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Utilizing *Sargassum polycystum* as Co-Feedstock to Enhance Methane Yield from Pig Dung in Anaerobic Digestion

Earl Francis A. Busilaoco[1], Wendyl M. Aligato[1]*, Jan Nico R. Gaslang[1], Hyrn G. Almoroto[1], Kristine Yhuri A. Libreta[1], Mervy Aretha Deon L. Loon[1], Camella A. Redulla[1], Darius Miguel Pederes[1], Angela Glaiza B. Pingcas[1], Rovie Joice M. Durante[1], Ricksterlie C. Verzosa[2]

[1]Immaculate Heart of Mary Academy, Manuel L. Quezon St., Central, City of Mati, 8200 Davao Oriental, Philippines. ORCID No.: Earl Francis A. Busilaoco 0009-0002-1168-8085, Wendyl M. Aligato0009-0008-7897-5736, Jan Nico R. Gaslang 0009- 0001-3675-7174, Hyrn G. Almoroto 0009-0004-9075-2376, Kristine Yhuri A. Libreta 0009-0003-9327-5432, Mervy Aretha Deon L. Loon 0009-0002-4134-7433, Camella A. Redulla 0009-0001-1450-87080, Darius Miguel Pederes0009-0000-5301-413X, Angela Glaiza B. Pingcas 0009-0005-3231-5191, Rovie Joice M. Durante 0009-0000-5443-9885
[2]Faculty of Agriculture and Life Sciences. Davao Oriental State University, Dahican, City of Mati, 8200 Davao Oriental, Philippines. ORCID: Ricksterlie C. Verzosa 0000-0003-1872-6232

*Corresponding author: aligatowendyl0706@gmail.com

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ABSTRACT

Anaerobic digestion (AD) is a promising technology for biogas production, but optimizing feedstock composition is still a key challenge. The present study investigated invasive macroalgae *Sargassum polycystum* as a co-feedstock source combined with pig dung to enhance methane production. Three feedstock groups were assessed: (1) mechanically pretreated *S. polycystum* + pig dung, (2) untreated *S. polycystum* + pig dung, and (3) pig dung alone. Seaweed feedstocks were collected in Dahican beachline, and pig manure was sourced from a livestock auction market in the City of Mati, Davao Oriental, the Philippines. Feedstocks were loaded and inoculated in an improvised biodigester. Methane concentrations were measured using a gas analyzer, and a flammability test was conducted to evaluate biogas quality. Kruskal-Wallis test revealed significant differences in methane production across treatments ($H(2) = 9.116$, $p = 0.010$). The pretreated group exhibited the highest methane concentration (>9,999.00 ppm), followed by the untreated group (8,931.75 ppm), while the control group produced the lowest yield (3,644.25 ppm). Post hoc analysis confirmed a significant difference in methane yield in the pretreated group compared to the control ($p = 0.010$). Only biogas from the pretreated group ignited, producing a blue flame indicating methane-rich, high-quality biogas. These findings highlight the dual benefit of using *Sargassum macroalgae as co-feedstock*, mitigating seaweed overgrowth in coastal areas and improving biogas production efficiency in pig manure. The study underscores the potential of seaweed-based co-digestion as an accessible, sustainable energy solution. Future research may explore long-term process stability, gas composition analysis, and the economic viability of large-scale applications.

Keywords: Anaerobic digestion, biogas systems, methane production, pig dung, Sargassum

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INTRODUCTION

Methane (CH_4) is a potent greenhouse gas with a global warming potential 28 times greater than carbon dioxide (CO_2) over a 100-year period (IPCC, 2013; Cheong et al., 2023). A significant portion of methane emissions originates from agricultural activities, particularly livestock manure management, which contributes to climate change (Grossi et al., 2019). Given the increasing demand for sustainable waste management solutions, the utilization of organic waste for renewable energy production through anaerobic digestion (AD) has gained attention (Dahunsi, 2019). AD is a biological process that decomposes organic matter in the absence of oxygen, producing biogas primarily composed of methane and carbon dioxide (Karthikeyan et al., 2024). This technology not only mitigates methane emissions from unmanaged waste but also provides an alternative energy source (Anacleto et al., 2024; Li et al., 2019). However, optimizing methane yield remains a challenge, necessitating the exploration of additional feedstocks to enhance biogas production efficiency.

Macroalgae, particularly Sargassum species, has emerged as a potential co-digestion substrate for biogas production (AP et al., 2021). This large brown seaweed is commonly found in tropical and subtropical coastal waters (Cunha dos Santos et al., 2022). While it plays an ecological role in marine ecosystems, uncontrolled proliferation has led to extensive “golden tides,” causing environmental and economic problems such as habitat degradation, reduced tourism, and increased waste accumulation (Devault et al., 2021; Tonon et al., 2022). Climate change and nutrient pollution have intensified the frequency and scale of seaweed blooms, demanding sustainable management strategies (Robledo et al., 2021).

Previous studies have investigated the use of Sargassum in pharmaceuticals, nutraceuticals, and cosmeceuticals (Cunha dos Santos et al., 2024; Farghali et al., 2023), however its potential as a biogas feedstock remains underexplored, particularly *Sargassum polycystum*. Macroalgae have been recognized for their high organic matter content, making them suitable for anaerobic digestion (Irfan et al., 2019; Milledge et al., 2018). Recent studies have underscored that utilizing invasive macroalgae for AD can effectively reduce the ecological footprint of coastal communities by transforming a problematic waste stream into a valuable energy resource (Bohutskyi et al., 2014; Jelani et al., 2023; Kumar et al., 2020). However, the co-digestion of *S. polycystum* with livestock manure, specifically pig dung, has not been extensively studied. Since pig manure is nitrogen-rich, combining it with Sargassum macroalgae, which has a high carbon content (Fauziee et al., 2021), may optimize the carbon-to-nitrogen (C/N) ratio (McKennedy and Sherlock, 2015). This in turn enhances microbial activity and methane production from co-digestion of feedstocks (Orhorhoro and Oghoghorie, 2024).

This paper expands on previous studies by evaluating the potential of *Sargassum polycystum* co-digested with pig dung to improve methane yield under controlled anaerobic digestion conditions. Specifically, it assessed methane production across different treatment groups: pig dung alone, untreated *S. polycystum* with pig dung, and pretreated *S. polycystum* with pig dung. By integrating invasive macroalgae into biogas systems, this study provides insights into sustainable waste management strategies while addressing methane emissions and energy production challenges. The findings aim to contribute to the development of renewable energy solutions for coastal and agricultural communities, answering the problem on waste reduction and bioenergy generation.

MATERIALS AND METHODS

Description of the study area

The study was conducted in the City of Mati, Davao Oriental, the Philippines from December 2023 – February 2024 (Figure 1). The experimental biodigesters were installed in an undisturbed, shaded area located at Madang, Barangay Central. This location was chosen to maintain an undisturbed environment during the decomposition process, ensuring accurate biogas production. Pig dung was collected from a nearby livestock auction market in Sitio Sudlon, Barangay Central. Samples of *Sargassum polycystum* was harvested from Dahican coastline where these seaweeds are abundant. The seaweed samples were authenticated by the Bureau of Fisheries and Aquatic Resources (BFAR).

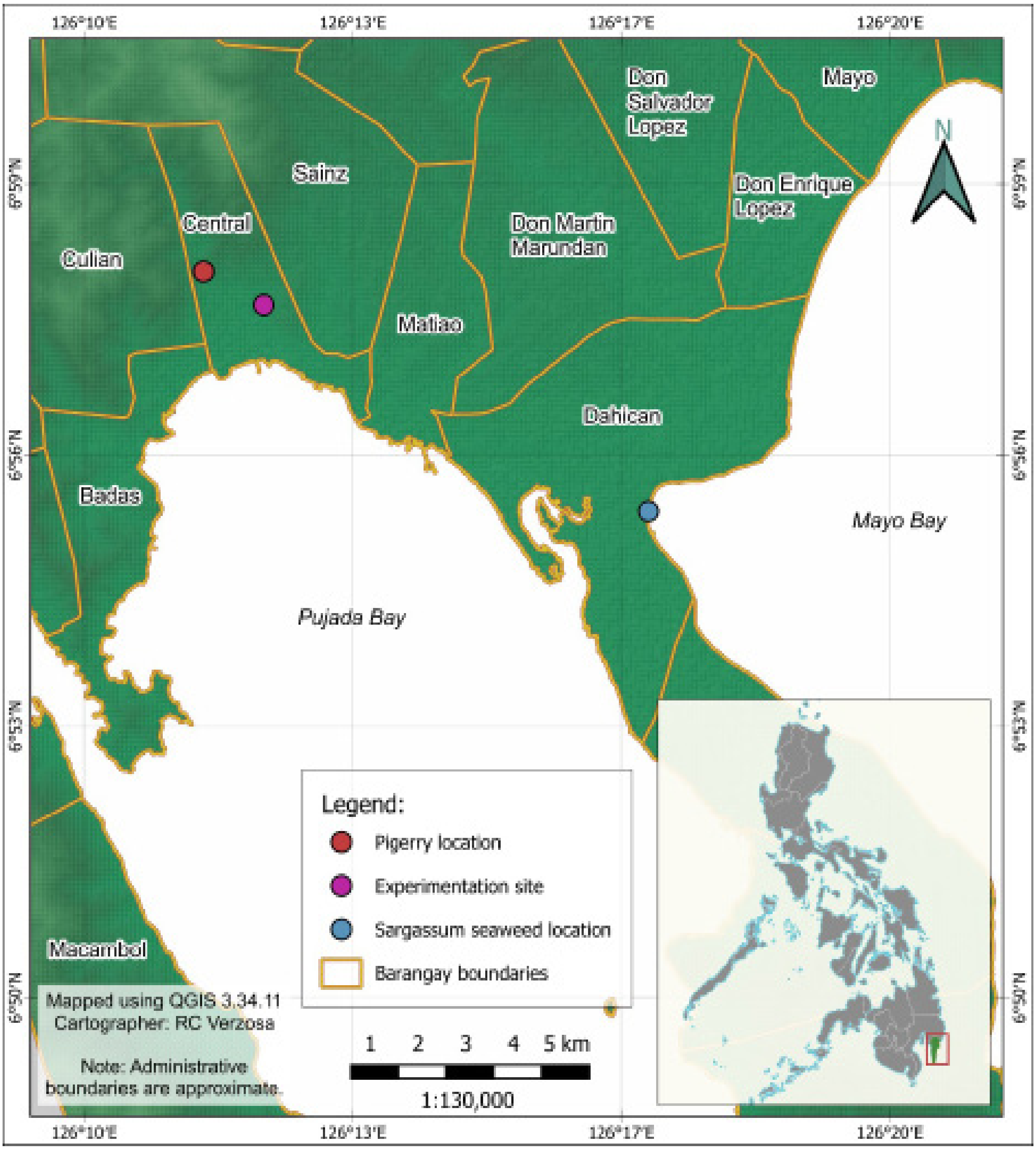

Figure 1. Map of the study area.

Biodigester installation

Improvised biodigesters were constructed using 20-liter water gallons as fermenting chambers. A schematic diagram (Figure 2) illustrates the biodigester prototype, which comprised key components including a fermenting chamber, feed tube, inlet and outlet pipes, on/off valves, and a tire tube (Interior F12) for biogas storage. All connections were rendered airtight using solvent cement, Exposeal epoxy, PVC L-shaped connectors, steel hose nipples, and garden hoses. The biodigesters were installed on a firm base and covered with tarpaulin to shield the system from direct sunlight. Drilled ports on the water gallon lids facilitated the attachment of pipes and hoses, following design adaptations from Naveed Zahir Creativity (2019).

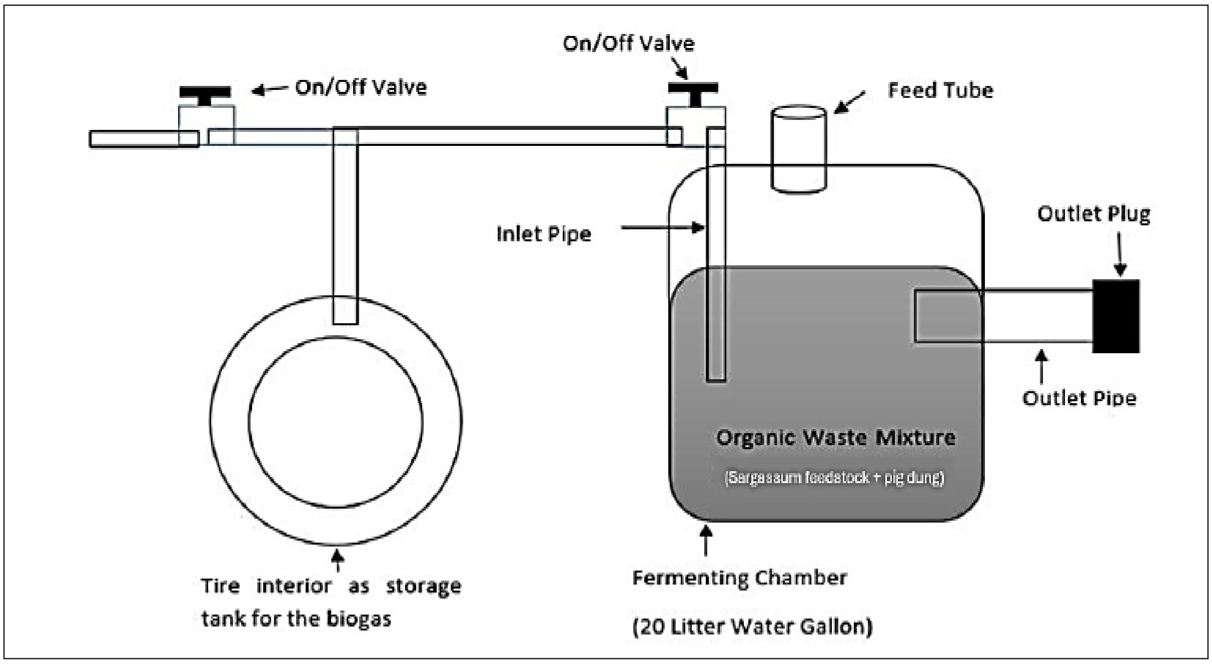

Figure 2. Schematic Diagram of the Biodigester Prototype.

Feedstock Collection and Preparation

Sargassum polycystum was collected manually from the Dahican coastline. After harvesting, the seaweed was thoroughly washed to remove sand and other debris, then it was air-dried. Pig dung was sourced fresh from the local livestock market. Both substrates were stored at ambient temperature and used within 24 hours of collection to preserve their organic integrity.

Figure 3. Collection of sargassum seaweed in Dahican coastline (A, B, C).

Three treatment groups were established for the experiment, pretreated - pig dung co-digested with pretreated *S. polycystum*, untreated-pig dung co-digested with untreated *S. polycystum* and control - pig dung only. For the pretreated grouped, the sliced seaweed was subjected to mechanical chopping aimed at enhancing the release of fermentable sugars (Irfan et al., 2019; Maneein et al., 2018). For the untreated group, the seaweed was mixed directly with the pig dung without any pretreatment. Whereas the control group, the pig dung alone was left without any pretreatment or mixture.

Biodigester Operation and Inoculation

The biodigesters were loaded with the respective feedstock mixtures and sealed to maintain anaerobic conditions (Figure 4). For substrate optimization, both pretreated and untreated groups were prepared at a 3:1 ratio (6 kilograms of *Sargassum polycystum* to 2 kilograms of pig dung). This specific feedstock composition was selected to achieve an optimal carbon-to-nitrogen (C/N) balance, a critical factor for efficient anaerobic digestion and enhanced methane production. A standardized inoculation protocol was followed by ensuring that the system was purged of air prior to starting the digestion process. Daily inspections were conducted to monitor for leaks, check system integrity, and ensure that oxygen intrusion was minimized. Environmental parameters such as ambient temperature and pH were recorded periodically to monitor system stability.

Figure 4. Improvised biodigester set-up (A, B, C, D).

Gas Sampling and Analysis

Gas samples were taken from the biodigesters using sealed balloons, which were labeled and brought to room temperature before analysis. A methane analyzer (HABOTEST® HT601 gas leak detector) was used for methane concentration determination with a maximum detection limit of 9,999 ppm. After calibrating the methane analyzer, the gas samples were measured to determine methane concentrations. The samples were analyzed and compared across different treatments to assess the effect of *S. polycystum* co-digestion on methane production of pig dung. Four gas samples were tested for each treatment.

Flammability Testing

In addition to gas concentration measurements, an open-air flammability test was performed to qualitatively assess the methane content of the biogas. A representative biogas sample was collected from the biodigester using rubber balloon. Approximately 50 mL of biogas was then slowly dispensed for controlled ignition and positioned in a well-ventilated outdoor area. Immediately following the gas release, a standard lighter was used to ignite the sample, and the flame characteristics were observed. A stable, blue flame was considered indicative of high methane content, while a weak or absent flame suggested a lower methane-to-CO_2 ratio (Ketut et al., 2018). This procedure was repeated in triplicate for each experimental group (control, untreated, and pretreated) to ensure reproducibility. All tests were conducted with appropriate safety precautions and with the presence of a fire extinguisher.

Data Analysis

Data from the anaerobic digestion (AD) experiment were obtained for three treatments—Control (pig dung alone), Untreated (pig dung co-digested with untreated *Sargassum polycystum*), and Pretreated (pig dung co-digested with pretreated *S. polycystum*). Given the small sample size and the non-normal distribution of the data as assessed by preliminary tests, nonparametric statistical methods were conducted.

A Kruskal–Wallis *H* test was employed to determine if there were statistically significant differences in methane concentration among the three treatment groups. The test was chosen because it does not assume normality and is appropriate for small sample sizes. For *post hoc* analysis, pairwise comparisons using Mann-Whitney U test with Bonferroni corrections were performed.

RESULTS

Methane Yield Across Treatments

Table 1 summarizes the mean concentrations of methane gas observed across the three treatment groups. The Pretreated group achieved the highest mean methane concentration (>9,999.00 ppm, maximum detection limit), followed by the Untreated group (8,931.75 ppm), while the Control group (pig dung alone) yielded evidently lower values (3,644.25 ppm). The data shows that incorporating *Sargassum polycystum* in pig dung increases methane production, with pre-treatment further improving the yield. These findings suggest that the inclusion of *S. polycystum* as a co-substrate enhances methane production, with pre-treatment of the seaweed can further improve the yield.

Table 1. Mean methane concentrations for AD treatments.

Treatment	Mean CH_4 Concentrations (ppm)
Pretreated *S. polycystum* + pig dung	> 9,999.00
Untreated *S. polycystum* + pig dung	8,931.75
Pig dung only (Control)	3,644.25

To account for significant variation in the methane concentration between treatments, Kruskal–Wallis *H* test was performed. The test revealed statistically significant differences in methane concentration across the three treatment groups ($H(2) = 9.116$, $p = 0.010$) (Table 2). This result indicates that the distribution of methane concentrations was not uniform across the treatment groups, rejecting the null hypothesis of equal methane yields.

Table 2. Kruskal-Wallis *H* test results for methane concentrations.

Treatment	*n*	Mean Rank	*H*	*p*
Pretreated *S. polycystum* + pig dung	4	9.50	9.116	.010
Untreated *S. polycystum* + pig dung	4	7.50		
Pig dung only (Control)	4	2.50		

[*n*] number of samples; [MR] Mean Rank; [*H*] H statistic or Chi-square value; [*p*] p–value

A *post hoc* pairwise comparison was conducted using the Bonferroni correction for multiple tests to identify specific group differences. The results revealed that the difference in methane yield between the Pre-treated Control groups was statistically significant (U = 7.00, adjusted p = 0.01). This confirms that the pre-treatment process substantially enhanced methane production compared to the control.

Furthermore, the comparison between the Untreated and Control groups showed a notable difference in methane yield (U = 5.00, adjusted p = 0.11), but this difference did not reach statistical significance after the Bonferroni adjustment. Although untreated *S. polycystum* contributed to increased methane production compared to pig dung alone, the lack of statistical significance suggests that the effect was not as pronounced as in the pre-treated group.

Flammability Test of Biogas Produced

The flammability test revealed that only the biogas produced from the pretreated *S. polycystum* + pig dung was ignitable, producing a blue flame (Figure 5), which is indicative of a methane-rich biogas suitable for combustion. In contrast, biogas from the control and untreated groups did not ignite, confirming the higher methane content of the pretreated group. This result further supports the high methane concentration observed in the pretreated group compared to control and untreated groups.

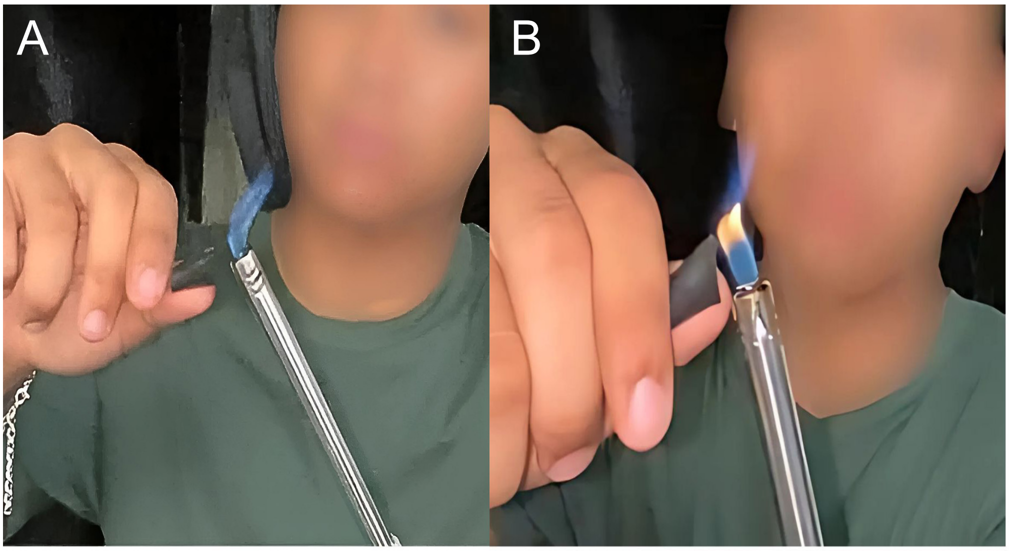

Figure 5. Blue flame produced by biogas sample in pretreated (A, B).

DISCUSSION

Enhancement of Methane Yield by *S. polycystum*

The results indicate that *Sargassum polycystum* serves as a viable co-feedstock for enhancing methane yield in anaerobic digestion (AD) of pig dung. The higher methane yield in the untreated and pretreated groups compared to the control aligns with previous studies demonstrating that macroalgae can enhance biogas production due to their high carbohydrate content (Oliveira et al., 2014).

The improved methane yields observed, particularly in the pretreated group, can also be attributed to the synergistic effects of co-digesting pig dung with *S. polycystum*. The high nitrogen content of pig dung complements the carbohydrate-rich *S. polycystum*, optimizing the carbon-to-nitrogen ratio necessary for microbial growth and methane production. This finding is consistent with the works of Thompson et al. (2021) and Rivera-Hernández et al. (2022), who emphasized the advantages of co-digestion in enhancing biogas yields by balancing the nutrient composition of the feedstocks. In the study of Orhorhoro and Oghoghorie (2024), the use of *Sargassum* spp. as co-feedstock improved the biogas yields of animal manure (chicken, goat, pig and cow) compared to sole digestion of the substrates.

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However, the difference between the control and untreated groups was not statistically significant, indicating that while *S. polycystum* enhanced methane production, its effectiveness was amplified by pre-treatment. This is likely due to the structural complexity of seaweed cell walls, which can limit microbial access to fermentable sugars if not pre-treated (Zou et al., 2018).

Effect of Pretreatment of *S. polycystum* on Methane Yield

Pre-treatment of *S. polycystum* significantly enhanced methane yield. This finding was evident in the statistically significant differences in methane yield between the control and pretreated groups, while no significant variations in untreated and control groups. This is primarily attributed to the mechanical pre-treatment of seaweeds, which effectively reduces particle size for faster anaerobic digestion. The resulting smaller particle size increases the surface area available for microbial attachment, thereby accelerating hydrolysis and enhancing substrate bioavailability for subsequent microbial digestion (Maneein et al., 2018; Rodriguez et al., 2018). By chopping the seaweed, the mechanical disruption facilitates the release of fermentable organic compounds from within the rigid cell walls, promoting efficient methanogenesis.

These results corroborate previous studies that mechanical pretreatment significantly enhances methane production and biodegradability of seaweed feedstock (Marshall and Oyekola, 2025). Similar outcomes have been observed in studies on other macroalgae, such as *Laminaria digitata* and various *Sargassum* species, where alternative pre-treatment methods, including hydrothermal, enzymatic, and acidic treatments, have also led to improved methane yields (Tabassum et al., 2017; Thompson et al., 2021; Vanegas et al., 2015). These findings collectively underscore the critical role of effective pre-treatment in improving the bioavailability of organic matter and, consequently, boosting biogas production in anaerobic digestion processes.

Flammability as an Indicator of Biogas Quality

The flammability test demonstrated that only the pretreated group's biogas was ignitable and with blue flame color. This indicates a higher methane content and better combustion efficiency in the co-digested pig dung and pretreated *S. polycystum.* Typically, biogas is composed of 50 - 70% CH_4 by volume, 30 - 50% CO_2, and other trace gases (Anggono, 2017; Jameel et al., 2024). A blue flame is generally associated with complete combustion and high thermal energy release (Suhaimi et al., 2017; Yusuf et al., 2020). This finding aligns with the study of Keita and Kamano (2024) where biogas from pig manure produced a stable yellow/blue flame after 15 to 20 days digestion.

On the other hand, the failure of the untreated and control group biogas to ignite suggests a lower CH_4-to-CO_2 ratio, possibly due to incomplete digestion or higher carbon dioxide levels. Similar observations have been reported in co-digestion studies of macroalgae with livestock manure or with food wastes (Castro et al., 2022; Xu et al., 2025), where feedstock composition influences the CH_4: CO_2 balance in biogas.

Environmental and Economic Implications

The integration of *Sargassum polycystum* into biogas production offers significant environmental benefits beyond reducing coastal waste accumulation and methane emissions from pig dung. The large-scale beaching of invasive seaweeds can disrupt coastal ecosystems, impair water quality, and diminish tourism revenue (Rodil et al., 2024; Thompson et al., 2021). Diverting seaweed biomass from shoreline accumulation into anaerobic digestion systems minimizes nutrient runoff and habitat degradation (Milledge et al., 2020). Moreover, the conversion process helps sequester carbon that would otherwise be released as methane. Using seaweed can achieve sufficiently high methane concentrations for efficient energy recovery and in turn contributing to reduced fossil fuel dependency and supporting climate change mitigation goals (Farghali et al., 2023).

In addition to these benefits, the use of *S. polycystum* in biogas production may also help alleviate coastal eutrophication. By removing excess macroalgae biomass from beaches, the risk of nutrient overload in adjacent waters is reduced, thereby protecting marine biodiversity and maintaining water quality (Warguła et al., 2021). This process indirectly supports the preservation of local fisheries and recreational areas, further contributing to a healthy coastal environment.

From an economic perspective, co-digestion of *S. polycystum* with pig dung presents a multifaceted opportunity for small-scale farmers and coastal communities. The process generates renewable energy in the form of biogas, which can be utilized locally for cooking or electricity generation (Kumar et al., 2020). This clean, renewable energy source replaces the use of firewood, especially in developing countries, and help reduce deforestation(Surendra et al., 2014). Biogas utilization also produces a nutrient-rich digestate that can be used as an organic fertilizer (McKennedy and Sherlock, 2015; Surendra et al., 2014). This reduces reliance on expensive synthetic fertilizers and supports a circular economy by turning waste into valuable resources. The integrated system may also lower waste management costs and creates additional revenue streams and job opportunities, thereby boosting local economic resilience.

CONCLUSION

The study highlights the potential of *Sargassum polycystum* as an effective feedstock for enhancing methane production in biogas systems, particularly when pretreated, in combination with pig dung. The results demonstrate that pretreatment significantly increases methane yield, making it a promising approach for both mitigating methane emissions and addressing the environmental issue of invasive seaweed accumulation. Additionally, the integration of *S. polycystum* into swine manure biogas production systems presents a sustainable alternative to conventional energy sources. These findings support the viability of using marine and agricultural waste to develop circular economy models that simultaneously reduce greenhouse gas emissions, promote sustainable agriculture, and contribute to local energy resilience. Future research could explore the long-term efficiency and scalability of *S. polycystum* biogas systems, including optimization of pretreatment methods, co-digestion ratios, and potential applications in large-scale bioenergy production

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AUTHOR CONTRIBUTIONS

Conceptualization, E.F.A.B., W.M.A., A.N.R. G., H.G.A., R.J.M.D.; methodology, E.F.A.B., W.M.A., A.N.R.G., H.G.A., R.J.M.D.; validation, K.Y.A.L., M.A. D.L.L., D.M.P., A.G.B.P., C.A.R.; formal analysis, E.F. A.B., W.M.A., A.N.R.G., H.G.A., R.J.M.D., R.C.V.; investigation, E.F.A.B., W.M.A., A.N.R.G., H.G.A., R.J. M.D.; resources, A.N.R.G., H.G.A., R.J.M.D., K.Y.A.L., M.A.D.L.L., D.M.P., A.G.B.P data curation, E.F.A.B., W.M.A., A.N.R.G., H.G.A.; writing—original draft preparation, E.F.A.B., W.M.A., R.C.V.; writing—review and editing, W.M.A., R.C.V.; project administration, E.F.A.B., W.M.A., A.N.R.G., H.G.A. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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