Anaerobic co-digestion of Batik wastewater with macroalgae

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Abstract. Macroalgae is one of renewable marine biomass feedstocks for substituting land biomass. With the use of anaerobic digestion (AD) technology, macroalgae has been greatly acknowledged for bioenergy production resources, including biogas. On the other hand, Batik wastewater contains chemical dyes and organic pollutants, which potentially cause environmental pollution if disposed without a proper treatment. The aims of this research were to evaluate the potential of biogas and methane production using different ratio of Batik wastewater: macroalgae (i.e. Gracilaria verrucosa fresh and dried samples) and to estimate the electrical potential generated. A biochemical methane potential (BMP) test was carried out at temperature of 37 °C for 28 days under batch condition. The results indicated that digesting Batik wastewater alone, without dilution, showed an inhibition process as indicated by a negative net biogas and methane production as well as the specific methane production (SMP). Co-digesting Batik wastewater with fresh marine G. verrucosa samples at ratio of 50:50 cannot produce a high amount of biogas or methane. All samples showed a similar biogas or methane volume compared to that of the inoculum sample. This indicated that an inhibition was occurred limiting the microbial consortia in inoculum to breakdown the organic matter in the feedstock material. This could be due to the salinity of macroalgae and the high organic pollutant concentration in Batik wastewater. However, co-digestion of Batik wastewater with dried marine G. verrucosa produced higher SMP. Further alternative options to enhance to utilisation of Batik wastewater are needed.

1. Introduction

Textile industry in Indonesia has positively contributed to the country’s economy, with the export income value of US$ 4.66 billion [1]; which predicted to continuously increase up to 4% annually. One of the textile industries in Indonesia is Batik industry, which also provided income generation due to an increase market demand [2]. This causes an increase in Batik production and the number of Batik industries which mainly composed of small and medium enterprises (SMEs) as Batik producers [3].

Many of Batik SMEs use chemical dyes in colouring process, resulted dyes-containing wastewater which are difficult to degrade [4]. Batik wastewater also contains high chemical oxygen demand (COD) concentration [5]. In addition, increasing demand in Batik production has contributed to an increase in Batik wastewater generation resulted from washing and soaking process [6]. Yet, most of Batik SMEs, due to lack of wastewater treatment facilities, directly disposed their wastewater into the nearby waterbody [7]. Such practices have led to a dangerous impact to environment due to water pollution, toxic chemicals accumulation or carcinogenic effect to human and aquatic life [8].
Therefore, the implementation of wastewater treatment technology that can effectively remove dyes or can valorise the organic matter in Batik wastewater is necessary.

Several methods and technology for treating Batik wastewater have been applied to enhance the effluent quality such as aerobic granular sludge [4]; Fenton and Electro-Fenton oxidation [9]; electrochemical coagulation [10]; sequencing batch reactor/SBR [11]; trickling filtration [12], and etc. However, Batik wastewater can also be potentially used for biogas production, due to its organic pollutants content, measured as COD or biochemical oxygen demand (BOD) [13]. In addition, macroalgae is one of renewable marine biomass feedstocks for substituting land biomass and has been greatly acknowledged for bioenergy production resources, including biogas [14]. The use of anaerobic digestion (AD) technology for biogas production is widely known. AD is a microbial degradation of biodegradable organic matter in an oxygen-free condition to produce biogas (i.e. a mixture consisting mainly of CH₄ and CO₂) and residual organic (known as digestate) [15].

Several studies have reported that using macroalgae as co-digestion feedstock in AD of wastewater or other biomass provide a great beneficial for enhancing biogas and methane production [16-17]. Karray et al. [16], for example, found that anaerobic co-digestion macroalgae (*Ulva rigida*) with sugar industry wastewater produced higher biogas and methane than digesting wastewater or macroalgae alone. Current research demonstrates that anaerobic co-digestion provides an improvement of nutrient balances, a more dilution potential toxic compounds, as well as a better synergistic effect of microorganisms, which lead to improving biogas and methane yields [18].

The aims of this research were to evaluate the potential of biogas and methane production using different ratio of Batik wastewater: macroalgae (i.e. *Gracilaria* sp. fresh and dried samples) and to estimate the electrical potential generated.

2. Materials and Method
2.1. Feedstocks and inoculums
Batik wastewater was freshly collected from ‘Batik Blimbing Malang’ SME and kept at room temperature upon arrival at Laboratory of Bioindustri, Dept. of Agro-industrial Technology, Universitas Brawijaya. *Gracilaria verrucosa* was collected from Ujung Pangkah Beach, Gresik Regency – Indonesia. Two types *G. verrucosa* was used include fresh and dried samples. Fresh *G. verrucosa* was kept at cold storage, while dried *Gracilaria verrucosa* was stored at room temperature. Both samples were grinded before use. No pre-treatments were applied either for Batik wastewater or *G. verrucosa* samples.

Inoculum for the biochemical methane potential (BMP) test used was digestate, which freshly collected 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 to remove any larger particles, followed by degassing process for 48 hours. This process was aimed to remove any residual biogas produced from the inoculum. The characterisation of inoculum includes pH, temperature (°C), moisture content (MC), ash, total solids (TS), and volatile solids (VS).

2.2. Experimental set-up for BMP test
The experiment was carried out using a manual BMP test under a batch condition with the ratio of inoculum to substrate (I/S) ratio of 6: 1. Samples tested include blank, positive controls (α-cellulose), Batik wastewater (BW), Batik wastewater: fresh *G. verrucosa* (50:50) or BW:FG 50:50) and Batik wastewater : dried *G. verrucosa* (50:50) or BW:DG 50:50), all prepared in triplicate. The blank sample is to measure the indigenous methane production from the inoculums. The positive control (α-cellulose) is to test the activity of the inoculum. The BMP test was carried out using 150-mL serum bottle with working volume of 40 mL, placed in a water bath at 37 °C for 28 days. The pressure was measured on a daily basis using a digital manometer (i.e. Digitron 2026P absolute pressure meter) (Electron Technology, UK), which then used to calculate biogas and methane produced.
2.3. Analysis
TS, VS, MC and Ash content were determined based on the Standard Method 2540 G [19]. 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). The specific methane potential (SMP) was calculated using the formula (1) as reported by Strömberg et al. [20]. Electrical potential estimation was calculated based on the assumption that 1 m³ biogas has a calorific value of 22 MJ, where 1 m³ CH₄ is equivalent to 36 MJ. In this study, the electrical conversion efficiency was assumed at the value of 35%, therefore 1 m³ CH₄ is equal with 10 kWh.

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BMP = \frac{V_S - V_B \frac{m_{IS}}{m_{VS,S}}} {m_{VS,S}}
\]

Where:
- SMP = the normalised methane volume (m³ CH₄/kg VS)
- V_S = the accumulated methane volume from reactor with inoculum and substrate
- V_B = the methane volume from reactor with blank sample (inoculum only)
- m_{IS} = the mass of VS of inoculum added in the sample
- m_{IB} = the mass of VS of inoculum added in the blank sample
- m_{VS,S} = the mass of substrate added in the reactor

3. Results and Discussion
3.1. Batik wastewater characterisation
As previously reported in Suhartini et al. [6], the Batik wastewater used in this study contains a high value of COD and BOD, indicating its potential for further valorisation using AD route. Despite its high organic matter content, however, it also has many limitations, for example, their high C/N ratio, which was found to be one of limiting factors to a stable AD process. Furthermore, our previous study was also demonstrated that a high COD value (54,700 mg/L) in Batik wastewater has caused an inhibition in AD system. Therefore, further measure was suggested to investigate its possibility to co-digest Batik wastewater with other biomass substrates, particularly marine biomass (i.e. marine G. verrucosa).

3.2. Biogas and methane potential
The cumulative biogas and methane production of single- and co-digestion of Batik wastewater with marine G. verrucosa are shown in Figure 1. The concentration of methane was assumed 50%. The figure shows that digesting Batik wastewater alone results a small amount of both cumulative biogas and methane production with the average value of 2.96 mL and 1.48 mL, which was much lower than that of the inoculum samples (13.83 mL and 6.92 mL), respectively. Such condition indicating that some inhibitions were occurred during the AD process, which may be possibly due to a high COD concentration or C/N ratio, as explained before. When co-digesting Batik wastewater with fresh marine G. verrucosa (BW:FG-50:50), there was a small increase in cumulative biogas and methane production, giving the average values of 11.82 mL (biogas) and 5.92 mL (methane). The trends in biogas and methane production for this sample were quite similar to the inoculum samples. However, when co-digesting Batik wastewater with dried marine G. verrucosa (BW:DG-50:50), a quite significant increase in cumulative biogas and methane production was observed, with the average values of 23.18 mL (biogas) and 11.59 mL (methane).
Figure 1. Cumulative biogas and methane production from the BMP test of single- and co-digestion of BW with *G. verrucosa*. BW = Batik wastewater, FG = fresh marine *G. verrucosa*, and DG = dried marine *G. verrucosa*. Error bars represent standard deviation on 3 measurements in the relevant period.

Net biogas and methane production were calculated from subtracting the cumulative biogas and methane production of samples with that of the inoculum only samples. As shown in Figure 2, single-digestion of Batik wastewater was not a good option, without any pre-treatment such as dilution to reduce COD concentration. A negative biogas and methane production indicated that the process was not stable and inhibited. Further investigation is needed to identify the root problems of such behaviour. Similarly, the samples of BW:FG (50:50) also indicated a negative net biogas and methane production, until day 24. Despite a slight increase in biogas and methane production which started on day 25, the values were still lower than expected. Inhibition from the salt content in fresh marine *G. verrucosa* could play a key factor in limiting the AD process [21]. While, the sample of BW:DG (50:50) results higher net biogas and methane production compared to that of the Batik wastewater only and BW:FG (50:50), with the average values of 9.34 mL and 4.67 mL, respectively.

Figure 2. Net biogas and methane production from the BMP test of single- and co-digestion of BW with *G. verrucosa*. Error bars represent standard deviation on 3 measurements in the relevant period.

In terms of the specific methane potential (SMP), Figure 3 shows that control blank (inoculum only) sample has SMP of 0.013 m³ CH₄/kgVS, indicating a further addition of nutrient or trace element are needed to improve its quality [22]. Similarly, the SMP of control positive α-cellulose also showed a low value (0.230 m³ CH₄/kgVS) compared the value reported in other studies [23, 24]. This
indicates that there were not enough anaerobic bacteria consortia in the inoculum to degrade the organic material in the substrate. Further in-depth study is required to evaluate the quality of the inoculum used.

Figure 3 also shows that Batik wastewater as single-digestion feedstock resulted negative SMP values. This was because the cumulative biogas and methane production from this sample was lower than the inoculum. The negative SMP values were again indicating that a high concentration of COD limit microorganism ability in AD system to breakdown COD into biogas or methane [6]. Xiang et al. [25] reported that the dyes contain in textile wastewater and their degradation products may influence the AD process. Furthermore, co-digesting Batik wastewater with fresh marine G. verrucosa did not give any significant improvement. Similarly, Malpei et al. [26] also reported that co-digestion of textile wastewater with milk-whey did not increase the biodegradation of organic content to biogas or methane. However, co-digesting Batik wastewater with dried marine G. verrucosa was found to enhance the SMP values. The findings from Opwis et al. [27] found that digestion textile wastewater with the addition of sugar-containing medium to the AD system was able to increase biogas generation.

![Figure 3. Specific methane production from the BMP test of single- and co-digestion of BW with G. verrucosa. Error bars represent standard deviation on 3 measurements in the relevant period.](image)

3.3. Further Implications

Based on the SMP value in Figure 3, it was estimated that the electriciy potential from co-digestion of Batik wastewater with dried marine G. verrucosa was 258.75 kWh. This value indicates a great potential for further implementation of AD treating Batik wastewater with biomass co-feedstocks.

However, given that the limitation on biogas and methane production from Batik wastewater alone or co-digest with fresh marine G. verrucosa was observed, pre-treatment to reduce COD content or salinity can promote higher decomposition and relief inhibition on AD process. Therefore, optimisation of pre-treatment of Batik wastewater and/or fresh G. verrucosa could be further studied.

Generation of bioenergy from Batik wastewater, either as single- or co-digestion with G. verrucose, could contribute to improve the environment quality, to enhance the economic development in Indonesia and to provide new business opportunities within a growing global bioeconomy. Such application can also contribute to better understand the potential of macroalgae as biomass feedstock in bioenergy production.
Another potential application is to grow macroalgae for removing the chemical dyes or organic pollutants in Batik wastewater [26-31]. Then, the macroalgae biomass can be further utilised either for biogas or for other value-added products [14]. Yet, this needs to be investigated in depth.

4. Conclusions
Higher COD and C/N ratio, as well as the presence of chemical dyes may impact the generation of biogas from Batik wastewater. Those values limit the microbial consortia in inoculum to breakdown the organic matter in the Batik wastewater. Co-digestion of Batik wastewater can be alternative solution to enhance its economic value and valorisation as bioenergy sources. However, using fresh marine G. verrucosa was not enhancing the biogas production. While, adding dried marine G. verrucosa was found to enhance biogas and methane production. This study confirms that macroalgae, i.e. G. verrucosa can be potential co-substrates to be added into Batik wastewater treatment, if further pre-treatment is applied to reduce the salt content. Yet, in depth study on effect of salt content in macroalgae and other potential co-substrates for biogas and methane generation from Batik wastewater is necessary.

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