Improvement in Methane Production from Pelagic Sargassum Using Combined Pretreatments

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Abstract: The constant golden tides of Sargassum spp., identified to be a mixture of Sargassum natans and Sargassum fluitans, observed recently in the Mexican Caribbean have affected the marine ecosystem and the local economy and have created the need for solutions for their management and use. The Sargassum arrivals have thus been considered as third-generation feedstock for biofuel. Their potential for energetic conversion to biomethane was investigated, with hydrolysis as the limiting step due to its complex composition; therefore, in the present study, different physical, chemical, and enzymatic pretreatments and a combination of them have been evaluated, with the additional use of granular activated carbon, to determine the best yield and methane quality. The combined pretreatments of 2.5% hydrogen peroxide, followed by an enzymatic pretreatment (enzymatic extract from Trametes hirsuta isolated from decomposing wood in the Yucatán Peninsula-Mexico), was the best option, reaching a biodegradability of 95% and maximum methane yield of 387 ± 3.09 L CH₄/kg volatile solid. The use of a conductive material, such as granular activated carbon, did not generate significant changes in performance and methane concentration.

Keywords: anaerobic digestion; pelagic Sargassum; pretreatments; direct interspecies electron transfer

1. Introduction

There are approximately 350 species of the genus Sargassum. Two of these holopelagic Sargassum species, S. natans and S. fluitans, are the major contributor to golden tides [1]. The first golden tides appeared in 2011 in the North Atlantic; during the following years, they spread from West Africa to the Caribbean Sea and Gulf of Mexico [2]. The recurrent golden tides have caused damage to the marine ecosystem, causing the death of fish, damage to coral reefs, preventing the nesting of turtles, and causing coastal dead zones [3,4]. In 2015, 10,000 tons of Sargassum were removed from the beach daily [1]. Although the influx of Sargassum decreased significantly during 2016 and 2017, in 2018 and 2019 the phenomenon was repeated, so it is expected that the arrivals will persist [5].

A solution for the disposal of Sargassum is the production of biogas, with hydrolysis as the rate-limiting step due to its composition: insoluble fibers (7.15–75%) [6,7], lignin (15.6% to 29.5% dry basis) [8,9], heavy metals, salts (15.2–23.1% dry basis) [10], polyphenols (25.4% dry basis) [8], and a low beneficial ratio of carbon and nitrogen (less than 20:1) [10,11]. Pretreatments break down structures that are difficult for hydrolytic microorganisms to digest [9,12]. For this reason, physical pretreatments such as maceration reduce the size of the particles, increasing the surface area that allows sugars to be released [12]. Generally, the physical pretreatments carried out on different macroalgae have positive results, with yields from 92 L CH₄/kg VS to 422 L CH₄/kg VS [13–18].
The use of chemical pretreatments, such as acids or alkalis, changes the structural solid fractions which facilitate the biodegradability of the biomass. In alkali pretreatment, the biomass simultaneously undergoes solvation and saponification, degrading the lignin and cellulose components, thereby increasing the concentration of sugars accessible for microbial digestion. Comparatively, the use of acids is more effective than alkalis at accelerating hemicellulose depolymerisation and delignifying biomass [19]. Yields of 148 to 365 L CH₄/kg VS have been reported for various macroalgae when using alkaline pretreatments such as NaOH [20–22], while for an acid pretreatment (HCl), the yields were from 52 to 312 L CH₄/kg VS [20–22]; however, severe pretreatments, such as the use of chemicals, can release inhibitors such as furfural, organic acids, and phenolic compounds and alter the pH, inhibiting anaerobic digestion [23].

The biological pretreatment applied in several macroalgae, including the use of enzymes such as alginate lyases, laminarinases, or glucanases, for the degradation of polysaccharides in brown algae, were obtained from marine microorganisms [24–26] or seaweed composts [26]. The phenolic compounds and lignin [8] commonly found in Sargassum spp. can be oxidized by laccases [8,9]. Many white rot fungi, including Trametes versicolor, have been used for the selective degradation of lignin and hemicellulose. These fungi produce extracellular lignolytic enzymes such as lignin peroxidase, manganese peroxidase, and laccase [27,28]. The fungal pretreatment from Trametes hirsuta has increased the methane production for a pelagic Sargassum consortium from the Mexican Caribbean by 17% [9].

The use of enzymes as pretreatments results in yields from 49 L CH₄/kg VS (Fucus vesiculosus) [14] to 232 L CH₄/kg VS (Laminaria digitata and Saccharina latissima) [29]. The use of extracellular enzymes is attractive due to their low cost, their extraction does not imply the use of chemicals that harm the environment, and they can be used as a pretreatment to improve the biodegradability of macroalgae [9].

Likewise, author Saratale et al., 2018, discuss in their review that the co-digestion of algal biomass with other waste substrates such as straws, waste paper, switch grass, glycerol, beet silage, and sewage sludge, among others, improve the C:N ratio which results in increased methane production [30].

A way to overcome the biochemical fermentative barrier in the process of increasing the production and quality of methane during anaerobic digestion is to promote the direct transfer of electrons between microorganisms (for example, Geobacter metallireducens and Methanosaeta harundinacea) from conductive materials such as granular activated carbon (GAC, low cost), biochar, nano-magnetite, and minerals [31,32]. Electrically conductive materials could substitute pilus and/or cytochromes to exchange electrons [33]. However, Vallejo et al. determined that the adsorption or promotion of DIET, when a conductive material was used, depended on the characteristics of the substrate; in the case of pot ale whiskey, the use of activated carbon did not increase performance in anaerobic digestion due to the adsorption of organic matter [34]. Shanmugam et al. used the GAC to adsorb inhibitors such as phenolic compounds and the concentration of COD before the anaerobic digestion begins; and, similar to other authors, the concentration of methane was mainly due to the characteristics of the substrate and the adsorption properties of the conductive material [35].

Therefore, the main objective of this work was to evaluate the potential of biogas production from pelagic Sargassum in the Mexican Caribbean, choosing the best pretreatment, whether physical, chemical, enzymatic, and/or combined, with and without granular activated carbon (DIET), that allows for improving production and quality methane. The use of hydrogen peroxide at low concentrations (2.5% v/v) has not yet been evaluated, similar to pretreatments in the production of methane from pelagic Sargassum spp.

2. Materials and Methods

2.1. Sample Collection

Sample mixes of Sargassum natans and Sargassum fluitans (Sargassum spp.) were manually collected offshore in Puerto Morelos, Quintana Roo (2050.9195° N, 08652.5743° W),
Mexico (2020 and 2021) during the autumn. The samples were washed superficially with tap water several times to remove impurities such as salts and sands and then dried at 80 °C (APHA 2005). The Sargassum spp. was stored in a cold room (4 °C) for their subsequent compositional analysis (moisture, ash, total solids, and volatile solids) and later use.

2.2. Enzymes

The enzymes were obtained from the methodology reported by Tapia-Tussell et al. [9] which consisted of a strain of T. hirsuta Bm-2 (GQ280373) that was isolated from decaying wood in Yucatán, Mexico. The strain was maintained by periodical subculturing on plates with 2% (w/v) malt extract and 2% bacteriological agar (w/v). The plates were incubated at 35 °C for 4 or 5 days. Subsequently, a mycelia suspension of T. hirsuta was obtained by inoculating four 1-cm diameter plugs in a 250 mL flask previously sterilized at 121 °C for 15 min in a culture medium whose composition was (g/L): glucose 10 g, malt extract 10 g, peptone 2 g, yeast extract 2 g, KH₂PO₄ 2 g, hepta-hydrated MgSO₄ 1 g, thiamine 1 g, and 2% (w/v) wheat bran. The fungus was incubated at 35 °C for 7 days at 150 rpm and subsequently filtered.

2.3. Inoculum

The inoculum consisted of a native mixed microbial consortium containing: 300 g/L bovine manure, 150 g/L pig manure, 30 g/L deep soil, and 1.5 g/L Na₂CO₃ [36].

2.4. Sample Characterization

Elemental analysis was determined using a Thermo Scientific Flash 2000 CHNS/O elemental organic analyzer and Thermo Scientific Flash 2000 software: EAGER Xperience version 1.2, Thermo Fisher Scientific, Waltham, MA, USA. From the results obtained from the elemental composition, the theoretical biochemical potential of methane from Sargassum spp. was determined according to Equations (1) and (2) reported by Ward et al. [37]. In Equation (1), the stoichiometric reaction of methane production, \(a\), \(b\), \(c\), and \(d\) correspond to the molar composition of carbon, hydrogen, oxygen, and nitrogen.

\[
(C_aH_bO_cN_d) + \left(\frac{4a - b - 2c + 3d}{4}\right)H_2O \rightarrow \left(\frac{4a + b - 2c - 3d}{8}\right)CH_4 + \left(\frac{4a - b + 2c + 3d}{8}\right)CO_2 + dNH_3 \tag{1}
\]

The theoretical methane yield can then be predicted by Equation (2) (L CH₄/kg VS).

\[
\frac{L \text{ de } CH_4}{kg \text{ SV}} = \left(\frac{4a + b - 2c - 3d}{12a + b + 16c + 14d}\right) V_m \tag{2}
\]

2.5. Biochemical Methane Potential (BMP) Test

The BMP tests followed protocols and calculations according to Valero et al. [38]. After the pretreatment of Sargassum spp., BMP tests were carried out in triplicate in 250 mL serum bottles capped with rubber septum sleeve stoppers, with a useful volume of 140 mL and a headspace volume of 110 mL. The tests were carried out for 30 days at 40 °C; all tests were manually shaken once a day. Before the BMP test, the inoculum was degassed for 5 to 10 days. The inoculum/substrate ratio was 2 g VS inoculum/g VS Sargassum spp. and the bottles were filled with distilled water to complement the volume to 140 mL. In the same way, granular activated carbon (GAC) was added (to some pre-treatments) at a concentration of 40 g/L for microorganisms to exchange electrons with and promote an increase in methane gas production. To avoid the presence of oxygen, each of the reactors underwent oxygen displacement with nitrogen flux in an anoxic chamber. Three blanks with inoculum were also tested to measure its potential for methane production. Accumulated methane gas production was measured for the blanks and each of the tests to perform the pertinent calculations according to the methodology described by Valero et al. [38].
2.6. Physical Pretreatment

The samples were washed several times with tap water and then dried at 80 °C. Subsequently, mechanical cutting was performed with a blade blender (waring commercial, Blender 51BL30) and sieved until particle sizes greater than 1 mm were obtained.

2.7. Chemical Pretreatment

The chemical pretreatment consisted of 10 g of Sargassum spp. physically treated with 200 mL of 2.5% (v/v) hydrogen peroxide solution. The pretreatment was performed in triplicate.

2.8. Enzymatic Pretreatment

The enzymatic pretreatment, conducted in triplicate, consisted of a suspension of Sargassum physically treated with 10% w/v buffer solution to maintain the pH balance at 5. The buffer solution consisted of a 40% citric acid solution (0.05 M) and 60% sodium citrate solution (0.05 M), to this suspension, the enzyme extract with initial laccase activity of 7000 U/ML for each g of Sargassum was added, according to the report by Tapia-Tussell and collaborators [9]. The resulting suspension was incubated at 40 °C, 150 rpm for 48 h.

2.9. Combined Pretreatments

Different combined pretreatments were performed in triplicate; chemical and enzymatic pretreatment (PE), which consisted of the previously physically treated Sargassum spp., followed by chemical and enzymatic pretreatment, and finally the PTE treatment which consisted of the previously physically treated Sargassum spp., then a chemical, thermal (120 °C in an autoclave for 15 min), and enzymatic pretreatment.

2.10. Scanning Electron Microscopy (SEM)

The effect of pretreatment was observed with a scanning electron microscope (SEM, model JSM-6360LV, JEOL, Tokyo, Japan). Flour samples were mounted on a metallic stub using double-sided adhesive tape coated with a 15 nm gold layer and observed at 20 kV.

2.11. Statistical Analysis

The effect of various pretreatments was evaluated by Tukey’s post-hoc test following one-way ANOVA at p-values lower than 5%. All values are presented as average ± standard deviation. Excel 2019 (Microsoft Office ProPlus 365 64 bit) was used. The effect of pretreatment was observed with a scanning electron microscope (SEM, model JSM-6360LV, JEOL, Tokyo, Japan). Flour samples were mounted on a metallic stub using double-sided adhesive tape coated with a 15 nm gold layer and observed at 20 kV.

3. Results

3.1. Compositional Analysis

The Sargassum spp. collected in Puerto Morelos were analyzed by the moisture content, ash, total solids (TS), and volatile solids (VS) (Table 1); the values correspond to the two periods described in Section 2.1.

Table 1. Moisture, total solids (TS), volatile solids (VS), and ash.

| Parameter (%) | Sargassum spp. |
|---------------|----------------|
| Moisture%     | 12.98 ± 4.34   |
| TS%           | 87.02 ± 4.34   |
| VS%           | 77.78 ± 1.95   |
| Ash           | 22.22 ± 1.95   |
| VS/TS         | 0.89           |
CHNS/O

The results of the CHNS analysis (Table 2), are indicated. Based on CHNS/O, the empirical formula (normalized) can be expressed as CH\textsubscript{1.66}O\textsubscript{0.81}N\textsubscript{0.03}.

Table 2. CHNS and theoretical methane potential (L CH\textsubscript{4}/kg VS).

| Parameter (%) | Sargassum spp. |
|---------------|----------------|
| Ash%          | 22.22 ± 1.95   |
| C%            | 33.84 ± 1.05   |
| H%            | 4.71 ± 0.22    |
| N%            | 1.39 ± 0.22    |
| S%            | 1.21 ± 0.22    |
| O%            | 36.62 ± 3.37   |
| C:N           | 24.26          |

3.2. Biochemical Methane Potential (BMP)

3.2.1. Methane Yield

The physical pretreatments evaluated with and without GAC (C and CC) (Figure 1A) rendered a methane accumulation of 224.19 ± 9.45 L CH\textsubscript{4}/kg VS and 152.89 ± 2.00 L CH\textsubscript{4}/kg VS, respectively. The chemical pretreatment (P and PC) resulted in a methane accumulation of 230.82 ± 11.65 L CH\textsubscript{4}/kg VS and 240.32 ± 3.04 L CH\textsubscript{4}/kg VS. The enzymatic pretreatments (E and EC) resulted in an accumulation of methane of 172.57 ± 0.56 L CH\textsubscript{4}/kg VS and 179.56 ± 0.50 L CH\textsubscript{4}/kg VS. The combined pretreatment of PEC, PTE, and PTEC rendered a methane accumulation of 385.73 ± 4.76 L CH\textsubscript{4}/kg VS; 364.95 ± 8.18 L CH\textsubscript{4}/kg VS; and 318.06 ± 10.24 L CH\textsubscript{4}/kg VS, respectively. The PE pretreatment (chemical and enzymatic treatment) resulted in a higher methane accumulation of 387.64 ± 1.41 L CH\textsubscript{4}/kg VS.

3.2.2. Concentration of Methane

During the 30 days of experimentation, the methane concentration, shown in Figure 1B), and the maximum accumulation of the methane test were observed after the ninth and twelfth day. The physical pretreatments C and CC presented a methane concentration of 46.44% ± 1.69 and 48.86% ± 3.41, respectively. The chemical pretreatments P and PC resulted in methane concentrations of 51.55% ± 3.77 and 51.11% ± 0.56. In the pretreatments E, EC, ET, and ETC the concentration of methane was 84.56% ± 0.15; 81.49% ± 1.02; 84.56% ± 0.1; and 88.60% ± 4.57, respectively. Finally, with the combined pretreatments PE, PEC, PTE, and PTEC, the methane concentration was 86.41% ± 0.04; 76.04% ± 0.8; 87.69% ± 1.49; and 85.26% ± 0.1, respectively.

3.2.3. Biodegradability Index

The biodegradability index (Table 3) results from dividing the biochemical potential of methane by the theoretical potential of methane. The combined pretreatment PE and PEC have a higher biodegradability index of 0.95, followed by the PTE pretreatment of 0.88.

3.3. Scanning Electron Microscopy (SEM)

The SEM results (Figure 2) of the air vesicles (1) that allow Sargassum spp. to float and the lanceolate leaves (2) without any previous pretreatment, indicated a completely smooth structure. After the physical pretreatment C, the air vesicle (3) has a hole in the upper part and its structure no longer looks as smooth; the same happens with the lanceolate leaves (4) which show cracks. The chemical pretreatments (P) with hydrogen peroxide provoke a higher number of cracks in the air vesicle (5) and lanceolate leaves of Sargassum spp. (6), indicating the treatment with 2.5% peroxide allowed the degradation of the Sargassum spp. structure. The enzymatic pretreatment (E), images (7) and (8) indicate there is no change in the structure of the Sargassum spp. For the combined PE pretreatments, images (9) and (10)
indicate structural changes in the pelagic *Sargassum* can be observed; the same occurs with the combined PTE pretreatment images (11) and (12).

**Figure 1.** (A) Concentration of methane; (B) Methane yield accumulation. C and CC: physical pretreatment; P and PC: chemical pretreatment (peroxide 2.5%); E and EC: enzymatic pretreatment; PE and PEC: combined pretreatment chemical and enzymatic; PTE and PTEC: combined chemical, thermal, and enzymatic pretreatment. C means pretreatment with GAC.
Table 3. Biodegradability index.

| Pretreatment | Test | BI   |
|--------------|------|------|
| PHYSICAL     | C    | 0.55 |
|              | CC   | 0.38 |
| CHEMICAL     | P    | 0.57 |
|              | PC   | 0.59 |
| ENZYMATIC    | E    | 0.42 |
|              | EC   | 0.44 |
| COMBINED     | PE   | 0.95 |
|              | PEC  | 0.95 |
|              | PTE  | 0.88 |
|              | PTEC | 0.78 |

BI = biodegradability index = BMP/TMP (potential/theoretical). C: physical pretreatment; CC: physical pretreatment with GAC; P: chemical pretreatment (peroxide 2.5%); PC: chemical pretreatment (peroxide 2.5%) with GAC; E: enzymatic pretreatment; EC: enzymatic pretreatment with GAC; PE: chemical and enzymatic pretreatment; PEC: chemical and enzymatic pretreatment with GAC; PTE: chemical, thermal and enzymatic pretreatment; and PTEC: chemical, thermal, and enzymatic pretreatment with GAC.

Figure 2. SEM: air vesicles without pretreatment (1) and lanceolate leaves without pretreatment (2); C: physical pretreatment (3) and (4); P: chemical pretreatment (5) and (6); E: enzymatic pretreatment (7) and (8); PE: combined pretreatment (chemical and enzymatic) (9) and (10); and PTE: combined pretreatment (chemical, thermal and enzymatic) (11) and (12).
4. Discussion

4.1. Composition of *Sargassum* spp.

*Sargassum* spp. collected in the Mexican Caribbean (autumn 2020 and 2021) contained 12.98% moisture after being dried at 80 °C for 24 h. This value is comparable to other *Sargassum* spp. (13.05% moisture) [39]. The pelagic *Sargassum* collected in Barbados (June 2018) resulted in 20.63% moisture after the *Sargassum* spp. was dried at 80 °C [11]. Generally, brown macroalgae such as *Sargassum* are high in moisture (80–90%) when they are not subjected to a drying process [10,12]. The ash content of *Sargassum* spp. collected in the Mexican Caribbean was 22.22%. Varied results have been found in the bibliography, for example, Oyesiku and Egunyomi found that *Sargassum* spp. collected in Nigeria contain 9.5% ash [7], while Morrison and Gray determined that *Sargassum* collected in the Caribbean contains 24% ash [15], similar to this study. The ash content of the pelagic *Sargassum* reported by other authors was 31.82% to 46.94% (dry basis) [10,11]. These differences are mainly because the composition of the *Sargassum* varies considerably from place to place, season to season, and species to species [40]. Furthermore, the chemical composition, growth, and pigmentation of the *Sargassum* are significantly affected by conditions such as light, temperature, salinity, available nutrients, and water movement [41,42]. In addition, the *Sargassum* is not only composed of *S. natans* and *S. fluitans*, but there are also microorganisms (bacteria, microalgae, and invertebrates), together with the remains of other contaminating compounds that have been trapped within its composition [43]. The TS and VS content in this study were 87.02% and 77.78%, respectively, and resulted in a VS/TS ratio of 0.89. Comparing these results with other brown macroalgae, the VS/TS ratio of this study is high. The VS/TS ratio of brown macroalgae (S. latissima) was 0.83 [44] and Thompson et al. determined that the VS/TS ratio of pelagic *Sargassum* was 0.47 [11]; Milledege et al. found similar results to that reported by Thompson, the VS/TS relationship for a mixture of pelagic *Sargassum* was 0.53 [10]. Previous studies have reported optimum conditions for biogas production at a VS/TS ratio of 0.70 [45]; since the ratio of the present study is above 0.7, the *Sargassum* spp. collected in the Mexican Caribbean is a good option for anaerobic digestion.

4.2. Elemental Analysis

Table 2 shows the elemental analysis of this study. The ultimate analysis presented 33.84% of carbon; this value is higher than the result found by Milledge et al. with a composition of 27.41% to 29.23% for different *Sargassum* samples collected in Turks and Caicos [10]. The nitrogen content of the present study was 1.39%; this value is close to that reported in the literature for pelagic *Sargassum* collected in Turks and Caicos, which was 1.57% to 1.71% [10], and the pelagic *Sargassum* collected in Barbados at 1.21% [46].

This marine biomass has a C:N ratio of 24:1 which lies within the ideal C:N range of 20–35:1 for optimum microbial digestion and fermentation [47]. Compared with other brown algae, this result is very similar to the C:N ratio reported by Thompson, which was 21.67 [46]. A C:N ratio above the optimum causes methanogens to rapidly consume nitrogen to meet their protein needs, but they will stop consuming the remaining carbon in the substrate, reducing biogas production [48]. If the ratio is below the optimum, this indicates that the substrate is made up mainly of proteins and is rich in nitrogen, so the ammonia content will increase, inhibiting anaerobic digestion due to the change in pH in the digester that results in a toxic effect of the methanogenic stage [47]. Therefore, the C:N ratio found in the pelagic *Sargassum* collected during the autumn in this study is adequate for anaerobic digestion.

Based on the elemental analysis of pelagic *Sargassum*, the theoretical methane potential of this marine biomass was 405 ± 18.68 L CH₄/kg VS, suggesting that pelagic *Sargassum* is a rich feedstock for mono-digestion and biomethane production. Compared with other macroalgae, this result was below that of *Ascophyllum nodosum*, *L. digitata*, *S. latissima*, and *Ulva lactuca*, which resulted in 488, 479, 422, and 465 L CH₄/kg VS, respectively [49]. The differences are due to macroalgae being collected in different regions and seasons of the
year, and being of different species [50]. Even when comparing the same species and the same collection region but collecting at different times of the year, the yield varies. This was described by Maneen et al., where the *Sargassum muticum* collected in the spring and summer present a theoretical yield of 397 to 463.8 L CH$_4$/kg VS, respectively [50]. The theoretical yield of *Sargassum* spp. collected in Conset Bay, Barbados (142.84 L CH$_4$/kg VS) [11] is below that reported in the present study (405 ± 18.68 L CH$_4$/kg VS), and when compared with the *Sargassum* spp. collected in Turks and Caicos (496 L CH$_4$/kg VS) [10] the theoretical yield of the present study turned out to be lower. The samples of *Sargassum* spp. collected in the Mexican Caribbean during the summer of 2019 determined that the theoretical potential of *Sargassum* resulted in 839.65 L CH$_4$/kg VS [51]; this value above the present study may be due to the time and area in which it was collected [50]. Finally, when compared with the ligno-cellulosic biomass, it was observed that the *Sargassum* yield was above that obtained from wheat straw (232 L CH$_4$/kg VS), corn stalk (206 L CH$_4$/kg VS), sorghum (242 L CH$_4$/kg VS), and barley straw (229 L CH$_4$/kg SV) and are among the yields obtained from organic waste such as feed residues, sewage sludge, and animal waste (200 to 500 L CH$_4$/kg VS) [45,52,53].

4.3. BMP

4.3.1. Physical Pretreatment

The physical pretreatments C and CC result in biochemical methane potential values of 224.19 ± 9.45 and 152.89 ± 2.00 L CH$_4$/kg VS (46.44% ± 1.69 and 48.86% ± 3.41 concentration of methane in the biogas). Table 4 shows the comparative yields reported. The vast majority of studies carried out anaerobic digestion under mesophilic conditions. The studies used different inoculums; in each of them, a blank was evaluated to correct the results obtained, and the methane accumulation reported is only from the macroalgae. The biochemical methane potential values of the literature are between 92 L CH$_4$/kg VS [14] and 422 L CH$_4$/kg VS [15], similar to those obtained in the present study. Milledge et al. determined a biodegradability of 17% to 39% for pelagic *Sargassum* samples when washed and cut [10]. Oliveira et al. determined that physical pretreatments increased biodegradability to 52% when compared to *Sargassum* without pretreatment [54]. Compared with these studies, the present work resulted in biodegradability of 55% for C and 38% for CC, close to the values reported by the literature. Regarding the increase in biogas production, the present study did not make a comparison with *Sargassum* without previous pretreatment, as described by other authors, for example, Nielsen and Heiske determined that *U. lactuca* resulted in a significant increase of 68% in methane from 152 to 255 L CH$_4$/kg VS when compared with the macroalgae without pretreatment [44]; Yuhendra et al. concluded that physical pretreatment carried out on *Sargassum fulvellum* with particle sizes of 0.075–0.85 mm resulted in an optimal pretreatment that improved methane yields by 52.34% [22], while Tedesco et al. found a 52% increase in biogas production and 53% methane concentration when physical pretreatment (*L. digitata*) was performed and compared with the substrate without pretreatment [18]. In this study, the methane concentration in the biogas turned out to be below that reported by Tedesco et al. and the methane obtained from biogas is not the optimal 60–70% required [55,56] to be used in equipment such as electric power generators, however, physical pretreatments are important because the increase in the surface area and release of the sugars are necessary for anaerobic digestion [12], therefore, this pretreatment is necessary to carry out anaerobic digestion and must be accompanied by another pretreatment to increase the biodegradability of the biomass.
Table 4. Results of the different pretreatments evaluated in this study and its comparison with other results in the literature.

| Pretreatment | Substrate | Operating Conditions | Pretreatment Characteristics | Yield (L CH\textsubscript{4}/kg VS) | % Methane | Literature |
|--------------|-----------|----------------------|-----------------------------|---------------------------------|----------|------------|
| Physical     | Sargassum spp. (C) | 40 °C | Washed, dried (80 °C), and particle size > 1 mm | 224.19 ±9.45 | 46.44% ± 1.69 | This study |
|              | Sargassum spp. (CC) | 38 °C | Inoculum: substrate: 2:1 | 350 | 44% | [22] |
|              | Laminaria spp. | 50 °C | Particle size 0.075 mm | 229 | 52% | [18] |
|              | 40 °C | 0.075 mm | 210 | 51% |
|              | 30 °C | 0.075 mm | 220 |
|              | Ulva lactuca | 55 °C | Wash and cut | 271 | - | [13] |
|              | S. latissima | Inoculum:substrate: 1:1 | Wash, dried (80 °C), and 2-3 mm | 422 | - | [15] |
|              | A. nodosum (brown seaweed) | Diary slurry and grass silage (37 °C and I:S;2:1) | Wash and 4 mm | 215-217 | - | [57] |
|              | L. Digitata | Diary slurry and grass silage (37 °C and I:S;2:1) | Wash and 4 mm | 267 | - | [17] |
|              | S. latissimi | 37 °C | 1000 bar 1 × 10 mm in the end (1 cm) | 92 | - | [14] |
|              | Ulva lactuca | 53 °C | Wash and macerated | 255 | - | [44] |
|              | Sargassum spp. (P) | 40 °C | Washed, dried (60 °C), and particle size > 1 mm + peroxide | 230.82 ±11.65 | 51.55% ± 3.77 | This study |
|              | Sargassum spp. (PC) | Inoculum: substrate: 2:1 | 40 mL/L | 240.32 ±3.04 | 51.11% ± 0.56 |
|              | S. fulvellum | 38 °C | 0.075-0.85 mm HCl 40 mL/L | 312 | 40% | [22] |
|              | Ulva sp. | 35 °C | Washed, dried (60 °C), and particle size > 1 mm + peroxide | 77 | 61% | [20] |
|              | Mixture of red and green macroalgae | 35-40 °C | 5–20 mm 0.05 M HCl 80 °C 5–20 mm 0.2 M HCl 80 °C 20 min | 66 | - | [58] |
|              | L. digitata and S. latissima | 35 °C | 1% lactic acid | 161 | - | [29] |
|              | Ulva sp. | 35 °C | Inoculum: substrate: 2:1 | 4% NaOH 20 °C | 148 | 57% | [20] |
|              | S. fulvellum | 38 °C | 0.075–0.85 mm NaOH 10mL/L | 282 | 43% | [22] |
|              | Palmaria palmata | 35 °C | Inoculum: substrate: 2:1 | 0.04 g NaOH gTS−1 at 20 °C | 365 | - | [21] |
Table 4. Cont.

| Pretreatment | Substrate Operating Conditions | Pretreatment Characteristics | Yield (L CH₄/kg VS) | % Methane | Literature |
|--------------|--------------------------------|-----------------------------|---------------------|-----------|------------|
| Sargassum spp. (E) | 40 °C Inoculum: substrate: 2:1 | Washed, dried (60 °C), and particle size > 1 mm \textit{T. hirsuta} | 172.57 ± 0.56 | 84.56% ± 0.15 | This study |
| Sargassum spp. (EC) | | | 179.56 ± 0.50 | 81.49% ± 1.02 | |
| Pelagic Sargassum spp. | 38 °C | 1 mL/L Viscomyl™ Flow cellulase enzyme | 133.27 | 44% | [22] |
| Ulva sp. | 35 °C Inoculum: substrate: 2:1 | Enzymes from \textit{T. hirsuta} | 153 | 58% | [20] |
| L. digitata and S. latissima | 35 °C | Cellulase and enzymes from \textit{Aspergillus fumigatus} | 232 | - | [29] |
| Rhizoclonium | 53 °C | Lipase and \textit{Xylanase} and \textit{α-amylase} | 97 | - | [60] |
| Oocystis sp. | | Commercial lacasse and \textit{Lacasse from T. versicolor} | 100 | - | [61] |
| F. vesiculosos | 37 °C | Hemicellulase, pectinase, protease, and cellulase | 49 | - | [14] |
| Sargassum spp. (PE) | | Washed, 60 °C particle size > 1 mm, peroxide and enzymes from \textit{T. hirsuta} | 387.64 ± 1.41 | 86.41% ± 0.04 | This study |
| Sargassum spp. (PEC) | | | 385.73 ± 4.76 | 76.04% ± 0.80 | |
| Sargassum spp. (PTE) | | Washed, 60 °C particle size > 1 mm, thermal 120 °C, peroxide and Enzymes from \textit{T. hirsuta} | 364.95 ± 8.18 | 87.69% ± 1.49 | |
| Sargassum spp. (PTEC) | | | 318.06 ± 10.24 | 85.26% ± 0.10 | |
| L. digitata and S. latissima | 35 °C | Cellulase 1% lactic acid | 161 | - | [29] |
| F. vesiculosos | 37 °C | 1000 bar + Hemicellulase, pectinase, protease, and cellulase | 49 | - | [14] |
| F. vesiculosus | 37 °C | 80 °C and 0.2 M HCl | 116 | - | [59] |

4.3.2. Chemical Pretreatments

Physical pretreatment was carried out first, then chemical pretreatment with hydrogen peroxide at a low concentration (solution at 2.5% v/v) was performed to obtain yields of 230.82 ± 11.65 and 240.32 ± 3.04 L CH₄/kg VS (51.55% ± 3.77 and 51.11% ± 0.56 concentration of methane in the biogas) for P and PC pretreatments, respectively. Comparing the pretreatments P and PC with the physical pretreatments (C and CC) resulted in an increased methane production of 3% and 57%, respectively. In this study, a slight improvement in methane production can be observed for P pretreatment, however, for PC pretreatment, the increase is greater. Biodegradability (57% and 59%) turned out to be higher than those obtained from physical pretreatment (C and CC). The results obtained were compared with other studies carried out on different macroalgae as shown in Table 4; the yields obtained with HCl pretreatment were 52 to 312 L CH₄/kg VS [20,22,58,59]. P and PC are within the reported yields; however, some authors reported a decrease in methane production due to the release of inhibitors such as furfural, organic acids, and phenolic compounds [23]. The use of an HCl-like pretreatment of \textit{S. fulvellum} resulted in a decrease of 8% in biogas production [22]; the same occurred with \textit{Ulva} sp. (HCl), i.e., methane reduction was 42% when compared to macroalgae without pretreatment [20]. Barbot et al. determined that yields are affected according to acid concentration; for example, a concentration of 0.05 M experienced a 24% reduction, however, when the HCl concentration was 0.2 M, there was a...
slight increase of 3% when compared to a substrate without pretreatment before anaerobic digestion [58]. The use of lactic acid for *L. digitata* and *S. latissima* resulted in a 37% decrease in methane production [29]. Table 4 shows that the yields of alkaline pretreatments (NaOH) vary from 148 to 365 L CH$_4$/kg VS for different macroalgae [20–22], with P and PC within the reported yields, as well as the use of acids in the use of NaOH, resulting in a 19% reduction in methane yield for *S. fulvellum* [22].

As shown in Table 4, there are no studies currently using hydrogen peroxide as a pretreatment in macroalgae anaerobic digestion, however, the production of bioethanol from *Ulva prolifera* used hydrogen peroxide as a pretreatment at a concentration of 0.5% v/v; this increased the presence of reducing sugars to 7.1 g/L and improved the efficiency of enzymatic hydrolysis by 31.4% [62]. The success of pretreatment with hydrogen peroxide is mainly due to its oxidative action since the derived radicals (OH and O$_2$) depolymerize lignin by attacking lignin side chains and fragmenting its macrostructure into a number of low-molecular-weight compounds [63]. Even though pretreatment P resulted in a slight improvement regarding the physical pretreatment, the pretreatment PC increased methane production by 57.18%; in the bibliography, it was observed that the use of peroxide pretreatment has been successful in the pretreatment of terrestrial plants since it allows the release and increase in reducing sugars, in addition to being carried out under moderate pressure and temperature conditions [64]. In this study, the pretreatment was carried out at room temperature, so high-energy requirements were not necessary, and the agitation was only carried out for 3 h, unlike the acid treatments that require temperatures of 150 °C to 80 °C [20,58]; by not using acids or other agents under severe conditions, the use of hydrogen peroxide resulted in the formation of minor inhibitors [64] and since it does not require high-energy demands, it is a good option for *Sargassum* pretreatment.

### 4.3.3. Enzymatic Pretreatment

The yields of pretreatments E and EC were 172.57 ± 0.56 and 179.56 ± 0.50 L CH$_4$/kg VS with an 84.56% ± 0.15 and 81.49% ± 1.02 concentration of methane in the biogas, respectively. Comparing the yields with other studies (Table 4), it was observed that the methane yields were within those reported in the literature, from 49 L CH$_4$/kg VS [14] to 232 L CH$_4$/kg VS [20]. However, the yields reported in Table 4 show the use of pretreatments under different conditions and substrates, however, only in the study carried out by Tapia-Tussell et al. were the enzymatic pretreatment (enzymes from *Trametes hirsuta* BM-2) and substrate (*Sargassum consortium in the Mexican Caribbean*) similar [9]. In the case of fungi, the present study was based on the methodology by Tapia-Tussell et al. with differences such as the culture medium in which the fungus was isolated and the cultivated time (4 to 5 days). Another difference was the time the fungus remained in the YMPG medium (7 days) at pH 5. In addition, the *Sargassum* used in Tapia-Tussell was collected in Progreso (Yucatán, Mexico), whose composition was affected by the location of origin and varies from season to season [40], i.e., the chemical composition, growth, and pigmentation of *Sargassum* are significantly affected by conditions such as light, temperature, salinity, available nutrients, and water movement [41,42]. The study carried out by Tapia-Tussell et al. resulted in 104 L CH$_4$/kg VS [9], below the yield obtained in this study of 172.57 ± 0.56 (L CH$_4$/kg VS).

Comparing the results obtained from E and EC with the physical pretreatment (C and CC), the methane yield for E and EC decreased by 23% and 17.44%, and compared with the chemical pretreatments (P and PC), E and EC have a lower yield (Table 4); the same occurs with biodegradability (for E and EC 42% and 44%, respectively). The methane production is affected by the transfer of electrons from the enzyme laccase to the substrate [65,66]; the oxidation of the phenylpropanoid units that lignin is made of, such as p-hydroxyphenyl [67], is involved in the formation of phenoms radicals [68] such as furfural, hydroxy-methylfurfural, or phenolic and/or aromatic compounds that inhibit anaerobic digestion [69,70]. Yuhendra et al. obtained a 62% decrease in biogas yield when using an enzymatic pretreatment [22]. Although the results obtained with the enzymes...
were not as favorable when compared to the physical and chemical pretreatments, this pretreatment is advantageous because *T. hirsuta* Bm-2 is a native fungus and does not involve the purchase of commonly expensive enzymes, in addition, enzymes are extracellular so they do not require the use of solvents or any extraction method, unlike other enzymes [71], and the culture medium was obtained at 35 °C, so the energy requirements are not high, contributing to the care of the environment.

4.3.4. Combined Pretreatments

The combined pretreatments consisted of a physical pretreatment, followed by 2.5% hydrogen peroxide, and finally, the use of enzymes (PE and PEC); in addition, a combined pretreatment with a physical, chemical, and thermal pretreatment was evaluated before carrying out the enzymatic hydrolysis. The evaluated pretreatments PE, PEC, PTE, and PTEC presented a methane yield of 387.64 ± 1.41; 385.73 ± 4.76; 364.95.64 ± 8.18; and 318.06 ± 10.24 L CH₄/kg VS, respectively (86.41% ± 0.04; 76.04% ± 0.80; 87.69% ± 1.49; and 85.26% ± 0.10 concentration of methane in the biogas, respectively), the biodegradability index was 95%; 95%; 88%; and 78%, respectively, as shown in Table 3. The results obtained from these pretreatments are very favorable; the yields obtained are very close to the theoretical (405 ± 18.68 L CH₄/kg VS), completely degrading the *Sargassum*, leading to a percentage of methane obtained from the biogas above the optimum (60–70%) [55,56]. Comparing these pretreatments with physical pretreatment C yields methane increases of 73% and 63% for PE and PTE, respectively. Compared with the chemical treatment (P and PC), the increase in methane production was 68%; 60.5%; 58%; and 32% for PE, PEC, PTE, and PTEC, respectively. The combined pretreatment (enzymes and acid) applied to *L. digitata* and *S. latissima* resulted in a yield of 161 L CH₄/kg VS [29], another combined pretreatment (pressure followed by an enzyme treatment) applied to *F. vesiculosus* resulted in an accumulation of 49 L CH₄/kg VS [14]; the yields obtained for the *Sargassum* spp. collected in the Mexican Caribbean were above what is cited in the literature.

Although the four pretreatments presented better results, PTE and PTEC had a reduction in methane accumulation of 6% and 17% compared to PE and PEC because the temperature has a profound influence on inhibitor reaction kinetics, in addition, the reaction kinetics of lignin are similar to the degradation of reducing sugars [72]. Another disadvantage of the thermal pretreatment used in PTE and PTEC is the high-energy demands, for this reason, PTE and PTEC are discarded as a good pretreatment for the methanization of *Sargassum* spp. Therefore, PE and PEC accumulated a higher yield of methane; the success of these combined pretreatments was because chemical pretreatment with peroxide depolymerizes lignin [63], additionally, peroxide is not as severe as acids or other chemical agents that favor the formation of inhibitors from lignin [64]. When enzymatic pretreatment is carried out after the use of peroxide, the laccase oxidizes the phenolic compounds released, allowing greater degradation of lignin and phenolic inhibitors [66,73,74]. These pretreatments have an advantage in that the use of hydrogen peroxide at a low concentration (solution of 2.5%) can be carried out at room temperature without requiring additional energy demand, in addition to the use of an enzymatic extract without the use of solvents, which helps reduce the generation of pollutants and is favorable to the economy of the process for scaling in real conditions.

4.3.5. DIET with Granular Activated Carbon (GAC)

Although the use of different conductive materials (GAC, biochar, nano-magnetite, and minerals) in the production of methane has been evaluated [31,32], this work has focused on GAC, mainly due to its high conductivity of 3,600 µS·cm⁻¹; this conductivity is higher than magnetite (160 µS·cm⁻¹) and biochar (5 µS·cm⁻¹) [75]. GAC is the ideal conductive material due to its surface area, high conductivity, and economically favorable low cost [76,77].

The evaluated pretreatments with GAC showed that there are no significant changes in the accumulated yield of methane (analysis of variance ANOVA). PE and PEC pretreatments
obtained the best yields with an average 86.14 ± 10% concentration of methane in the biogas, being above the optimum (60–70%) [55,56]. The same effect was determined by Cheng et al., who obtained a reduction of 40 to 45% in methane yields when adding biochar and GAC-like conductive material (a pig wastewater-like substrate). This effect is due to the GAC adsorption properties, this conductive material removed 11 to 17% of COD, in addition, the adsorption of organic compounds was observed [78]. The higher adsorption of volatile fatty acids from GAC has been observed with substrates whose volatile fatty acid (VFA) profile consists of hydrophobic compounds and long chains (above C5), for which a decreasing adsorption affinity was determined in the order of butyric, propionic, and acetic acid, which can cause a decrease in methane yields [79,80]. On the other hand, this can help keep VFAs below the inhibitory limits (less than 1.5 g/L) [81,82]; Valero et al. determined that the adsorption or promotion of DIET when a conductive material was used depended on the characteristics of the substrate; in the case of pot ale whiskey, the use of activated carbon did not increase anaerobic digestion due to the adsorption of organic matter, which in turn reduced the production of methane, while the powdered activated carbon (PAC) improved anaerobic digestion using brewery spent yeast as substrate and provided better resistance to inhibitory conditions with a yield of 699 L CH$_4$/kg VS [34]. Conductive materials prevent the accumulation of volatile fatty acids, maintaining the optimal pH for biogas production (6.8 to 7.4) [83]. Shanmugam et al. used the GAC to adsorb inhibitors such as phenolic compounds and alter the concentration of COD before the anaerobic digestion begins, and, similar to other authors, the reduction in the concentration of methane was mainly due to the characteristics of the substrate and the adsorption properties of the conductive material [35]. Florentino et al.; 2019, found that the characteristics of the substrate can promote the adsorption of organic matter and correspond to a profile of COD and low free fatty acids, so when using GAC in the anaerobic digestion of blackwater at concentrations of 2.6 to 4.6 g COD/L (corresponding to 35 and 37% soluble COD), a reduction in soluble COD from 27 to 66% was obtained during the first 3 days, unlike when anaerobic digestion was carried out without GAC (increase in soluble COD of 60%). This demonstrated the adsorption of organic matter; regarding volatile fatty acids, the same behavior was observed when GAC was not used, acetate was 1277 mg/L, however, when GAC was added, acetate was 280 and 242 mg/L. However, at a concentration of 18.5 g COD/L, acetate was 1578 mg/L [84]. Although the use of GAC has been shown to promote DIET to increase methane production and quality, its use can also help improve pH and maintain redox potential within the range of methane formation (values below −250 mV) [34], in addition, its adsorption property could help reduce inhibitors [35]; however, the promotion of DIET depends on the concentration of GAC [85–87], as well as the addition of COD, that can favor the adsorption properties of GAC [84], so it is necessary to continue evaluating the concentration ratios and specific characteristics of each type of GAC, in anaerobic digestion tests when Sargassum spp. collected in the Mexican Caribbean was used as substrate.

5. Conclusions

The use of a conductive material such as GAC did not generate significant changes according to the analysis of variance performed (ANOVA) regarding performance and the concentration of methane. The main highlight of this research is that the best results were obtained from the combined pretreatments of 2.5% hydrogen peroxide, followed by an enzymatic pretreatment, resulting in a biodegradability of 95%, and an accumulated yield of 387 ± 3.09 L CH$_4$/kg VS. However, it is necessary to continue evaluating the concentration of GAC and its characteristics when using Sargassum spp.

Likewise, the pelagic Sargassum used in this study is promising for bio-methane production in the Mexican Caribbean zone and solves the problem of its handling. Future research is needed to support these results to scale up continuous anaerobic reactors, in addition, techno-economic and environmental studies are necessary.
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References
1. Smetacek, V.; Zingone, A. Green and golden seaweed tides on the rise. Nature 2013, 504, 84–88. [CrossRef] [PubMed]
2. Wang, M.; Hu, C.; Barnes, B.B.; Mitchum, G.; Lapointe, B.; Montoya, J.P. The great Atlantic Sargassum belt. Science 2019, 364, 83–87. [CrossRef] [PubMed]
3. Louime, C.; Fortune, J.; Gervais, G. Sargassum Invasion of Coastal Environments: A Growing Concern. Am. J. Environ. Sci. 2017, 13, 58–64. [CrossRef]
4. Rasher, D.B.; Hay, M.E. Chemically rich seaweeds poison corals when not controlled by herbivores. Proc. Natl. Acad. Sci. USA 2010, 107, 9683–9688. [CrossRef]
5. Chávez, V.; Uribe-Martínez, A.; Cuevas, E.; Rodríguez-Martínez, R.E.; van Tussenbroek, B.L.; Francisco, V.; Estévez, M.; Celis, L.B.; Monroy-Velázquez, L.V.; Leal-Bautista, R.; et al. Massive influx of pelagic Sargassum spp. On the coasts of the mexican caribbean 2014–2020: Challenges and opportunities. Water 2020, 12, 2908. [CrossRef]
6. De Jesus Raposo, M.F.; De Morais, A.M.M.B.; De Morais, R.M.S.C. Emergent sources of prebiotics: Seaweeds and microalga. Mar. Drugs 2016, 14, 27. [CrossRef]
7. Oyesiku, O.; Egunyomi, A. Identification and chemical studies of pelagic masses of Sargassum natans (Linnaeus) Gailllon and S. fluitans (Borggescen) Borggscen (brown algae), found offshore in Ondo State, Nigeria. Afr. J. Biotechnol. 2014, 13, 1188–1193.
8. Alzate-Gaviria, L.; Domínguez-Maldonado, J.; Chablé-Villacís, R.; Olguín-Maciel, E.; Leal-Bautista, R.M.; Canché-Escamilla, G.; Caballero-Vázquez, A.; Hernández-Zepeda, C.; Barredo-Pool, F.A.; Tapia-Tussell, R. Presence of polyphenols complex aromatic “Lignin” in Sargassum Spp. From Mexican Caribbean. J. Mar. Sci. Eng. 2021, 9, 6. [CrossRef]
9. Tapia-Tussell, R.; Avila-Arias, J.; Maldonado, J.D.; Valero, D.; Olguín-Maciel, E.; Pérez-Brito, D.; Alzate-Gaviria, L. Biological pretreatment of mexican caribbean macroalgae consortiums using Bm-2 strain (trametes hirsuta) and its enzymatic broth to improve biomethane potential. Energies 2018, 11, 494. [CrossRef]
10. Milledge, J.J.; Maneein, S.; Arríbas López, E.; Bartlett, D. Sargassum Inundations in Turks and Caicos: Methane potential and proximate, ultimate, lipid, amino acid, metal and metalloid analyses. Energies 2020, 13, 1523. [CrossRef]
11. Thompson, T.M.; Young, B.R.; Baroutian, S. Efficiency of hydrothermal pretreatment on the anaerobic digestion of pelagic Sargassum for biogas and fertiliser recovery. Fuel 2020, 279, 118527. [CrossRef]
12. Barbot, Y.N.; Al-Ghaili, H.; Benz, R. A review on the valorization of macroalgal wastes for biomethane production. Mar. Drugs 2016, 14, 120. [CrossRef]
13. Bruhn, A.; Dahl, J.; Nielsen, H.B.; Nikolaisen, L.; Rasmussen, M.B.; Markager, S.; Olesen, B.; Arias, C.; Jensen, P.D. Bioenergy potential of Ulva lactuca: Biomass yield, methane production and combustion. Bioresour. Technol. 2011, 102, 2595–2604. [CrossRef]
14. Li, H.; Kjerstadius, H. Evaluation of pretreatment methods for increased biogas production from macro algae. SGC Rapprot 2013, 278, 34.
15. Morrison, M.; Gray, D. Anaerobic Digestion Economic Feasibility Study: Generating Energy from Waste, Sewage and Sargassum Seaweed in the OECS. Caribbean Council. 2017. Available online: https://www.caribbean-council.org/wp-content/uploads/2017/02/CPI-The-Caribbean-Council-Anaerobic-Digestion-Sargassum-Economic-Feasibility-Study-January-2017.pdf (accessed on 20 June 2022).
16. Tabassum, M.R.; Xia, A.; Murphy, J.D. Potential of seaweed as a feedstock for renewable gaseous fuel production in Ireland. Renew. Sustain. Energy Rev. 2017, 68, 136–146. [CrossRef]
17. Tabassum, M.R.; Wall, D.M.; Murphy, J.D. Biogas production generated through continuous digestion of natural and cultivated seaweeds with dairy slurry. Bioresour. Technol. 2016, 219, 228–238. [CrossRef]
18. Tedesco, S.; Marrero Barroso, T.; Olabi, A.G. Optimization of mechanical pre-treatment of Laminariaceae spp. biomass-derived biogas. *Renew. Energy* 2014, 62, 527–534. [CrossRef]

19. Ariunbaatar, J.; Panico, A.; Esposito, G.; Pirozzi, F.; Lens, P.N.L. Pretreatment methods to enhance anaerobic digestion of organic solid waste. *Appl. Energy* 2014, 123, 143–156. [CrossRef]

20. Ben Yahmed, N.; Carrere, H.; Marzouki, M.N.; Smaali, I. Enhancement of biogas production from Ulva sp. by using solid-state fermentation as biological pretreatment. *Algal Res.* 2017, 22, 206–214. [CrossRef]

21. Jard, G.; Dumas, C.; Delgenes, J.P.; Marfaing, H.; Sialve, B.; Steyer, J.P.; Carrère, H. Effect of thermochemical pretreatment on the solubilization and anaerobic biodegradability of the red macroalga *Palmaria palmata*. *Biochem. Eng. J.* 2013, 79, 253–258. [CrossRef]

22. Yuhendra, A.P.; Farghali, M.; Mohamed, I.M.; Iwasaki, M.; Tangtaweewipat, S.; Ihara, I.; Sakai, R.; Umetu, K. Potential of biogas production from the anaerobic digestion of *Sargassum* fulvellum macroalgae: Influences of mechanical, chemical, and biological pretreatments. *Biochem. Eng. J.* 2021, 175, 108140. [CrossRef]

23. Jeong, G.T.; Kim, S.K.; Park, D.H. Application of solid-acid catalyst and marine macro-algae *Gracilaria verrucosa* to production of fermentable sugars. *Bioreour. Technol.* 2015, 181, 1–6. [CrossRef] [PubMed]

24. Kim, E.J.; Fathoni, A.; Jeong, G.T.; Jeong, H.D.; Nam, T.J.; Kong, I.S.; Kim, J.K. Microbacterium oxydans, a novel alginate- and laminarin-degrading bacterium for the reutilization of brown-seaweed waste. *J. Environ. Manag.* 2013, 130, 153–159. [CrossRef] [PubMed]

25. Gerken, H.G.; Donohoe, B.; Knoshaug, E.P. Enzymatic cell wall degradation of *Chlorella vulgaris* and other microalgae for biofuels production. *Plantas* 2013, 237, 239–253. [CrossRef]

26. Jegatheesan, A.; Sudhakar, M.P.; Poonam, C.; Perumal, K.; Arunkumar, K. Isolation and characterization of alginate-degrading bacteria *Sinorhizobium oceani*. *Biomass Convers. Biorefinery* 2017, 7, 51–58. [CrossRef]

27. Pazarlio˘glu, N.K.; Sarii¸ sik, M.; Telefoncu, A. Laccase: Production by *Trametes versicolor* and application to denim washing. *Biochem. Eng. J.* 2005, 40, 1673–1678. [CrossRef]

28. Wesenberg, D.; Buchon, F.; Agathos, S.N. Degradation of dye-containing textile effluent by the agaric white-rot fungus *Clitocybula fulvellum* *macroalgae*: Influences of mechanical, chemical, and biological pretreatments. *Bioresour. Technol.* 2016, 208, 319–332. [CrossRef]

29. Vanegas, C.H.; Hernon, A.; Bartlett, J. Enzymatic and organic acid pretreatment of seaweed: Effect on reducing sugars production and on biogas inhibition. *Int. J. Ambient Energy* 2015, 36, 2–7. [CrossRef]

30. Ganesh Saratale, R.; Kumar, G.; Banu, R.; Xia, A.; Periyasamy, S.; Dattatraya Saratale, G. A critical review on anaerobic digestion of microalgae and macroalgae and co-digestion of biomass for enhanced methane generation. *Bioresour. Technol.* 2018, 262, 319–332. [CrossRef]

31. Holmes, D.E.; Tang, Y.; Walker, D.J.F.; Lovley, D.R. The electrically conductive pili of Geobacter species are a recently evolved feature for extracellular electron transfer. *Microb. Genom.* 2016, 2, e000072. [CrossRef]

32. Malvankar, N.S.; Lovley, D.R. Microbial nanowires for bioenergy applications. *Curr. Opin. Biotechnol.* 2014, 27, 88–95. [CrossRef]

33. Rotaru, A.E.; Shrestha, P.M.; Liu, F.; Shrestha, M.; Shrestha, D.; Embree, M.; Zengler, K.; Wardman, C.; Nevin, K.P.; Lovley, D.R. A new model for electron flow during anaerobic digestion: Direct interspecies electron transfer to Methanoseta for the reduction of carbon dioxide to methane. *Environ. Sci. Policy* 2014, 7, 408–415. [CrossRef]

34. Valero, D.; Alzate-Gaviria, L.; Montes, J.A.; Rico, C. Influence of a Conductive Material and Different Anaerobic Inocula on Biochemical Methane Potential of Substrates from Alcoholic Beverage Production. *Waste Biomass Valorization* 2020, 11, 5957–5964. [CrossRef]

35. Shanmugam, S.R.; Adhikari, S.; Wang, Z.; Shyaka, R. Treatment of aqueous phase of bio-oil bygranular activated carbon and evaluation of biogas production. *Bioresour. Technol.* 2017, 223, 115–120. [CrossRef]

36. Poggi-Varaldo, H.M.; Valdés, L.; Esparza-García, F.; Fernández-Villagómez, G. Solid substrate anaerobic co-digestion of paper mill sludge, biosolids, and municipal solid waste. *Water Sci. Technol.* 1997, 35, 197–204. [CrossRef]

37. Ward, A.J.; Lewis, D.M.; Green, F.B. Anaerobic digestion of algae biomass: A review. *Algal Res.* 2014, 5, 204–214. [CrossRef]

38. Valero, D.; Montes, J.A.; Rico, J.L.; Rico, C. Influence of headspace pressure on methane production in Biochemical Methane Potential (BMP) tests. *Waste Manag.* 2016, 48, 193–198. [CrossRef]

39. Zhang, Y.; Li, L.; Kong, X.; Zhen, F.; Wang, Z.; Sun, Y.; Dong, P.; Lv, P. Inhibition Effect of Sodium Concentrations on the Anaerobic Digestion of *Sargassum* species. *Energy Fuels* 2017, 31, 7101–7109. [CrossRef]

40. McKenney, J.; Sherlock, O. Anaerobic digestion of marine macroalgae: A review. *Renew. Sustain. Energy Rev.* 2015, 52, 1781–1790. [CrossRef]

41. Jung, K.A.; Lim, S.R.; Kim, Y.; Park, J.M. Potentials of macroalgae as feedstocks for biorefinery. *Bioresour. Technol.* 2013, 135, 182–190. [CrossRef]

42. Marinho-Soriano, E.; Fonseca, P.C.; Carneiro, M.A.A.; Moreira, W.S.C. Seasonal variation in the chemical composition of two tropical seaweeds. *Bioresour. Technol.* 2006, 97, 2402–2406. [CrossRef]

43. Milledge, J.J.; Harvey, P.J. Golden Tides: Problem or golden opportunity? The valorisation of *Sargassum* from beach inundations. *J. Mar. Sci. Eng.* 2016, 4, 60. [CrossRef]

44. Nielsen, H.B.; Heske, S. Anaerobic digestion of macroalgae: Methane potentials, pre-treatment, inhibition and co-digestion. *Water Sci. Technol.* 2011, 64, 1723–1729. [CrossRef]

45. Xia, A.; Cheng, J.; Murphy, J.D. Innovation in biological production and upgrading of methane and hydrogen for use as gaseous transport biofuel. *Biotechnol. Adv.* 2016, 34, 451–472. [CrossRef]
46. Thompson, T.M.; Young, B.R.; Baroutian, S. Pelagic Sargassum for energy and fertiliser production in the Caribbean: A case study on Barbados. Renew. Sustain. Energy Rev. 2020, 118, 109564. [CrossRef]

47. Kwietniewska, E.; Tys, J. Process characteristics, inhibition factors and methane yields of anaerobic digestion process, with particular focus on microalgal biomass fermentation. Renew. Sustain. Energy Rev. 2014, 34, 491–500. [CrossRef]

48. Chandra, R.; Takeuchi, H.; Hasegawa, T. Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. Renew. Sustain. Energy Rev. 2012, 16, 1462–1476. [CrossRef]

49. Allen, E.; Wall, D.M.; Herrmann, C.; Xia, A.; Murphy, J.D. What is the gross energy yield of third generation gaseous biofuel sourced from seaweed? Energy 2015, 81, 352–360. [CrossRef]

50. Maneein, S.; Milledge, J.J.; Harvey, P.J.; Nielsen, B.V. Methane production from Sargassum muticum: Effects of seasonality and of freshwater washes. Energy Built Environ. 2021, 2, 235–242. [CrossRef]

51. López-Aguilar, H.; Kennedy-Puentes, G.; Gómez, J.; Huerta-Reynoso, E.; Peralta-Pérez, M.D.R.; de la Serna, E.Z.D.; Pérez-Hernández, A. Practical and theoretical modeling of anaerobic digestion of Sargassum spp. In the mexican caribbean. Pol. J. Environ. Stud. 2021, 30, 3151–3161. [CrossRef]

52. Boțheu, D.; Lie, B.; Bakke, R. Oxygen effects in anaerobic digestion—II. Model. Identif. Control 2010, 31, 55–65. [CrossRef]

53. Song, M.; Pham, H.D.; Seon, J.; Woo, H.C. Marine brown algae: A conundrum answer for sustainable biofuels production. Renew. Sustain. Energy Rev. 2015, 50, 782–792. [CrossRef]

54. Oliveira, J.V.; Alves, M.M.; Costa, J.C. Optimization of biogas production from Sargassum sp. using a design of experiments to assess the co-digestion with glycerol and waste frying oil. Bioresour. Technol. 2015, 175, 480–485. [CrossRef] [PubMed]

55. Nadaleti, W.C.; Przybyla, G. Emissions and performance of a spark-ignition gas engine generator operating with hydrogen-rich syngas, methane and biogas blends for application in southern Brazilian rice industries. Energy 2018, 154, 38–51. [CrossRef]

56. Thompson, T.M.; Young, B.R.; Baroutian, S. Advances in the pretreatment of brown macroalgae for biogas production. Fuel Process. Technol. 2019, 195, 106151. [CrossRef]

57. Tabassum, M.R.; Xia, A.; Murphy, J.D. The effect of seasonal variation on biomethane production from seaweed and on application as a gaseous transport biofuel. Bioresour. Technol. 2016, 209, 213–219. [CrossRef]

58. Barbot, Y.N.; Thomsen, L.; Benz, R. Thermo-acidic pretreatment of beach macroalgae from rügen to optimize biomethane production—Double benefit with simultaneous bioenergy production and improvement of local beach and waste management. Mar. Drugs 2015, 13, 5681–5705. [CrossRef]

59. Barbot, Y.N.; Falk, H.M.; Benz, R. Thermo-acidic pretreatment of marine brown algae Fucus vesiculosus to increase methane production—a disposal principle for macroalgae waste from beaches. J. Appl. Phycol. 2015, 27, 601–609. [CrossRef]

60. Ehimen, E.A.; Holm-Nielsen, J.B.; Poulsen, M.; Boelsmand, J.R. Influence of different pre-treatment routes on the anaerobic digestion of a filamentous algae. Renew. Energy 2013, 50, 476–480. [CrossRef]

61. Hom-Diaz, A.; Passos, F.; Ferrer, I.; Vicent, T.; Blánquez, P. Enzymatic pretreatment of microalgae using fungal broth from Trametes versicolor and commercial laccase for improved biogas production. Algal Res. 2016, 19, 184–188. [CrossRef]

62. Li, Y.; Cui, J.; Zhang, G.; Liu, Z.; Guan, H.; Hwang, H.; Aker, W.G.; Wang, P. Optimization study on the hydrogen peroxide pretreatment and production of bioethanol from seaweed Ulva prolifera biomass. Bioresour. Technol. 2016, 214, 144–149. [CrossRef]

63. Cabrera, E.; Muñoz, M.J.; Martín, R.; Caro, I.; Curbelo, C.; Díaz, A.B. Alkaline and alkaline peroxide pretreatments at mild temperature to enhance enzymatic hydrolysis of rice hulls and straw. Bioresour. Technol. 2014, 167, 1–7. [CrossRef]

64. Rabelo, S.C.; Andrade, R.R.; Maciel Filho, R.; Costa, A.C. Alkaline hydrogen peroxide pretreatment, enzymatic hydrolysis and fermentation of sugarcane bagasse to ethanol. Fuel 2014, 136, 349–357. [CrossRef]

65. Messerschmidt, A.; Huber, R. The blue oxidases, ascorbate oxidase, laccase and ceruloplasmin modelling and structural relationships. Eur. J. Biochem. 1990, 187, 341–352. Available online: http://onlinelibrary.wiley.com/doi/10.1111/j.1432-1033.1990.tb15311.x/pdf (accessed on 20 June 2022). [CrossRef]

66. Strong, P.J.; Claus, H. Laccase: A review of its past and its future in biomediation. Crit. Rev. Environ. Sci. Technol. 2011, 41, 373–434. [CrossRef]

67. Wong, D.W.S. Structure and action mechanism of ligninolytic enzymes. Appl. Biochem. Biotechnol. 2009, 157, 174–209. [CrossRef]

68. Kudanga, T.; Nemadziva, B.; Le Roes-Hill, M. Laccase catalysis for the synthesis of bioactive compounds. Appl. Microbiol. Biotechnol. 2017, 101, 13–33. [CrossRef]

69. Carlsson, M.; Lagerkvist, A.; Morgan-Sagastume, F. The effects of substrate pre-treatment on anaerobic digestion systems: A review. Waste Manag. 2012, 32, 1634–1650. [CrossRef]

70. Chen, Y.; Cheng, J.J.; Creamer, K.S. Inhibition of anaerobic digestion process: A review. Bioresour. Technol. 2008, 99, 4044–4064. [CrossRef]

71. Baldrian, P. Fungal laccases—occurrence and properties. FEMS Microbiol. Rev. 2006, 30, 215–242. [CrossRef]

72. Lenihan, P.; Orozco, A.; O’Neill, E.; Ahmad, M.N.M.; Rooney, D.W.; Walker, G.M. Dilute acid hydrolysis of lignocellulosic biomass. Chem. Eng. J. 2010, 156, 395–403. [CrossRef]

73. Ma, L.; Li, A.; Li, T.; Li, M.; Wang, X.; Hussain, M.A.; Qayum, A.; Jiang, Z.; Hou, J. Structure and characterization of laccase-crosslinked α-lactalbumin: Impacts of high pressure homogenization pretreatment. LWT 2020, 118, 108843. [CrossRef] [PubMed]

74. Riva, S. Laccases: Blue enzymes for green chemistry. Trends Biotechnol. 2006, 24, 219–226. [CrossRef] [PubMed]
75. Valero, D.; Rico, C.; Canto-Canché, B.; Domínguez-Maldonado, J.A.; Tapia-Tussell, R.; Cortes-Velazquez, A.; Alzate-Gaviria, L. Enhancing biochemical methane potential and enrichment of specific electroactive communities from nixtamalization wastewater using granular activated carbon as a conductive material. *Energies* 2018, 11, 2101. [CrossRef]

76. Arami-Niya, A.; Wan Daud, W.M.A.; Mjalli, F.S.; Abnisa, F.; Shafeeyan, M.S. Production of microporous palm shell based activated carbon for methane adsorption: Modeling and optimization using response surface methodology. *Chem. Eng. Res. Des.* 2012, 90, 776–784. [CrossRef]

77. Hesas, R.H.; Arami-Niya, A.; Daud, W.M.A.W.; Sahu, J. Preparation and characterization of activated carbon from apple waste by microwave-assisted phosphoric acid activation: Application in methane blue adsorption. *BioResources* 2013, 8, 2950–2966.

78. Cheng, Q.; De Los Reyes, E.L.; Call, D.F. Amending anaerobic bioreactors with pyrogenic carbonaceous materials: The influence of material properties on methane generation. *Environ. Sci. Water Res. Technol.* 2018, 4, 1794–1806. [CrossRef]

79. Freitas, A.F.; Mendes, M.F.; Coelho, G.L.V. Thermodynamic study of fatty acids adsorption on different adsorbents. *J. Chem. Thermodyn.* 2007, 39, 1027–1037. [CrossRef]

80. Da Silva, A.H.; Miranda, E.A. Adsorption/desorption of organic acids onto different adsorbents for their recovery from fermentation broths. *J. Chem. Eng. Data* 2013, 58, 1454–1463. [CrossRef]

81. Bernard, O.; Polit, M.; Hadj-Sadok, Z.; Pengov, M.; Dochain, D.; Estaben, M.; Labat, P. Advanced monitoring and control of anaerobic wastewater treatment plants: Software sensors and controllers for an anaerobic digester. *Water Sci. Technol.* 2018, 2, 175–182. [CrossRef]

82. Milquez-Sanabria, H.; Blanco-Cocom, L.; Alzate-Gaviria, L. A fast linear predictive adaptive model of packed bed coupled with UASB reactor treating onion waste to produce biofuel. *Microb. Cell Fact.* 2016, 15, 167. [CrossRef]

83. Mao, C.; Feng, Y.; Wang, X.; Ren, G. Review on research achievements of biogas from anaerobic digestion. *Renew. Sustain. Energy Rev.* 2015, 45, 540–555. [CrossRef]

84. Florentino, A.P.; Xu, R.; Zhang, L.; Liu, Y. Anaerobic digestion of blackwater assisted by granular activated carbon: From digestion inhibition to methanogenesis enhancement. *Chemosphere* 2019, 233, 462–471. [CrossRef] [PubMed]

85. Johnravindar, D.; Liang, B.; Fu, R.; Luo, G.; Meruvu, H.; Yang, S.; Yuan, B.; Fei, Q. Supplementing granular activated carbon for enhanced methane production in anaerobic co-digestion of post-consumer substrates. *Biomass Bioenergy* 2020, 136, 105543. [CrossRef]

86. Calabrò, P.S.; Fazzino, F.; Folino, A.; Scibetta, S.; Sidari, R. Improvement of semi-continuous anaerobic digestion of pre-treated orange peel waste by the combined use of zero valent iron and granular activated carbon. *Biomass Bioenergy* 2019, 129, 105337. [CrossRef]

87. Martínez, E.J.; Rosas, J.G.; Sotres, A.; Moran, A.; Cara, J.; Sánchez, M.E.; Gómez, X. Codigestion of sludge and citrus peel wastes: Evaluating the effect of biochar addition on microbial communities. *Biochem. Eng. J.* 2018, 137, 314–325. [CrossRef]