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Valorization of brown macroalgae *Sargassum plagiophyllum* for biogas production under different salinity conditions

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ABSTRACT

This study investigates the biogas production from *Sargassum plagiophyllum* under different salinity using a semi-continuous reactor. Inoculum preparation was conducted by mixing the two prepared *S. plagiophyllum* juice (deionized and saline water) with the cow manure using a ratio of 1:1. Once every two days, 5 % of the substrate was replaced with 5 % of fresh macroalgae juice continuously. The results found that cumulative biogas production in deionized water was 3.7 times higher than in saline water after 30 days. It could be attributed to anaerobic bacteria growth inhibited by salt. The maximum cumulative methane and biogas yields in the deionized water digester were 266.18 mL/g-VS and 371.76 mL/g-VS, respectively. The kinetics of the methane production was also determined, and the experimental data fitted well with the modified Gompertz model. These results indicate that *S. plagiophyllum,* under low salinity conditions, can be developed as a feedstock for biogas production.

1. Introduction

Fossil fuel has been the primary energy source globally since the 20th century. This dependency is causing environmental problems and making world energy vulnerable because fossil fuel is not renewable at a human scale (Capellán-Pérez et al., 2014). The world has been facing an unprecedented energy crisis in recent years, and the increasing share of renewable energy, including bioenergy, is a global necessity. Since it is classified as naturally replenishing resources and carbon neutral, bioenergy plays an essential role in contributing to the Net Zero Emission (NZE) target by 2050 (Handayani et al., 2022). Many countries, including Indonesia, have made significant progress on net zero emission pledges (NZE). Yet, the implementation is still far from the target, including the renewable energy share in the energy mix. With the Indonesian target of a renewable energy share of 23 % in 2025, only 11.5 % will be achieved in 2021 (Farobie and Hartulistiyoso, 2022). Therefore, increasing renewable energy share needs to be more intensive.

Among bioenergy, biogas has enormous potential in Indonesia, which is 32 GWe, but its implementation was just 6.5 % in 2022. Mostly, biomass wastes such as cattle dung and palm oil mill waste generated on land are used for biogas production in Indonesia. On the other hand, with 3.2 million km^2 of ocean covering two third of its area, Indonesia has a high potential for marine biomass, including macroalgae. Macroalgae are known for their rapid growth exceeding terrestrial plants; some species can grow up to 60 m in length (Costa et al., 2013). Moreover, macroalgae habitat is expansive with high space-use efficiency, and their cultivation does not require arable land, freshwater,

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and fertilizer, making them a very attractive biomass energy source (Costa et al., 2013).

Indonesia has more than 700 species of macroalgae, and among them is *Sargassum* sp. which is widely found in different areas from the west to the east coasts (Farobie et al., 2022b). There are 12 species of *Sargassum* have been identified from various regions in Indonesia. They grow especially in clear waters with the substrate of coral rock, dead coral, and volcanic rock at the bottom of the ocean. However, the current utilization of *Sargassum* is still limited. Thus, *Sargassum* utilization by converting it into biogas via anaerobic digestion is an attractive option (Hughes et al., 2012). Furthermore, the diversification of products from *Sargassum*, not only for food or pharmaceutical but also for energy, can increase its economic value.

Biogas potential from macroalgae ranges from 8.6 million kL to almost 400 million kL of methane, depending on their locations (Farobie et al., 2022b). Several literatures have explored the technique of using *Sargassum* sp. for biogas. A study on the pretreatment technique by AP et al. (2021) showed that the mechanical method gives higher effectiveness with potential biomethane production from *Sargassum* sp. improved by 48.7 % compared with chemical pretreatment using HCL and NaOH. Chikani-Cabrera et al. (2022) investigated the combinations of different pretreatments recently. It was found that 2.5 % hydrogen peroxide followed by enzymatic process showed the highest biodegradability of 95 % and maximum methane yield of 387 ± 3.09 L CH_4/kg volatile solid. Co-digestion technique was also applied by mixing *Sargassum* and food waste using hydrothermal pretreatment that promoted the hydrolysis of organics, thus increasing methane recovery by 212.57 % (Thompson et al., 2021). An increase of biochemical methane potential by 56 % and 46 % was also observed when *Sargassum* was co-digested with glycerol and waste frying oil, respectively (Oliveira et al., 2015). *The review on biogas from macroalgae is discussed quite comprehensively by* Hughes et al. *(*2012*), who noted that inoculum,* feedstock composition, and digester system configuration are essential aspects in determining biogas production and quality.

On the other hand, water as the medium for biogas production is also an important limiting factor in the anaerobic digestion of biogas (Bansal et al., 2017). Moreover, the wise use of water resources is essential in ensuring the sustainability of biogas production. The use of wastewater, recycled water, or seawater as a substitute for fresh water in biogas production is an attractive option for reducing pressure on fresh water (Misrol et al., 2021). Some literatures have studied the effect of saline water on biogas production. Faisal et al. (2022) reported that using seawater as the liquid substrate, biomethane production is possible when appropriate microbial communities are used. Ogata et al. (2016) found that biogas can be generated from landfill waste using saline water at a particular concentration. However, methane production was reduced when the salinity was too high, i.e., 80 mS cm^{-1} of electrical conductivity (EC). Similarly, Letelier-Gordo et al. (2020) investigated the effect of water salinity on biogas production from wastewater. It was found that methane production decreased by 50 % when salinity increased from 0 to 4.37 g/L.

In the case of macroalgae, the research on the impact of seawater (saline water) on biogas production from *Sargassum* is still limited found. Knowing this effect will be beneficial for determining the possibility of using seawater instead of freshwater for biogas production from macroalgae. Furthermore, the kinetic model for the anaerobic digestion of *Sargassum* for methane production has not been elucidated well. To *the best of our knowledge, such a study is not available yet in the literature. Therefore, the objectives of this study are to investigate the biogas production from Sargassum under different salinity and to deduce the kinetic model for anaerobic digestion of Sargassum for methane production. Sargassum plagiophyllum was employed in this study since it is still unutilized and abundantly available in Indonesia. Meanwhile, the significance of this study is not only to use unutilized feedstock S. plagiophyllum but also to provide an innovative way to valorize Sargassum for biogas production as well as to tackle the environmental issue. The result of this study is expected to contribute to fundamental knowledge on the sustainability approach to harvesting biogas from macroalgae.*

2. Materials and methods

2.1. Feedstock preparation and analysis

Brown macroalgae *S. plagiophyllum* was obtained from East Lombok beach, Indonesia. The freshly collected *S. plagiophyllum* was first cleaned with tap water to discharge the debris and impurities, such as sand and coral. The wet biomass was then stored in a cool box insulated with styrofoam at around 3.5 °C and transported to IPB University, Bogor, Indonesia, for further use. Next, the wet *S. plagiophyllum* biomass was dried under the sunlight for around 6 h and then stored in sacks at room temperature, around 25 °C.

The detailed proximate and ultimate analysis of raw biomass have been reported in our previous studies (Farobie et al., 2022a). In brief, the proximate analysis of macroalgae *S. plagiophyllum* was performed following the ASTM E1131-08 using a thermogravimetric analyzer TGA 4000 (Perkin Elmer, United States). Meanwhile, the ultimate analysis was determined using a CHN628 and CHN632 analyzer (Leco). Furthermore, the macroalgae's higher heating value (HHV) was determined following ASTM D 5865-04 using a bomb calorimeter (Parr 6200 Isoperibol). All the analysis was conducted at least three times to obtain reproducible data. Furthermore, carbohydrates, lipids, and protein content were analyzed by PT. Saraswanti Indo Genetech (SIG)–Bogor, a laboratory service in Indonesia accredited with ISO/IEC 17025. It should be noted that the protein content was analyzed using an automated Kjeldahl analyzer (Kjeltec 8400, Foss, Denmark). In addition, scanning electron microscopy-energy dispersive X-ray (SEM-EDX) analysis was performed using a scanning electron microscope (SEM, Hitachi, SU 3500).

2.2. Substrate and inoculum preparation

The macroalgal feedstock was soaked in clean water for 20–30 min and drained. Next, 250 g of macroalgal feedstock was weighed and mixed with 500 g of water (macroalgae: water ratio was 1:2), then blended and mashed using a blender to make the macroalgae juice. There were 2 types of water used as treatments in this study, i.e., deionized and saline water.

Two types of substrates were prepared using *S. plagiophyllum* macroalgae juice suspended in deionized or saline water. Herein, the preparation was carried out by mixing the two prepared brown macroalgae *S. plagiophyllum* juice (deionized and saline water) with the cow manure using a ratio of 1:1. The substrate mixture was then stirred for 10 min or until evenly mixed.

2.3. Acclimatization

The acclimatization process was conducted in a 5 L semi-continuous reactor (Fig. 1). Meanwhile, the photographic view of the experimental apparatus is presented in the Supplementary Material (Fig. S1). Briefly, the prepared inoculum substrate was transferred around 2/3 of the reactor capacity (1500 g) into the reactor. Furthermore, 5 % of the total slurry (75 g, 5 % of the total 1500 g inoculum) was taken from the outlet once every two days, to measure COD, TSS, and pH values. Subsequently, 5 % new feedstock (a mixture of macroalgae with the two types of water in a ratio of 1:2) was fed again into the reactors. This acclimatization process was carried out for a month. Once every 2 days, the volume of produced gas was recorded and then transferred into a gas bag with a capacity of 1 L for further analysis using a gas chromatograph (GC). The standard temperature and pressure for normalization of biogas and methane volumes were generally set at 0 °C and 1 atm (101.325 kPa). This standard is known as the "normal temperature and pressure" or NTP and is used as a reference for measuring and comparing

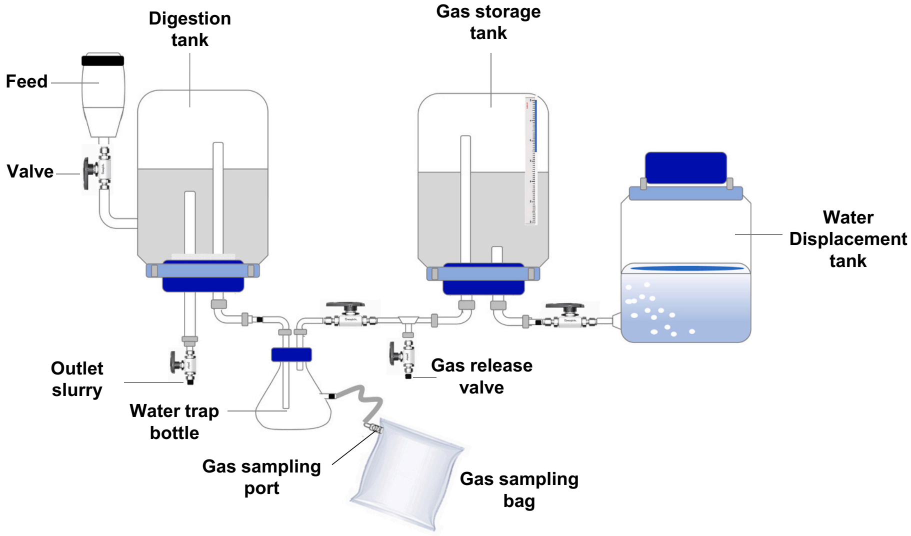

Fig. 1. Schematical diagram of the semi-continuous reactor for anaerobic digestion of *S. plagiophyllum*.

volumes of gases. The hydraulic retention time (HRT) of the reactor was 3.3 days.

2.4. Anaerobic biodegradation

Anaerobic biodegradation was conducted as similar to acclimatization. Here, the inoculum was obtained from the acclimatization process, while the substrate used was fresh macroalgae juice. The volume of produced gas was recorded and then transferred into a gas bag with a capacity of 1 L for further analysis using a gas chromatograph (GC) once every 2 days. Parameters such as COD, TSS, and pH values were also determined from the slurry.

2.5. Product characterization

The gaseous products were analyzed using a Hewlett Packard 6890 Series gas chromatograph (GC) equipped with a thermal conductivity detector (TCD) and a flame ionization detector (FID). The column used was HP-PLOT Q (0.53 mm × 30 m i.d., 40 μm, part No. 19095P-Q04) with a TCD detector temperature of 250 °C. The inlet was 250 °C split mode with 0.25 cc valve and split the flow of 100 mL min^{-1}. H_2 was detected by GC-TCD with N_2 as the carrier gas, CO_2 and CO were detected by GC-TCD with He as the carrier gas, and CH_4, C_2H_4, and C_2H_6 were detected by GC-FID with He (pressure of 9.0 psi at 60 °C) as the carrier gas.

Before starting the analysis, the GC was turned on for conditioning. The calibration process was also carried out prior to sample analysis. Standard gas (PT. Air Liquide, Jakarta) was injected for the calibration. Standard gas contains a mixture of methane (49.89 %), hydrogen (20.16 %), carbon monoxide (5 %), and carbon dioxide (balance). Standard gas was taken using a syringe, and then 0.1 mL, 0.2 mL, and 0.3 mL were injected in duplicates. After the measurement was complete, a calibration curve was made. After that, the instrument was ready to be used according to the gas refinery's temperature program: start at 60 °C for 5 min, then increase 20 °C min^{-1} up to the final temperature of 200 °C, which was maintained for 1 min. After the GC-TCD was calibrated, the collected gas sample from the gas bag was taken as much as 0.2 mL using a syringe and injected into the GC system. Measurements were performed 2 times. The sample measurement results were compared with the standard gas measurement results, and the peak area of each gas component was used to determine the gas composition and content (mL) using the standard calibration curve.

Furthermore, the liquid samples were analyzed for pH, volatile solid (VS), and total chemical oxygen demand (COD). The slurry samples' pH was measured using a Mettler Toledo pH meter. VS of the slurry was measured following the APHA method (APHA, 2005). Meanwhile, the total COD was conducted using Hach standard kit (Hach Lange, Düsseldorf, Germany).

3. Results and discussion

3.1. Chemical characteristics of S. plagiophyllum

Firstly, the chemical constituent, proximate, and ultimate analysis of *S. plagiophyllum* were determined. Please note that the sun-dried *S. plagiophyllum was used to determine its chemical characteristics.* The proximate, chemical constituent, and ultimate analysis of sun-dried *S. plagiophyllum* are presented in Table 1. As can be seen, sun-dried *S. plagiophyllum* contains a moister content of around 7.02 ± 0.02 wt %. The moisture content of dried *S. plagiophyllum* obtained from this study is nearly the same as that of pelagic *Sargassum* from the Mexican Caribbean coast, with a range of 5–7 % (Saldarriaga-Hernandez et al.,

Table 1
Proximate, chemical constituent, and ultimate analysis of sun-dried *S. plagiophyllum*.

Parameters	*S. plagiophyllum*
Proximate analysis (wt%)	
Moisture	7.02 ± 0.02
Ash content[a]	9.32 ± 0.04
Fixed carbon[a]	26.71 ± 0.03
Volatile matter	56.95 ± 0.12
Chemical constituent (wt%)	
Carbohydrates	53.23 ± 0.17
Proteins	12.71 ± 0.14
Lipids	2.05 ± 0.04
Others[b]	32.01
Ultimate analysis (wt%)	
C	42.40 ± 0.38
H	5.86 ± 0.03
N	1.45 ± 0.01
S	2.78 ± 0.05
O[c]	38.19 ± 0.30
C/N ratio	29.24 ± 0.18
HHV (MJ kg^{-1})	14.46 ± 0.08

[a] Dry base.
[b] Calculated by the difference.
[c] O = 100 % – C – H – N – S – ash.

2021). This value is also comparable with the moisture content of sun-dried pelagic *Sargassum* in Jamaica, which accounts in the range of 8.48–11.6 %, depending on the location (Machado et al., 2022). Meanwhile, Thompson et al. (2020) determined a moisture content of around 20.63 % for dried pelagic *Sargassum* from Barbados. Saldarriaga-Hernandez et al. (2021) reported that the moisture content of macroalgae varies depending on diverse factors, such as species, a year's season, the pre-drying process, and the collection place.

In addition, *S. plagiophyllum* has a significant amount of ash content, namely 9.32 ± 0.04 wt%, compared to the typical lignocellulosic biomass, such as coconut shell, which has an ash content of 1.05 wt% (Mohamed Noor et al., 2019). Based on the scanning electron microscopy-energy dispersive X-ray (SEM-EDX) analysis result (Fig. S2 in Supplementary Material), calcium (Ca) was the major macronutrient in *S. plagiophyllum*, followed by potassium (K), magnesium (Mg), and sodium (Na). This result agrees with the previous studies regarding the major mineral contents in other taxa *Sargassum* (Milledge et al., 2020). The macro-nutrients in macroalgae (K, Mg, Na, and C) are essential for metabolic activity and anaerobic growth of the methanogens population (Thompson et al., 2020). In addition, Chen et al. (2008) reported that the Na content in the substrate might also be beneficial for the stability of biodigester by minimizing the toxicity of NH_3-N. However, extremely high levels of Na may cause cell dehydration via osmotic pressure due to methanogen proliferation inhibition (Wall et al., 2014). Having said that, the presence of other macro-nutrients, such as Ca, K, and Mg, in the raw biomass may give an antagonistic effect of Na-induced biodigester toxicity (Chen et al., 2008).

Regarding the chemical constituent, *S. plagiophyllum* contains high carbohydrate fractions, i.e., 53.23 ± 0.17 wt%. The high carbohydrate content in macroalgae makes it easy to decompose by bacteria-producing biogas via anaerobic degradation (Costa et al., 2013). The carbohydrate content obtained from this study is almost similar to that found in *S. natans* I and *S. fluitans* from the Turks and Caicos Islands, which can account for 55.97 and 58.56 % of the dry weight, respectively (Milledge et al., 2020). In the meantime, *S. natans* from Trinidad and Tobago Islands are more affluent in carbohydrates, reaching up to 77.63 % of the dry weight (Mohammed et al., 2020). Rioux et al. (2007) reported that the chemical composition of macroalgae might be affected by the season of the year and the geographical origin.

Nevertheless, it should be noted that macroalgae also contain hydrocolloids included in the carbohydrate. Farghali et al. (2021) reported that alginate and high polyphenols in *Sargassum* spp. could inhibit the biodegradation process into simple sugars, lowering methane yield. These unique hydrocolloids in macroalgae may require a different substrate-specific pathway for better biogas production performance. Hence, it is interesting to investigate the effect of the pretreatment of different species of macroalgae for biogas production in the future.

Protein content in *S. plagiophyllum* was around 12.71 ± 0.14 wt% in dry mass. This content is nearly equal to the protein found in *S. fluitans* from Brazil, which was around 12.80 % (Ramos et al., 2000). Meanwhile, Mohammed et al. (2020) found a protein content of 5.80 % in *S. natans* from the Trinidad and Tobago Islands. Furthermore, the protein content found in *S. natans* I and *S. fluitans* from the Turks and Caicos Islands was much lower, i.e., 3.81 and 3.25 % of the dry weight, respectively (Milledge et al., 2020). The protein content found in marine macroalgae can vary depending on the species and environmental factors, such as geographical location, season, and harvesting time (Øverland et al., 2019). In addition, Øverland et al. (2019) also reported that seasonal changes in the protein content of different macroalgae are apparently due to water temperature, wave force, and light intensity at the harvesting location and time.

Ganesh Saratale et al. (2018) reported that macroalgae are a promising feedstock for biogas production since they have abundant carbohydrates and fewer proteins. However, high protein content in the feedstock is not endorsed since it will decompose into NH_3^+, leading to a toxic effect on methanogens bacteria (Kovács et al., 2013).

Moreover, the lipid content in *S. plagiophyllum* was determined to be around 2.05 ± 0.04 wt%. This content is slightly lower than the value reported by Milledge et al. (2020), who determined the lipid content around 3.58 % of the dry weight for *S. natans* VIII from the Turks and Caicos Islands. Meanwhile, Mohammed et al. (2020) reported that the lipid content in *S. natans* from Trinidad and Tobago Islands accounts for only 0.01 % of the dry weight. In any case, the lipid content in *S. plagiophyllum* is still acceptable as the biogas substrate. However, Cirne et al. (2007) reported that if the lipid content of the substrate is higher than 30 %, it will inhibit the anaerobic digestion process.

It is also interesting to note that *S. plagiophyllum* has a C/N ratio of 29.24 ± 0.18, comparable with the pelagic *Sargassum* from Conset Bay, Barbados, having a C/N ratio of 21.67 ± 0.21 (Thompson et al., 2020). The ultimate values of macroalgae are reported to vary and are affected by several factors, such as species, harvesting period, geographical origin, physiological variations, and environmental growth (Karray et al., 2015). In any case, the C/N ratio of raw feedstock used in this study is in the range of the ideal C/N ratio (i.e., 20–30) for optimum fermentation and anaerobic digestion (Thompson et al., 2020). The low ratio of C/N results in the accumulation of nitrogen in the form of ammonium ion (NH_4^+), which can increase biodigester's pH levels, producing a toxic effect on microbes (Chandra et al., 2012).

The analysis results of the chemical constituent of *S. plagiophyllum* reveal that *S. plagiophyllum* is a promising feedstock for biogas production. In this study, *S. plagiophyllum* was mixed with cow manure for inoculum preparation with a ratio of 1:1. The physicochemical properties of the substrate after mixing were determined as follows: VS of 31.02 g/L, C/N ratio of 22.84, and pH of 7.27. Therefore, it confirms that the mixture employed in this study is suitable for the substrate of the anaerobic digestion process since the C/N ratio is 20–30.

3.2. Gas product during the acclimatization process

The most effective and economical microbial source for biogas production is cow manure because it contains consortium bacteria that are effective for anaerobic digestion. However, since mammals eat terrestrial plants, these consortium bacteria should not be effectively used for direct anaerobic digestion of marine resources like macroalgae that contain different chemical compounds than those plants, such as high minerals and different types of polysaccharides. Herein, the adaptation of bacteria contained in cow manure should be performed first to enable them to live and degrade the organic compounds in marine macroalgae. Then, acclimatization is necessary to obtain inoculum derived from cow manure that has adapted to the condition of marine macroalgae.

Fig. 2 shows the gas composition and pH value during acclimatization under (a) deionized and (b) saline water. The results revealed that methane production started after 2 days for the inoculum using deionized water as a medium. The methane production increased significantly with the fermentation time. The maximum methane production, as much as 64.21 vol%, was achieved at 20 days of anaerobic degradation. The methane production remains stable after that. This result is comparable with the previous.

Unlike the deionized water media, methane production was not observed even after 2 days of fermentation for the inoculum with saline water. The methane was observed initially at a longer fermentation time of 4 days. The highest methane yield, as much as 45.59 vol%, was achieved after 28 days of anaerobic digestion. It means that biogas production from *S. plagiophyllum* in deionized water gave higher methane production than in saline water. It could be attributed to anaerobic bacteria growth inhibited by salt. It is in line with the previous finding of Oren et al. (1992), who observed that high salinity leads to low methane production due to anaerobic-microbial inhibition.

Apart from the gas composition, the pH value was also investigated during the acclimatization process. Both treatments had different days for stabilizing the pH. Under the deionized condition, the inoculum was stabilized on the 14th day with a pH range of 7.0 to 7.5. Meanwhile, the

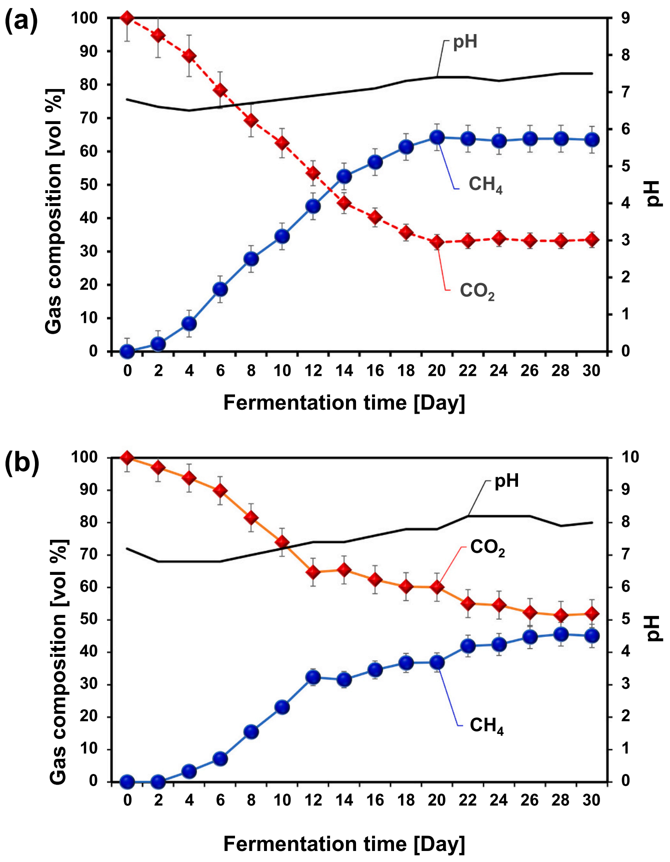

Fig. 2. Gas composition and pH value during the acclimatization process under (a) deionized water and (b) saline water.

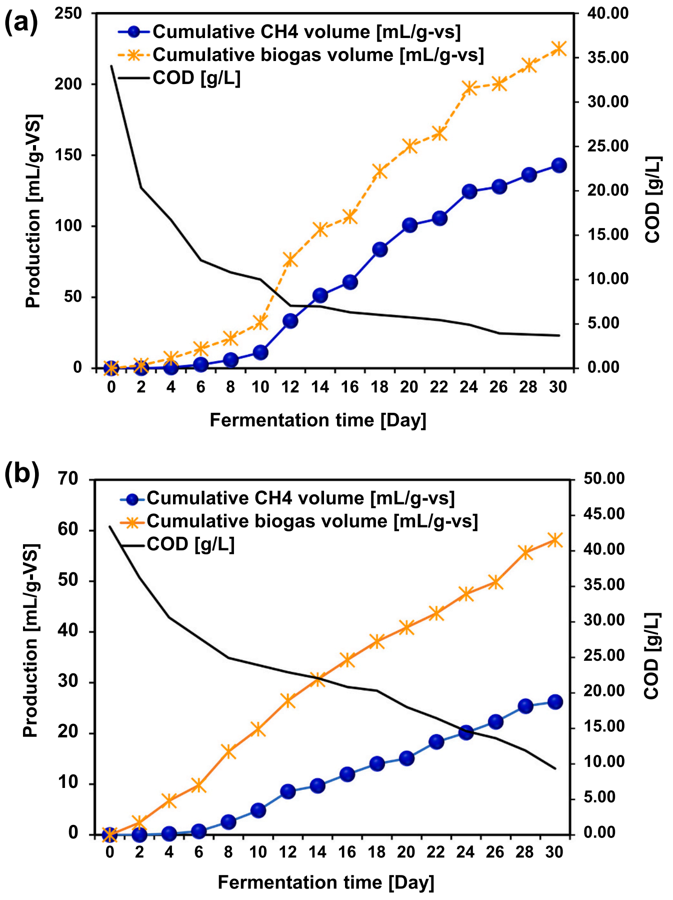

Fig. 3. Cumulative methane and biogas volume and COD during the acclimatization process under (a) deionized water and (b) saline water.

pH fluctuated more under the saline water condition from 7.4 to 8.2. It was reported by Hilkiah Igoni et al. (2008) that the range of pH levels between 6 and 8 is the optimum pH for microorganisms to conduct the anaerobic process.

During acclimatization, cumulative methane and biogas production versus COD was also investigated. Fig. 3 shows the average cumulative methane and biogas volume versus COD during acclimatization under (a) deionized and (b) saline water. Under the deionized water condition, the cumulative methane production of 142.94 mL/g-VS and biogas production of 225.14 mL/g-VS was achieved after 30 days of fermentation. Meanwhile, the methane and biogas production under saline water conditions were merely 26.22 and 58.16 mL/g-VS, respectively. It can be confirmed that high salinity inhibits methane composition and biogas volumes. This finding is in line with some previous works reporting that high salinity concentration of substrate could suppress the methane yield. Sun et al. (2017) investigated the effect of salinity on the anaerobic digestion of *Macrocystis pyrifera* by varying the salinity concentration to 38 ‰, 41 ‰, 48 ‰, 56 ‰, 63 ‰, 70 ‰, and 80 ‰. They found that the maximum value of 211.04 mL/g-VS substrate was obtained at a salinity of 38 ‰, and the cumulative methane yield decreased slightly at a salinity of 41 ‰. At the same time, it dropped drastically at a salinity above 48 ‰. Similarly, Marquez et al. (2013) also reported that salinity above 42 ‰ could inhibit methanogenic activity and methane production of an anaerobic digestion system treating sea wrack as substrate.

Furthermore, it is interesting to note that there is a negative correlation between methane volume and COD. COD significantly decreased from 34.06 g/L to 3.70 g/L after 30 days of fermentation for deionized water. In the meantime, for the case of saline water, the COD decreased from 43.41 g/L to 9.34 g/L. The COD removal during anaerobic digestion of *S. plagiophyllum* equals 89.1 % and 78.5 % for deionized and saline water, respectively. A decrease in COD can be correlated to bacterial activity in breaking down organic substances into methane gas. Since the degradation of organic compounds was easier under low salinity conditions, the COD in deionized water dropped significantly compared to that in saline water. This trend follows the previous work of Picos-Benítez et al. (2019), who reported that the COD removal decreased from 94 % to 89.8 % as an increase in salinity from 0 to 10 g/L for anaerobic digestion of saline wastewater.

3.3. Anaerobic biodegradability after the acclimatization process

As a second step, the anaerobic biodegradability after the acclimatization process was also studied by adding macroalgal feedstock (a mixture of macroalgae with the two types of water in a ratio of 1:2) into the inoculum. Please note that the two types of water used in this process were the same as the acclimatization process: deionized water and saline water. The cumulative methane and biogas volume results after the acclimatization process are presented in Fig. 4.

After 30 days of fermentation in the deionized water digester, the cumulative methane and biogas volumes were 266.18 mL/g-VS and 371.76 mL/g-VS, respectively, higher than those in the saline water digester, whose volumes were 63.77 mL/g-VS and 101.1 mL/g-VS, respectively. Again, this difference is due to the salinity concentration level, which inhibits bacterial activity (Oren et al., 1992).

Furthermore, it is noted that in a deionized water digester, biogas production remains increasing by prolonging the fermentation time, even using the *S. plagiophyllum* solely. Indeed, increased biogas production is influenced by the high carbohydrate and low lignin content of *S. plagiophyllum*. Macroalgae *S. plagiophyllum* contains high polysaccharides and cellulose contents, which bacteria can easily decompose

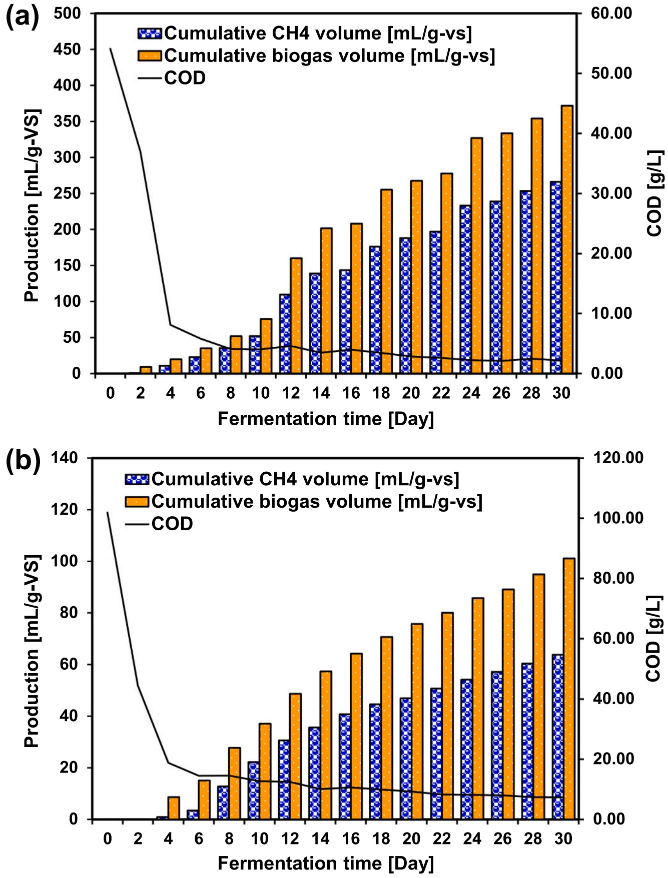

Fig. 4. Cumulative methane and biogas volume and COD after acclimatization process using (a) deionized water and (b) saline water.

(Alreshidi et al., 2022). In addition, the low lignin content enables microorganisms easily decompose the organic material to generate biogas (Song et al., 2014).

The methane yield obtained from this study was also compared with the previous studies using several types of micro- and macroalgae. Table 2 compares methane yield obtained from this study with other species of micro- and macroalgae. As shown, the highest methane yield obtained from this study (i.e., 266.18 mL/g-VS) is comparable with the previous works using other species of micro- and macroalgae. This finding can be compared with the methane yield achieved from anaerobic digestion of Caribbean pelagic *Sargassum* by Thompson et al. (2021), where the maximum methane yield was 292.18 ± 8.70 mL/g-VS from a blend of co-pretreated pelagic *Sargassum* and food waste at the weight ratio 25:75. For the case of other species of *Sargassum*, Chikani-Cabrera et al. (2022) recently reported that maximum methane yield of 387 ± 3.09 L/kg-VS was achieved from anaerobic digestion of *S. natans* and *S. fluitan*. However, the pretreatments using 2.5 % hydrogen peroxide followed by an enzymatic process were needed. Furthermore, AP et al. (2021) investigated the anaerobic digestion of *S. fulvellum* from Indonesia to understand better the effect of mechanical, chemical, and biological pretreatments on methane production. They reported that mechanical pretreatment of *S. fulvellum* without chemical addition resulted in 142.91 ± 0.004 mL CH_4/g-VS, higher than chemically pretreated reduced-sized macroalgae.

Table 2
Comparison of methane yield obtained from this study with the other species of micro- and macroalgae.

Group	Species	Methane yield [ml/g-VS]	Reference
Green macroalgae	*Codium tomentosum*	158	(Jard et al., 2013)
	Ulva lactuca	270–480	(Barbot et al., 2016)
Red macroalgae	*Gigartina* spp.	266	(Maia et al., 2016)
	Gracilaria	280–400	(Barbot et al., 2016)
	Palmaria palmate	279	(Jard et al., 2013)
Brown macroalgae	*Laminaria ochroleuca*	472	(Maia et al., 2016)
	Saccharina latissima	425	(Maia et al., 2016)
	Saccorhiza polyschides	232	(Jard et al., 2013)
	Sargassum plagiophyllum	266.18	Present study
	Sargassum spp.	387	(Chikani-Cabrera et al., 2022)
	Undaria pinnatifida	283	(Jard et al., 2013)
Microalgae	*Chlorella pyrenoidosa*	264.71	(Prajapati et al., 2014)
	Chlorella vulgaris	195.64	(Prajapati et al., 2014)

It is interesting to note that methane yields obtained from anaerobic digestion of micro- and macroalgae were higher than that of lignocellulosic biomass, such as corn stover (0.107–0.241 m^3/kg-VS), switchgrass (0.125 m^3/kg-VS), and pine wood (0.02 m^3/kg-VS) (Xu et al., 2014). This could be attributed to the fact that lignocellulosic biomass has a complex structure and high lignin content which is highly resistant to hydrolysis and toxic for some microorganisms (Zabed et al., 2020). On the other hand, micro- and macroalgae have no lignin or are present in a negligible amount, resulting in a higher biodegradability than lignocellulosic biomass (Ganesh Saratale et al., 2018).

Besides the chemical composition, the methane yield of anaerobic digestion was also affected by algae habitats. For instance, studying 15 freshwater and 5 marine microalgae species, Frigon et al. (2013) reported that methane yield varied between 298 ± 83 mL/g-VS in marine algae and 329 ± 43 mL/g-VS in freshwater algae.

3.4. Reaction kinetics of methane production

Finally, the reaction kinetics of methane production under different salinity conditions are determined. The methane production from macroalgae was modeled using a modified Gomperz equation, the popular semi-empirical model for the kinetic study of methane production (Syaichurrozi et al., 2013). The modified Gomperz equation for methane production is presented in Eq. (1) as follows:

$$M_t = A.exp\left\{-exp\left[\frac{\mu.e}{A}(\lambda - t) + 1\right]\right\} \tag{1}$$

where M_t is methane yield at t time [−], A is the methane production potential [−], μ is the maximum specific methane rate [ml/g], λ is the lag phase period or minimum time to produce methane [days], and e is mathematical constant (2.718282).

The kinetic constants of A, μ, and λ were calculated using the least-squares-error (LSE) method. The comparison of experimental data and the modified Gompertz model for methane production under deionized water and saline water conditions is presented in Fig. 5 (a) and (b), respectively. As observed, modified Gompertz simulated the methane production for the anaerobic digestion of *S. plagiophyllum*. Moreover, it was also confirmed by the parity plots, which resulted in the high coefficient of determination (R^2), i.e., 0.9992 and 0.9967, as shown in Fig. 5 (c) and (d).

The highest methane yield of 0.642 for anaerobic digestion of *S. plagiophyllum* using deionized water was achieved after 20 days of fermentation, corresponding to 0.615 of calculated methane yield using the modified Gompertz model. Meanwhile, the highest methane yield of 0.456 for anaerobic digestion of *S. plagiophyllum* using saline water was obtained after 28 days of fermentation, corresponding to 0.436 of calculated methane yield using the modified Gompertz model.

By employing the Gompertz model for the anaerobic digestion of

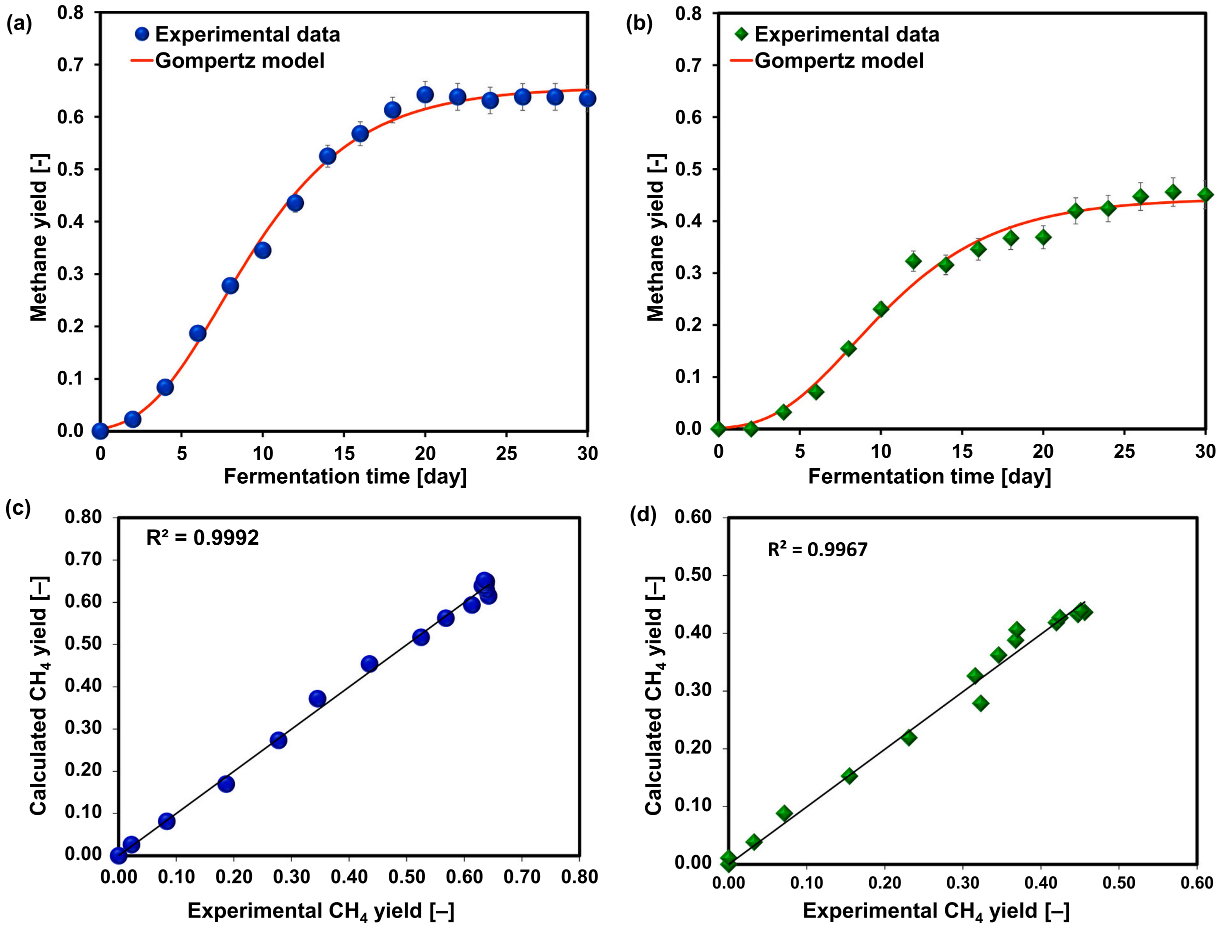

Fig. 5. Comparison of experimental data and modified Gompertz model for methane production under (a) deionized water and (b) saline water and its corresponding parity plots under (c) deionized water and (d) saline water.

S. plagiophyllum, the methane production potential (*A*) and the lag phase period (*λ*) were also successfully determined. The methane production potential for the anaerobic digestion of *S. plagiophyllum* under deionized water conditions was higher (i.e., 0.24) than that under saline water conditions (i.e., 0.16). Furthermore, the lag phase period (λ) value was determined around 7.4 and 8.3 days for anaerobic digestion of *S. plagiophyllum* under deionized water and saline water, respectively. This lag phase period or minimum time to produce methane was comparable with the previous work of Pardilhó et al. (2022), who calculated the lag phase period of 6.6 and 9.8 days for anaerobic digestion of marine macroalgae waste at different total solid contents of 0.9 and 1.7 %, respectively.

4. Conclusions

Sargassum plagiophyllum has the potential to be used as a feedstock of biogas since they have high carbohydrate content (53.23 ± 0.17 wt%) and a balanced C/N ratio (29.24 ± 0.18). The acclimatization process of *S. plagiophyllum* in manure requires 20 days until a neutral pH is reached. After 30 days of fermentation, the cumulative biogas volumes in the deionized water digester were 371.76 mL/g-VS, higher than those in the saline water digester, whose volumes were only 101.1 mL/g-VS. The highest methane yield of 266.18 mL/g-VS was achieved for the anaerobic digestion of *S. plagiophyllum* after 30 days of fermentation under low salinity conditions. It confirmed that biogas production under low salinity conditions is better than that under high salinity conditions. The kinetic evaluation using a modified Gompertz model simulated the methane production satisfactorily. The minimum time to produce methane (λ) was around 7.4 and 8.3 days for anaerobic digestion of *S. plagiophyllum* under deionized water and saline water, respectively.

CRediT authorship contribution statement

Obie Farobie: Conceptualization, Methodology, Writing – original draft, Resources, Formal analysis, Data curation, Project administration, Funding acquisition. **Apip Amrullah:** Investigation, Methodology, Formal analysis. **Latifa Aisya Anis:** Investigation, Data curation, Formal analysis. **Edy Hartulistiyoso:** Conceptualization, Writing – review & editing, Supervision. **Novi Syaftika:** Writing – review & editing, Resources. **Ganjar Saefurahman:** Investigation, Methodology. **Asep Bayu:** Conceptualization, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2023.101403.

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