of the initial and final digestion was important to be analyzed in order to determine the potential of macroalgae as a source of biogas energy. The advantages of batch system were a reduced risk of contamination from the outside, easy to estimate the biogas production from substrate loading and easy to control the process because the input will be loaded one time. However, there was a deficiency that gas production decreased over time due to availability of the substrate in digester as a result of digestive process undertaken by anaerobic bacteria. Sludge from output tank can be developed for fertilizer and have to analyze before.

Analytical Methods: Proximate analyses consist of water content, ash content, carbohydrates, protein, lipid and fiber content (SNI 01-2891-1992). H2S scrubber to compare their concentrations collected biogas from the digester valve with gas sampler plastic bags before and after filtration. The bags were allowed to sample for 10–15 sec. before shutting the outlet valve to insure that sample was representative of digester gas [24]. The biogas composition was determined using a gas chromatograph Shimadzu GC-14A equipped with Flame Ionization Detector (FID) for CH₄ and Thermal Conductivity Detector (TCD) for CO₂. Methane and CO₂, trap box size are 40 cm x 40 cm x 60 cm and 40 cm x 20 cm x 15 cm respectively. Gas sample (0.5 ml) was injected into the chromatograph with a column temperature of 45°C using helium as the carrier gas. The gases concentration was compared between before and after filtering by H₂S scrubber.

The FID is the most popular detector for gas chromatography because it is highly sensitive to almost all organic compounds. Meanwhile, being little influenced by changes in temperature and flow rate ensures a high signal-to-noise ratio and wide dynamic range. During high-sensitivity analysis, it is recommended to use high-purity gas (Hydrocarbon less than 1 ppm). The sensitivity for TCD was 40,000 mV.ml/mg (Built-in pre-amplifier, with 10⁻⁴ amplification). The calibration of GC used digital setting by electronic flow controller (AFC). Temperature, flow rate, pressure of each part inside GC was programmed digitally. Standard gas CO₂ (10600 ppm) was used to determine CO₂ concentration from the injection sample and Nitrogen 99.99% (Ultrahigh purity) was used as carrier gas. The temperature for each part on GC was 100 °C for injector and column, then 150 °C for detector.

Hydrogen sulfide was analyzed by blue metilen method (SNI-7117.7-2005). Gas sample was corrected in normal condition (25 °C, 760 mm Hg) and passed to absorber solution using the vacuum and reacted with p- amino dimetil aniline and Fe (III) in acid condition to form blue metilen solution. The absorbent was measured in spectrometer with ε=670 nm for concentration range 5-1.000 ppm (7 mg/Nm⁻³-1390 mg/Nm⁻³). Spectrometer was calibrated using blank solution and to make sure the accuracy of measurement, all of the equipment must be sterilized and standardized. Chemical Oxygen Demand, TS, VS and water content were analyzed based on APHA [25]. Total solid (TS) and volatile solid (VS) were analyzed according to APHA [25]. The temperature was 105 °C during 1 hour for TS analysis and 600°C for VS during 3 hours.

RESULTS AND DISCUSSIONS

Macroalgae Characteristics: Gracilariaspp.is red algae (Rhodophyta) [26, 27] and has nutritional contents. Especially living people in the coastal area extensively consume fresh and dried macroalgae. In recent years, macroalgae has increasingly attracted interest for drugs exploration and has been shown to be a primary source of natural bioactive compounds and biomaterials [28]. However, some country use cultivated macroalgae for the production of biogas [2] as an effort to fill sustainable energy necessity and environmental friendly. Each species has different potential and characteristic. The characteristic compound of some Gracilaria spp. is shown in Table 1.

Fresh G. verrucosa directly was taken from fishpond in Tanara Village, Banten Province, Indonesia. This sample was rejected by industry because has low quality performed (pale color, small size, limp thalli) and mud or sand substrate attach to the sample. The distributors of algae (The one who buy algae from fisherman before industry) determined and sort the algae based on experience and general observation. In Indonesia, there is no exact standard to determine physical quality of
Table 1: Characteristic compound of *Gracilaria* spp.

<table>
<thead>
<tr>
<th>Characteristic (%)</th>
<th><em>Gracilaria crassa</em>&lt;sup&gt;a)&lt;/sup&gt;</th>
<th><em>Gracilaria salicornia</em>&lt;sup&gt;b)&lt;/sup&gt;</th>
<th><em>Gracilaria verrucosa</em>&lt;sup&gt;c)&lt;/sup&gt;</th>
<th><em>Gracilaria verrucosa</em>&lt;sup&gt;d)&lt;/sup&gt;</th>
<th><em>Gracilaria verrucosa</em>&lt;sup&gt;e)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>-</td>
<td>15.63</td>
<td>10.17</td>
<td>11.7</td>
<td>16.65</td>
</tr>
<tr>
<td>Protein</td>
<td>5.18</td>
<td>11.21</td>
<td>18.7</td>
<td>20.28</td>
<td>9.33</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>42.0</td>
<td>24.47</td>
<td>33.67</td>
<td>-</td>
<td>21.46</td>
</tr>
<tr>
<td>Lipid</td>
<td>1.3</td>
<td>0.35</td>
<td>7.6</td>
<td>2.66</td>
<td>0.41</td>
</tr>
<tr>
<td>Fiber</td>
<td>-</td>
<td>30.94</td>
<td>8.35</td>
<td>-</td>
<td>11.84</td>
</tr>
<tr>
<td>Ash</td>
<td>43.18</td>
<td>17.37</td>
<td>30.72</td>
<td>12.8</td>
<td>40.31</td>
</tr>
</tbody>
</table>

<sup>a)</sup><sub>[29]</sub>, <sup>b)</sup><sub>[30]</sub>, <sup>c)</sup><sub>[14]</sub>, <sup>d)</sup><sub>[31]</sub>, <sup>e)</sup>This study

Table 2: Chemical Oxygen Demand (COD), Total Solid (TS), Volatile Solid (VS) and water content of substrate

<table>
<thead>
<tr>
<th>Day</th>
<th>COD (mgL&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>TS (%w.w)</th>
<th>VS (%w.w)</th>
<th>Water (%w.w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6.95</td>
<td>9.73</td>
<td>6.15</td>
<td>90.41</td>
</tr>
<tr>
<td>15</td>
<td>4.70</td>
<td>5.64</td>
<td>3.05</td>
<td>95.57</td>
</tr>
<tr>
<td>25</td>
<td>4.16</td>
<td>3.21</td>
<td>0.76</td>
<td>98.70</td>
</tr>
</tbody>
</table>

The result showed that *G. verrucosa* as rejected optimum pH value in the process of anaerobic macroalgae sample in this study has lower carbohydrate content than some other sample. The carbohydrate content of *G. verrucosa* ranged from 21.46-42%. Ash content of rejected sample was 40.31%, mean the highest then others and described mineral compound in biomass was high, nevertheless this compound could not be degraded by microorganism including bacteria. Macroalgae generally contain 8-40% of minerals and trace elements [32]. The mineral contents of macroalgae are reported to vary according to such factors as species, geographical origin, seasons, environmental and physiological variations [29,33,34]. The ash contents in most marine macroalgae are usually much higher than those in terrestrial plants (5–10% d.w) [35].

The carbohydrates content and quality of *Gracilaria sp*. determine the number and strength of gel that contained therein [36]. All of organic substrate compounds are biogas resources. Soluble carbohydrate component is correlated with high methane yield production and also agar content. *Gracilaria* sp. is easier degraded in anaerobic digestion [37]. The carbohydrate content of the macroalgae depend on the species. Various environmental parameters influenced the chemical composition of Kappaphycus alvarezii and these parameters demonstrated seasonal fluctuations [38]. Besides that, harvest period influence the composition of macroalgae, the optimum period for harvest is 45 days.

**Inoculum and Substrate Acclimatization Process:**

The ranges of pH was 7.0-8.3 during acclimation process and fluctuated due to the addition of macroalgae every 4 days into digester. The tendency of the pH value raised after the addition of the substrate macroalgae. The optimum pH value in the process of anaerobic macroalgae ranged between 7.0-7.2 [39] to increase the yield of biogas, while the low pH (<6) cause a negative impact on the production of gas. The temperature was fluctuated depend on environmental condition. We used this condition to know the real result of biogas production in the field. The range of temperature in the field was 28-37°C.

**Anaerobic Digestion in Batch System:** The result showed that COD value decreased 40% from 6.95 mgl<sup>-1</sup> in the beginning to 4.16 mgl<sup>-1</sup> at the last day. Degradation of substrate indicated that microorganism activities could decompose organic materials and converts them into biogas. The decreasing of COD demonstrated of acid consumption for the methane production [40]. Degradation was visualized by the changing of substrate from a more solid to liquid form. Water content (%) of substrate definitely increased from 90.41% (w.w) to 98.7%w.w, otherwise TS and VS declined in passing day. The results of substrate analysis are shown in Table 2.

TS and VS described loading rate to be degraded by microorganism in hydrolysis process. Microorganisms adapted to macroalgae substrate then TS and VS tended to decline during the biogas production process. TS value at 5<sup>th</sup> day was 9.73% (w.w), decreased 77% to 3.21% (w.w) at 25<sup>th</sup> day, as well as VS decreased 92% from 6.15% (w.w) at 5<sup>th</sup> day to 0.76% (w.w) at 25<sup>th</sup> day. That was indicated by elevated levels of methane produced. VS was substrate (food source) for non-methanogenic microorganisms that work in the early stages of biogas production.

Table 3 shows the concentration of biogas composition before and after filtration by H<sub>2</sub>S scrubber.
Table 3: Methane, carbon dioxide and hydrogen sulfide concentration before and after filtration by H2S scrubber

<table>
<thead>
<tr>
<th>Day</th>
<th>CH4 (mg/l) BS</th>
<th>CH4 (mg/l) AS</th>
<th>CO2 (mg/l) BS</th>
<th>CO2 (mg/l) AS</th>
<th>H2S (mg/l) BS</th>
<th>H2S (mg/l) AS</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>176,426.0</td>
<td>551,408.0</td>
<td>10,950.5</td>
<td>28,541.0</td>
<td>7.98</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>603,900.0</td>
<td>636,472.0</td>
<td>76,814.5</td>
<td>94,919.0</td>
<td>52.05</td>
<td>-</td>
</tr>
<tr>
<td>25</td>
<td>428,992.0</td>
<td>460,017.0</td>
<td>192,248.5</td>
<td>237,846.0</td>
<td>29.96</td>
<td>-</td>
</tr>
</tbody>
</table>

BS = Before H2S scrubber  
AS = After H2S scrubber  
(-) = Undetected

At the beginning, methanogenesis was not effective and bacteria need to acclimate with macroalgae substrate and increased at day 15th then reached optimum concentration. However, methane concentration depressed at the last observation. The average composition of CH4 from biogas during batch system was 69% before scrubber and 66% after scrubber. Macroalgae is a rapidly degradable material, gas production stopped quickly when digesters were no longer fed, the methane content also reduced when biogas decreasing.

The concentration of CO2 increased in the middle and end of measurement. It was caused by bacteria activity that hydrolyzed a substrate, however did not balanced by methanogen production stage as an effect of the H2S presence in digester that can impede the process [16]. In addition, CO2 gas was produced along with methane when methanogen bacteria broke down the acidogenesis compounds. The high levels of CO2 indicated the lower methane content that influenced to energy value and its availability decreased the heating value of biogas. The average composition of CO2 from biogas were 31% before scrubber and 17% after scrubber. The enhancement concentrations of methane and CO2 was not the effect of the scrubber. The filtration process led to diminish one of the gas and accumulated others gas concentration.

The concentration of H2S increased from 7.98 mg/l (days 5th) to 52.05 mg/l (Days 15th) then reduced to 29.96 mg/l (25th days). The composition of this gas was less than 1% on biogas. The gas was very disturbing even though in slight concentration then methane and carbon dioxide. The methanogenesis may be limited by disturber gas when H2S content more than 6% [41]. The concentration of H2S after scrubber was not detected by gas chromatography that indicated by disappearance of biogas bad smell. Hydrogen sulfide was derived from proteins and sulfur of substrate degradation. The concentration depended on the raw materials and varied between 0.1 to 2%. The physical and biological process were involved in gas filtration mechanisms. Gas passed the hollow space of marbles and spread to other side evenly, then degrading bacteria on coir oxidize H2S and reduced its concentration. The continually utilization of H2S scrubber may be restricted by bacterial ability that decreased concomitant with nutrient supply on scrubber.

**Biogas Production:** After acclimatization process, rumen bacteria adapted to macroalgae substrate and produced gas. The most rapidly degradation of macroalgae fraction and rising of biogas production occurred during day 10th - 19th. The highest gas production was 132 l in day 15th, while the lowest gas production was 21.5 l in day 30th and the average of biogas daily production was 72 l/day (Fig 2). Cumulative biogas production of macroalgae during 31 days observation was 2305.5 l (Fig 2).

![Fig. 2: Biogas production of macroalgae G. verrucosa on batch system; (a) Daily and (b) Cumulative.](image-url)
The biogas production from washed Ulva sp. in batch digester was 87.4 l for 100 g substrate and 1.5 L water. This result was lower than non-washed substrate that produced 116.7 l for the same amount of substrate and water [9]. In our case the substrate was washed before the process in order to remove the sand and mud. On the other hand, the washing process changed the osmotic pressure and also removed some easily fermentable soluble metabolites and consequently can decrease methane production [9]. The gas production process slowed down in the beginning and slightly increased after 10 days. In the end of process the production decreased again, it means the availability of substrate inside digester was degraded and converted become biogas. There was no input of new substrate on batch proses. Total gas production in anaerobic digestion was depending on substrate materials and the process inside digester. Digestion performance also was affected by species and composition of macroalgae [23].

CONCLUSIONS

The conversion of macroalgae G. verrucosa biomass into biogas requires bacteria from cow manure as starter using a digester in anaerobic conditions. The maximum biogas volume was produced on day $10^{6}$-19$^{a}$ and the daily production volume was 72 l.day$^{-1}$. Biogas consisted of 6-69% methane, 17-31% carbon dioxide and <1% hydrogen sulfide. Results of this study could be the beginning of the implementation study of the use of macroalgae as a source of bioenergy in the field scale. However, further research can be done by adding a filter to reduce the CO$_2$ implies that the quality of biogas produced could be better. In addition to research about the saturation level of H$_2$S scrubber.

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