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Effects of the Chemical and Mechanical Pre-Treatment of Brown Seaweed on Biomethane Yields in a Batch Configuration

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Abstract: Brown seaweed could be a viable option for biogas production, with the added advantage of not competing with land-based crops, which negates the food vs. fuel argument. To optimise the process, this research investigates using mechanical and chemical pre-treatment to increase the biomethane yield of seaweed. The biomethane potential, biodegradability index, and biomethane yields were determined as well as the kinetics based on the hydrolysis of the anaerobic digestion process. Mechanical pre-treatment showed the highest increase in methane yield for the smaller size (<1.7 mm), recording yields of 126.16 mL/g VS after 28 days when compared to 31.54 mL/g VS for the control (2–3 mm). Chemical pre-treatment yielded higher methane rates (34.59–60.33 mL/g VS) than the control, but not as high as the mechanical pre-treatment processes. First-order kinetics described the anaerobic digestion process, with k-values between 0.050 and 0.106. The biodegradability index was between 0.145 and 0.580. The research increased the knowledge base of the potential of the *Ecklonia Maxima* seaweed to produce biogas. Careful consideration of the impact on the overall process must be completed to determine the advantages or disadvantages of including a pre-treatment step in the process under consideration.

Keywords: *Ecklonia Maxima*; biogas; anaerobic digestion; kinetics

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1. Introduction

Global warming affects everyone on the planet. It primarily results from fossil fuel usage, producing carbon dioxide emissions [1]. These emissions have increased steadily despite climate change agreements [2], leading to increased extreme weather phenomena. Examples include increased flash floods, rising ocean temperatures, and melting ice caps [3]. There is a need for alternate renewable and sustainable energy sources, which could provide energy security for vulnerable countries. Significant attention has been paid to the production and usage of biofuels as an alternate energy feedstock. Biofuels compete well with fossil fuels as they are renewable, and when grown sustainably, they are carbon neutral, which could lead to a net reduction in greenhouse gas emissions [4].

Feedstocks for biofuel production include sugarcane, corn, agricultural wastes and algal-derived feedstocks [2]. The problem is that land-based crops, even though they are well documented and have shown promising results in biofuel production, may take away from the food security of a country, especially in countries already battling with food insecurity [1]. Macroalgae, which are third-generation biomasses, could be a viable alternative as they do not require agricultural land for cultivation [5] and contain high levels of carbohydrates, which can be converted to biogas and low levels of lignin [6].

Research has shown that seaweed could be utilised to produce biomethane [5,7–9]. Pre-treatment processes are investigated to improve the efficiency and yield of the biomethane. They are used to destroy or remove recalcitrant materials or increase the bioavailability of polysaccharides, which will improve the biogas yield while at the same time reducing costs [10]. Pre-treatment processes include biological, chemical, physical, and heat processes [11], and are used before the start of the anaerobic digestion process to increase the digestibility of the feedstock and, therefore, speed up the hydrolysis stage of anaerobic digestion [12], which is widely accepted as the rate-determining step [13].

Mechanical pre-treatment could include beating, milling, or sonication. It is used to reduce the particle size of the biomass, therefore increasing the biomass's surface area to volume ratio, which increases the rate of hydrolysis in the anaerobic digestion process [10,13]. A biomaterial may also be treated chemically, using either an acid or an alkaline to break down the chemical structure of the biomass. Alkaline pre-treatment causes swelling in the pores of the biomaterial, leading to simultaneous solvation and saponification processes, which effectively release sugars faster for microbial digestion [13]. Whether an acid or alkaline pre-treatment is used, it invariably increases the costs of the overall process, mainly if very harsh chemicals are used at elevated temperatures (80–120 °C) [11]. Acid pre-treatment has the added cost of utilising acid-resistant equipment in the process and recycling the used acid [10].

- Seaweed has proven to be a suitable feedstock for biogas production. However, the differences in operating conditions and the differing compositions resulting from growth conditions make predicting and optimising biogas yields challenging based on previous studies performed. The macroalgae under consideration is a brown benthic seaweed found in abundance along the West Coast of South Africa (SA). *Ecklonia Maxima* has been collected as beach-cast seaweed since the 1950s, and harvested from the ocean floor since the 1970s [14] according to a maximum sustainability yield for the zone it is grown in. It can grow up to 15 m high and can be harvested up to 8 times per year if the frond is harvested. There is limited research on biomethane production utilising *Ecklonia Maxima*, as it is primarily utilised as an abalone feed and in the production of plant growth stimulants. Since 2003, approximately 6000 to 7000 tonnes per annum of *Ecklonia Maxima* was harvested as abalone feed alone. On a dry basis, it consists of mostly carbohydrates (25–50%), proteins (7–15%), and a small amount of lipids (1–5%), making it an ideal feedstock for biomethane production. In SA., *Ecklonia Maxima* has the potential to be commercially farmed, which makes it a viable alternative to land-based crops for energy production.
- The hypothesis of the research is that pre-treatment of the macroalgae will increase the biomethane yield when compared to a control sample using an anaerobic digestion process. To optimise the biogas yield from brown seaweed, this research aims to investigate the use of size reduction and chemical pre-treatment to determine its effects on the biogas yield using *Ecklonia Maxima* in a semi-batch configuration. The research will develop new insights into, and add to the limited data availability for the pre-treatment of *Ecklonia Maxima* to improve biomethane yield, and to explore the macroalgae as a viable feedstock for an industrial process for cleaner energy production.

2. Materials and Methods

2.1. Materials

The seaweed feedstock, *Ecklonia Maxima*, was bought in the dried form as chips from a local supplier in the Western Cape Province of South Africa. Once harvested, the seaweed was rinsed, dried, chipped, and packaged in 25 kg bags. The inoculum that provided the initial microbial community for biomass degradation was a synthetic all-purpose blended organic fertiliser with high nitrogen content (Atlantic Bioganic). The anaerobic digestion reactor consisted of a double-walled 1 L glass reactor maintained at mesophilic temperatures by running water through the jacket (Figure 1). The reactor inlet tube was connected to a flexible tube with a clamp, and the outlet tube (which could also be clamped) was connected to a gas bubbler to measure the gas yield using a water displacement method, and finally to a gas bag where the biomethane produced was collected. The pH was measured using a Hanna Edge pH meter (model HI2002) with HI11311 Electrode. Dissolved oxygen was measured using a Hanna Edge DO meter (model HI2004) with HI764080 Electrode. All the gases produced during the AD process were collected in a 1 L Supel inert foil SCV gas bag. The gases produced were analysed using a Biogas 5000 Portable Gas Analyser (Biogas 5000 ATEX,CSA.IECEx, Serial no G507348), which can analyse methane, carbon dioxide, and hydrogen sulphide. The water temperature was controlled using a water bath and measured using a digital temperature probe in the reactor.

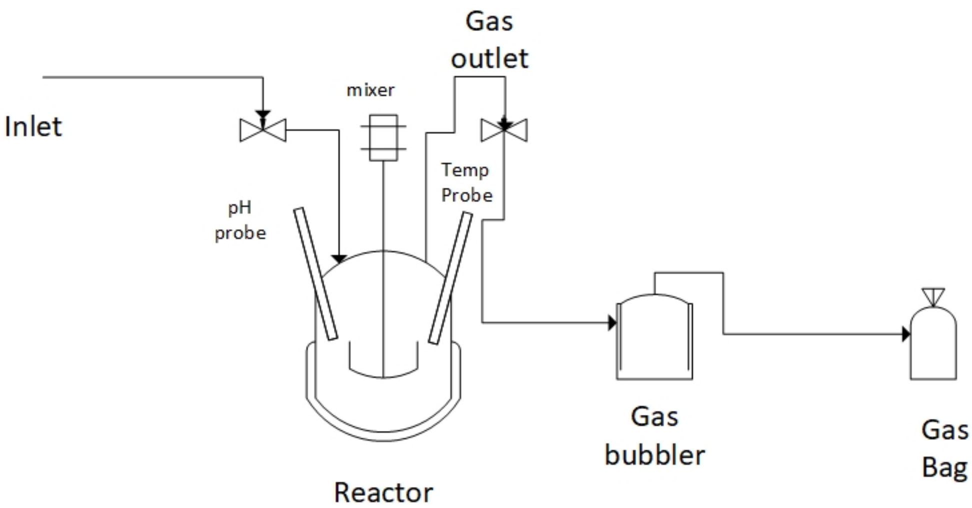

Figure 1. Experimental setup.

2.2. Methods

2.2.1. Feedstock Preparation and Analysis

The seaweed feedstock was ground to approximately 3 mm for the control experiments. The seaweed was rinsed with distilled water, dried at 80 °C until a constant weight was obtained, and stored in an airtight container until required. The seaweed samples were sent to an outside laboratory for chemical analysis to determine the C, H, O, N, and S composition using inductively coupled plasma mass spectrometry (ICP-MS). The carbohydrate and protein content were provided by the company that supplied the *Ecklonia Maxima* as part of a certificate of analysis. The lignin content of the seaweed was determined using the Klason Method [15]. The total solids (TSs), total volatile solids (TVSs), and ash content were determined using method 1684 from [16].

2.2.2. Analytical Methods and Calculations

The seaweed's biomethane potential (BMP) was theoretically determined based on the chemical composition of the seaweed using a modified version of the Buswell equation, which included the sulphur in the seaweed [17]

$$\mathrm{BMP} = \frac{22.4\mathrm{x}\left(\frac{\mathrm{a}}{2} + \frac{\mathrm{b}}{8} + \frac{\mathrm{c}}{4} + \frac{3\mathrm{d}}{8} + \frac{\mathrm{e}}{4}\right)}{12.017\mathrm{a} + 1.0079\mathrm{b} + 15.999\mathrm{c} + 14.0067\mathrm{d} + 32.065\mathrm{e}} \quad (1)$$

where a, b, c, d, and e are coefficients based on the chemical composition of the seaweed, corresponding to the fraction of carbon, hydrogen, oxygen, nitrogen, and sulphur, respectively.

The biodegradability index (BI) of the process was determined as

$$\mathrm{BI} = \frac{\text{methane yield after pre} - \text{treatment}}{\text{theoretical yield of methane}} \times 100 \quad (2)$$

The carbon conversion to methane was estimated as follows:

$$\text{Carbon conversion to methane} = \frac{\text{fraction of methane formed}}{\text{methane fraction} + \text{carbon dioxide fraction}} \quad (3)$$

The kinetics were compared assuming first-order kinetics and assuming that hydrolysis was the rate-determining step [18]:

$$\mathrm{B(t)} = \mathrm{B_0}\left(1 - \exp^{-\mathrm{kt}}\right) \quad (4)$$

where B is the cumulative methane yield (mL/g VS); B_0 is the ultimate methane yield (mL/g VS); k is the hydrolysis rate constant (days^{-1}); and t is the time (days).

The cumulative methane yield can be estimated at different hydraulic retention times using the following equation [18] assuming a continuous system:

$$\mathrm{B}(\tau) = \mathrm{B_0}\left(1 - 1/(1 + \mathrm{k}\tau)\right) \quad (5)$$

where B(τ) is the cumulative methane yield (mL/g VS); B_0 is the ultimate methane yield (mL/g VS); k is the hydrolysis rate constant (days^{-1}); and τ is the hydraulic retention time (days).

2.2.3. Anaerobic Digestion Process

The process considered for biogas production is anaerobic digestion. It is a biological process that occurs due to microorganisms degrading organic matter in the absence of oxygen to form biogas. Anaerobic digestion can process wet biomass, which is advantageous and cost-effective as the macroalgae will not have to be dried beforehand. The process consists of four stages, as shown in Figure 2. Stage one is hydrolysis, where proteins and fats are decomposed. Stage two is acidogenesis, where acidifying bacteria convert hydrolysis products to short-chain organic acids. Stage three is acetogenesis, where acetogenic bacteria produce acetic acid, hydrogen, and carbon dioxide. Stage four is methanogenesis, where methanogenic bacteria produce methane and carbon dioxide [19].

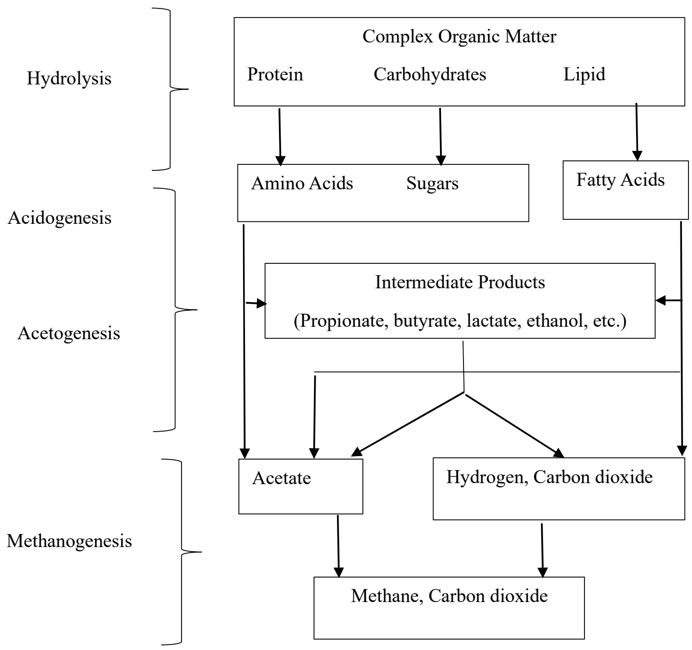

Figure 2. Anaerobic digestion process (adapted from [20]).

2.2.4. Experimental Procedure

Anaerobic digestion experiments were performed over 28 days at mesophilic conditions to determine the methane production of the seaweed with no pre-treatment (control). The seaweed to inoculum load was 1:1 based on the volatile solids. The reaction volume was 800 mL, which consisted of 400 mL seaweed slurry (20% *w*/*w*) and 400 mL inoculum slurry (20% *w*/*w*). Seaweed slurry, inoculum slurry, and the reactor were separately homogenised at 37 °C, and the pH of the slurries was adjusted to 7 ± 0.2. The reactor was purged with nitrogen to remove any oxygen in the system. The homogenised substrate and inoculum were added to the reactor, and the pH was adjusted to 7 ± 0.2. The system was once again purged with nitrogen until the dissolved oxygen reading was less than one ppm. The reactor was fed a slurry mixture of macroalgae on the sampling days. On days 8, 11, 15, 19, and 23, 80 mL of slurry was removed from the reactor. The TVS of the slurry was determined, and an equivalent amount of volatile solids was added to the reactor as seaweed slurry. As this is a semi-batch system, it was important to maintain the VS ratio in the reactor as the methane produced is determined per gram of VS present in the reactor. On sampling days, the methane yield was collected from the gas bags and analysed for methane, hydrogen sulphide, and carbon dioxide.

These experiments were repeated and then duplicated twice for each pre-treatment method (either mechanical or acid-rinsed or alkaline-rinsed) and analysed for methane yield.

2.2.5. Biomass Pre-Treatment

The mechanical pre-treatment of the seaweed included grinding the seaweed to 2 additional size fractions (<1.7 mm and 1.7–3 mm) before the AD process. After grinding, the seaweed was rinsed with distilled water and dried to a constant weight. The same operating conditions of 37 °C for 28 days were utilised for the AD experiments. The chemical pre-treatment included both acid and alkaline pre-steps. Before the AD process, the seaweed was soaked for 1 h using HCl at two different concentrations (0.15 M and 0.3 M). The seaweed was then rinsed with distilled water and dried until a constant weight was obtained. The alkaline pre-treatment included soaking the seaweed in NaOH at two concentrations (0.15 M and 0.3 M) for one hour before the AD processes. The NaOH-soaked seaweed was rinsed with distilled water and dried until a constant weight was reached. All the pre-treatment processes were performed on individual batches of feedstock and conducted as separate anaerobic digestion experiments at 37 °C for 28 days. The methane yielded for each experiment was collected in gas bags and analysed for methane, hydrogen sulphide, and carbon dioxide.

2.2.6. Statistical Analysis

One-way ANOVA with a 95% confidence interval was utilised to compare the data collected. The null hypothesis stated that the pre-treatment processes did not influence the biomethane yield for the anaerobic digestion process of the *Ecklonia Maxima*. If the calculated f-value was more than 1, the null hypothesis would be rejected, and if the f-value was less than 1, the null hypothesis would be valid.

3. Results and Discussion

3.1. Seaweed Analysis and Characterisation

The elemental analysis for raw seaweed is given in Table 1. The seaweed has a high carbon content, which is advantageous for methane production. The C:N ratio is 10.84, which is lower than the ratio of 14.11 reported by [21]. It should be noted that the seaweed in this study by [21] was subjected to a different process of polysaccharide extraction. Polysaccharide extraction involves extracting long-chain carbohydrates from a molecule using methods such as steam, alkaline treatment, microwaving, or ultrasonic-assisted extraction. The seaweed was enzymatically hydrolysed, and the solid hydrolysis residue was used as the raw material. The entire *Ecklonia Maxima* frond was, therefore, not used; hence, the ratio of C:N would be different. The global average for C:N ratios for seaweed is 20.2 ± 14.5 SD with a range between 5.6 and 122.5 [22]. The ratio indicates the amount of nitrogen the seaweed assimilates per mole of carbon via photosynthesis. It is influenced by the growth conditions and the area of harvesting, which account for the wide range identified previously [23]. There could be distinct differences in composition between the frond and the holdfast because they sequester nutrients differently. The time of the year the seaweed is harvested could also influence the overall composition.

Table 1. Analysis of *Ecklonia Maxima*.

Elemental Composition		Organic Analysis	
Element	**Mass % (m/m)**	**Component**	**Mass % (m/m)**
C	51.15	Protein	12.01%
H	19.99	Carbohydrate	51.83%
O	3.789	Ash	25.52%
N	4.719		
S	11.512		

Ecklonia Maxima has a high carbohydrate content, which is favourable for its conversion to biogas. The average carbohydrate content of brown seaweed is between 30% and 50% [23]. *Ecklonia Maxima* is at the upper limit of this range. High-yield biogas production is expected to be achieved, although the ease of carbohydrate conversion is not indicated. A high nitrogen concentration in the biomass could inhibit the formation of methane. If the C:N ratio is low, it could cause the ratio of the ammonium to nitrogen to increase, therefore inhibiting the formation of methane [24]. The TVS of the seaweed biomass was determined to be 0.72 g/g. The TVS of the inoculum was determined as 0.72 g/g as well. The lignin content was determined to be 29% (m/m), higher than expected. The lignin content of Sargassaceae, which is a brown seaweed, was determined using a modified version of the Klason method as between 6% and 12.9% of the dry weight of the seaweed [25].

The differences could be attributed to the different processing conditions of the modified Klason method versus the original Klason method. Due to the high lignin content, a pre-treatment step is needed for the *Ecklonia Maxima* to release fermentable sugars to optimise biogas production.

3.2. Calculated Variables

The calculated biomethane potential using Equation (1) was 499.64 mL/g seaweed. This is based on the following stoichiometric equation of the *Ecklonia Maxima* for 1 mole of seaweed:

$$C_{0.0426}H_{0.0472}O_{0.0024}N_{0.0143}S_{0.0036} + 0.0422H_2O \rightarrow 0.0204CH_4 + 0.0223CO_2 + 0.0143NH_3 + 0.0036H_2S$$

The corresponding expected carbon conversion to methane based on the coefficients of CO_2 and CH_4 is 47.78%. These calculations do not consider the hydrolysis stage and are, therefore, a higher estimate than the actual yield. The calculation also assumes that the mass fractions of carbon, hydrogen, oxygen, nitrogen, and sulphur constitute the entire composition of the seaweed feedstock, which is not the case. These components only account for approximately 91% of the seaweed composition. Taking into account the carbon conversion, as well as the composition of the seaweed, the theoretical BMP can be estimated as 217.24 mL/g VS. The literature reports actual biomethane yields of between 19% and 81% of the BMP [26]. The expected biomethane yields for the experiments should, therefore, be in the range of 41.28 mL/g VS to 175.96 mL/g VS.

The calculated BI is shown in Table 2. The highest BI is seen for the <1.7 mm size distribution, with the lowest BI calculated for the control sample. A high BI value indicates high amounts of organic matter available for biodegradation. Therefore, the methane yield is expected to be highest based on the highest BI value determined. The varying BI indicates the difficulty digesting the seaweed, influenced by the varying pre-treatment processes.

Table 2. Biodegradability index for *Ecklonia Maxima*.

Seaweed	Biodegradability Index
Control	0.145
Mechanical (<1.7 mm particle size)	0.580
Mechanical (1.7–3 mm particle size)	0.243
Acid (0.15 M HCl)	0.278
Acid (0.3 M HCl)	0.190
Alkaline (0.15 M NaOH)	0.159
Alkaline (0.3 M NaOH)	0.260

The BI values align with the documented results [27] of 0.19 to 0.81 for varying seaweeds. The mechanically pre-treated seaweed has the highest BI values, with the weak acid and strong base comparable to the larger particle distribution for the seaweed. It is still almost 50% less than the smaller particle size distribution, which indicates that the smaller particle size has more organic material available to be degraded. This could be attributed to the pH of the system for the acid pre-treated and alkaline pre-treated seaweed. At low pH values, the volatile fatty acids accumulate, which inhibits degradation. At higher pH values, free ammonia increases in the system, which is toxic to methanogenesis [28].

3.3. Methane Yields

Cumulative methane yield was calculated for all the scenarios, as shown in Table 3. Methane accounted for between 20% and 40% of the total gas collected. Initially, the methane produced was high, but as the AD system approached the stationary phase, the daily methane formation reduced significantly. All the pre-treatment processes showed increased methane yields of between 9% and 300%. This corresponded to between 15.92% and 58.07% of the BMP for *Ecklonia Maxima*. The maximum methane yield was obtained using mechanical pre-treatment using the smaller particle size distribution of less than 1.7 mm, accounting for a 300% increase in methane yield compared to the control experiments.

Table 3. Cumulative methane yields for un-treated and pre-treated *Ecklonia Maxima*.

Substrate	Methane Yield (mL/g VS)	% Change in Methane Yield	% of Theoretical Methane Yield
Untreated (control)	31.54	-	14.51
Mechanical (<1.7 mm particle size)	126.16	+300	58.07
Mechanical (1.7–3 mm particle size)	52.74	+67.2	24.28
Acid (0.15 M HCl)	60.33	+91.28	27.77
Acid (0.3 M HCl)	41.30	+30.94	19.01
Alkaline (0.15 M NaOH)	34.59	+9.67	15.92
Alkaline (0.3 M NaOH)	56.53	+79.23	26.02

The control sample yielded 31.54 mL/g VS of methane, which corresponds to 14.51% of the BMP. This is lower than expected. However, it was previously mentioned that the seaweed had a high ash content and would need a pre-treatment step to release the sugars required for hydrolysis. The HRT for the control sample is 26 days, after which the AD system stabilises and the AD process enters a stagnant phase. When comparing this trend to the mechanically pre-treated seaweed, it is seen that the exponential growth phase of the AD process is still evident after 28 days, as shown in Figure 3, with the continued upward trend of both the cumulative methane curves for the two size distributions. This indicated that methanogenesis was still taking place after 28 days of reaction, inferring that the seaweed could still produce methane. The biomethane production could, therefore, be estimated at higher HRT using the kinetic parameters of the system, or the HRT increased through experimentation to determine the actual methane yield. Consequently, the percentage change in methane yield and the percentage of the theoretical yield could be a lot higher than the tabulated values in Table 3 for the mechanically pre-treated feedstock. Ref. [21] reported a yield of 26% of the BMP for *Ecklonia Maxima*, which compared well with the higher particle size distribution shown below.

The acid pre-treatment process showed better results when compared to the control. Methane yield was initially slow, but it increased as the system's pH stabilised. The 0.15 M HCL pre-treatment yielded better results, almost doubling the methane yield compared to the control. Pre-treating the seaweed with acid caused a drop in pH for the system, taking it below the optimal pH operating range of between 7 and 7.6 for the AD process. The number of protons increased due to acid pre-treatment, which prolonged the acidogenic effect, delaying the methanogenesis stage of AD. This phenomenon could also explain the lag phase for all the processes as the system took some time to reach the optimum pH range. While the acid pre-treatment caused a decrease in pH, the alkaline pre-treatment caused the pH to increase beyond the optimum range. Ref. [29] found that as the pH increased from 9 to 13, the methane yield decreased from 363 to 213 mL/g VS using a pre-treated NaOH–seaweed blend. At a pH of 9, the yield was already less than the raw seaweed results, emphasising that the methane yield is negatively affected once the AD process operates outside its optimum pH range. The control of the alkaline pre-treatment process was also complex as residual NaOH in the system after degradation destroyed the bicarbonate buffer of the system, leading to a higher pH, which not only inhibited the microorganisms in the system but also negatively affected the AD process. There was insufficient biomass breakdown at low doses of NaOH pre-treatment, which affected hydrolysis. At higher doses of NaOH, the AD process was inhibited; therefore, the methane yield was lower than expected due to an increase in pH. The 0.15 M NaOH pre-treated seaweed only started producing methane after 15 days. The resultant methane yield, while more than the control sample, was less than the acid pre-treatment and the size reduction. These observations were also noted by [30] when using chemical pre-treatment concentrations of between 0.05 and 0.5 M NaOH, where the 0.3 M NaOH methane yield was higher at 181% of the raw seaweed yield.

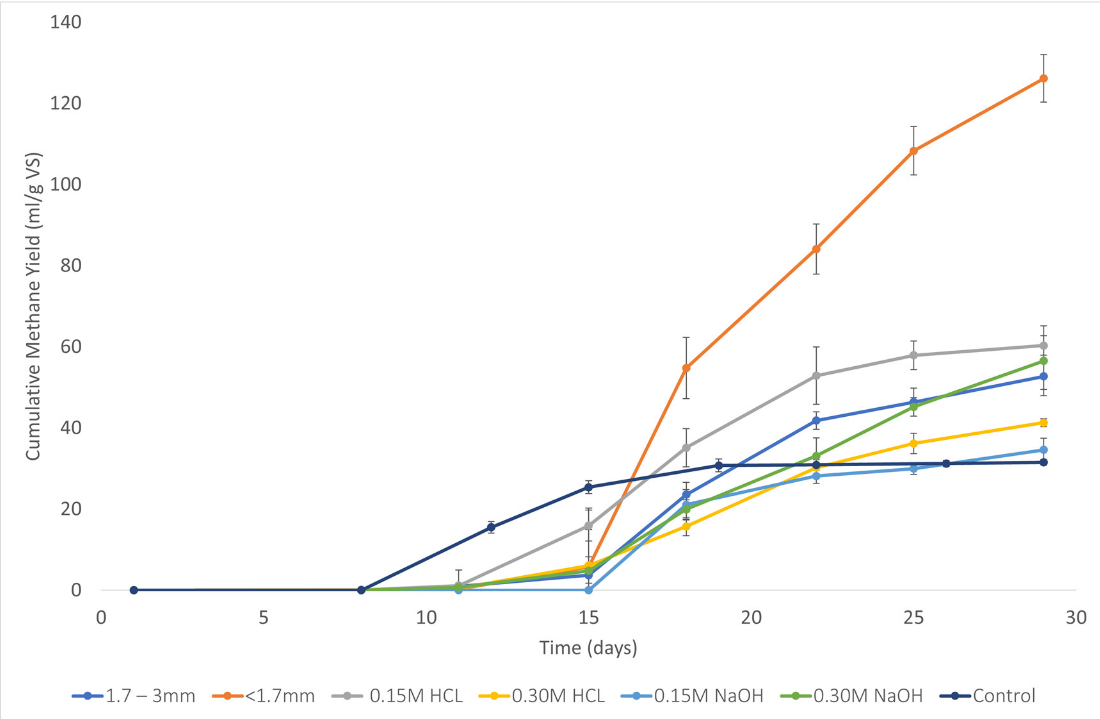

Figure 3. Cumulative methane yield for pre-treated *Eckonia Maxima*.

Biogas yields and operating conditions using macroalgae in the cited literature vary significantly, as shown in Table 4, making comparing and predicting process performance difficult. The maceration results were conflicting using *Ulva Lactuca*. Methane yields were reported to decrease with mechanical pre-treatment by [31]. They demonstrated an increase by [32], as well as [33] following mechanical pre-treatment. A slight decrease was reported for *Saccharina Latissimi* [32], whereas increases were shown for *Laminariacea* spp. [34] and *P. canaliculate* [35]. The bigger size distribution of 1.7 mm–3 mm was comparable to that obtained for the washed *Ulva Lactuca* biomass. A mixture of smaller and larger particles would benefit the methane yield during anaerobic digestion as the smaller particles might be easier to digest at the beginning of the AD process [13]. However, they would not necessarily increase the overall biogas production as factors such as pH, reactor blockages, and inoculum must be considered.

For *Laminaria* spp., the percentage increase varies even though the seaweed was harvested from the same area. The seaweed was, however, harvested at different times of the year, meaning the seaweed had different chemical compositions. This would affect the calculation for the biomethane potential of the control feedstock and, therefore, affect the overall yield increase or decrease in the methane produced. This, once again, emphasises the importance of seaweed composition based on harvesting time and conditions as described by [23].

Table 4. Overview of biomethane yields where mechanical and chemical pre-treatment methods were utilised on seaweed feedstock.

Feedstock	Pre-Treatment	Methane Yield (% Change)	Reference
	Mechanical Pre-Treatment		
Ulva Lactuca	Maceration	-	[31]
	Maceration (washed)	+67.7%	[32]
	Maceration	+32%	[33]
Saccharina Latissima	Maceration	−2.1%	[32]
P. canaliculata	Beaten	+74%	[35]
	Beaten	+179%	[13]
	Beaten	+70%	[36]
Laminaria spp.	Beaten	+53%	[34]
	Ball milled; particle size 1–2 mm	−26.5%	[31]
	Beaten—harvested Nov 2013	+2.1%	[31]
	Beaten—harvested May 2014	+8.6%	[37]
Gracilaria gracilis	Beaten	+52%	[33]
G vermiculophylla	Maceration—unwashed	+14.6%	[38]
	Maceration—washed	+11.9%	[38]
	Maceration—washed and dried	+7.7%	[38]
	Maceration—washed	+11.4%	[32]
F. vesiculosus	Chopped; washed	+95%	[36]
		+220%	[13]
Ulva spp.	Grinding	+18%	[33]
	Chemical Pre-treatment		
Ulva spp.	0.1 M HCl at 90 °C	+12.7%	[39]
	0.04 g HCL/g TS at 150 °C	+12.1%	[40]
	0.1 M NaOH @ 90 °C	−0.7%	[39]
	0.04 g NaOH/g TS at 20 °C	−41.7%	[40]
P. palmata	0.04 g NaOH/g TS at 160 °C	−8.4%	[41]
L. digitata	2.5% citric acid	+3.9%	[42]
	2.5% citric acid	+4%	[13]
	6% citric acid	−69.7%	[42]
	6% citric acid	−330%	[13]
G. vermiculophylla	0.05 g NaOH/g seaweed at 90 °C	−21%	[39]

There were mixed results for *L. digitata* using citric acid. A slight increase in biomethane yield was observed using 2.5% citric acid, followed by a more than 69.7% reduction in biomethane yield using 6% citric acid [42]. This indicates that low pH values inhibit the anaerobic digestion process, which has an optimum operating pH of between 7 and 7.6 [23,30,43–46]. Chemical pre-treatment shows limited benefits in terms of increased solubility based on the results shown in Table 4. The acid pre-treatment in this study showed an overall increase in biomethane yield due to the fact that pH adjustments were carried out whenever a sample was taken to ensure the pH range was within the optimum pH range for anaerobic digestion.

The alkaline pre-treatment decreased the methane yield for *Ulva* spp., *P. palmata*, and *G. vermiculophylla* [39,41,42].

The one-way ANOVA results are shown in Table 5. The f-value determined was 1.32, and the f-critical value is more than this value at 2.29. The null hypothesis is, therefore, rejected. Pre-treatment processes do influence the methane yield. This is corroborated by the results discussed above.

Table 5. One-way ANOVA results.

ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	***p*-Value**	**F Crit**
Between Groups	5934.29	6	989.048	1.319	0.2662	2.290
Within Groups	36,719.48	49	749.377			
Total	42,653.77	55				

3.4. Kinetics and Methane Yield Predictions

First-order kinetics was determined using Equation (4). The rate constant values ranged between 0.05 and 0.1 (Table 6). The rate constant indicates the rate at which methane is produced. As can be seen, the pre-treated smaller size fraction of seaweed showed the highest reaction rate for hydrolysis, which also corresponded to the highest methane yield. The lowest reaction rate constant corresponds to the control sample, which had the lowest methane yield and also the lowest calculated BI.

Table 6. Rate constants for pre-treated *Ecklonia Maxima*.

Seaweed	**B_o (mL/g VS)**	**% Theoretical CH_4**	**k (-Day)**	**R^2**
Control	31.537	14.51	0.050407	81.59
Size < 1.7 mm	126.157	58.07	0.10615	89.62
Size 1.7–3 mm	52.740	24.28	0.073578	89.54
Acid pre-treated 0.15 M HCL	60.325	27.77	0.080572	92.69
Acid pre-treated 0.3 M HCl	41.3	19.01	0.062585	91.73
Alkaline pre-treated 0.15 M NaOH	56.533	15.92	0.056908	86.04
Alkaline pre-treated 0.3 M NaOH	52.740	26.02	0.073071	90.5

To estimate the methane yield in a continuous system, Equation 5 is applied using the calculated hydraulic rate of retention and the ultimate methane yield. It is a valuable tool once the ultimate methane yield is determined. Based on the data provided and the upward trend for the cumulative methane, as seen in Figure 3, it is clear that the pre-treated seaweed could have a higher ultimate methane yield if the HRT was increased beyond 28 days. The <1.7 mm size distribution was subjected to further experimentation by extending the HRT to 42 days, which resulted in an ultimate methane yield of 190.06 mL/g VS, corresponding to 87.48% of the BMP.

Figure 4 shows the cumulative methane yield assuming a continuous system for the pre-treated *Ecklonia Maxima* based only on the batch-determined ultimate methane yields. The <1.7 mm size fraction still shows better methane production, which is expected based on the maximum methane produced at 28 days. The system is predicted to undergo a growth phase for the first 100 days, and then stabilise.

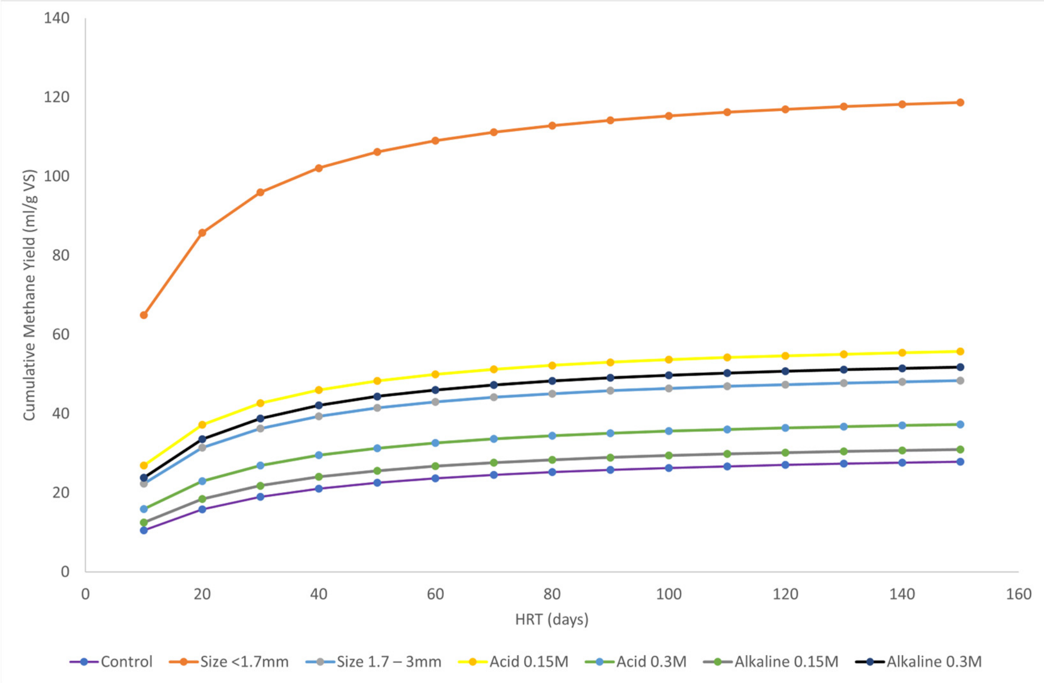

Figure 4. Cumulative methane yields at different HRTs.

4. Conclusions

Ecklonia Maxima is a promising potential feedstock for biogas production using anaerobic digestion. All the pre-treatment processes increased the biomethane yield between 9% and 300% of the control experiments. The most significant increase was recorded for the mechanically smaller size distribution (<1.7 mm), which yielded 126.16 mL/g VS methane over the first 28 days. Increasing the HRT to 42 days saw an ultimate biomethane yield of 190.06 mL/g VS, corresponding to 87.5% of the BMP determined, compared to the 58.1% BMP after 28 days. This smaller distribution also showed a high biodegradability index of 0.580, indicating the proportion of degradable organic matter that can be converted to biogas.

pH control was an important consideration along with challenging effluent treatment, which could have additional cost implications. It is important to maintain a pH within the operating pH to promote methanogenesis, which was not always easy due to the acid or alkaline used to pre-treat the seaweed.

The research conducted not only gives insight into the use of *Ecklonia Maxima* as a possible feedstock for biomethane production but is also the first step in identifying parameters for the modelling of the AD process specifically for macroalgae. The composition of the seaweed plays an important role and should be included in any modelling framework developed for the process. Size distribution is a bigger factor to consider than chemical treatment in this instance. A thorough techno-economic study needs to be completed to incorporate the size reduction costs and to determine if collecting and drying the seaweed would be more cost-effective than purchasing the seaweed.

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